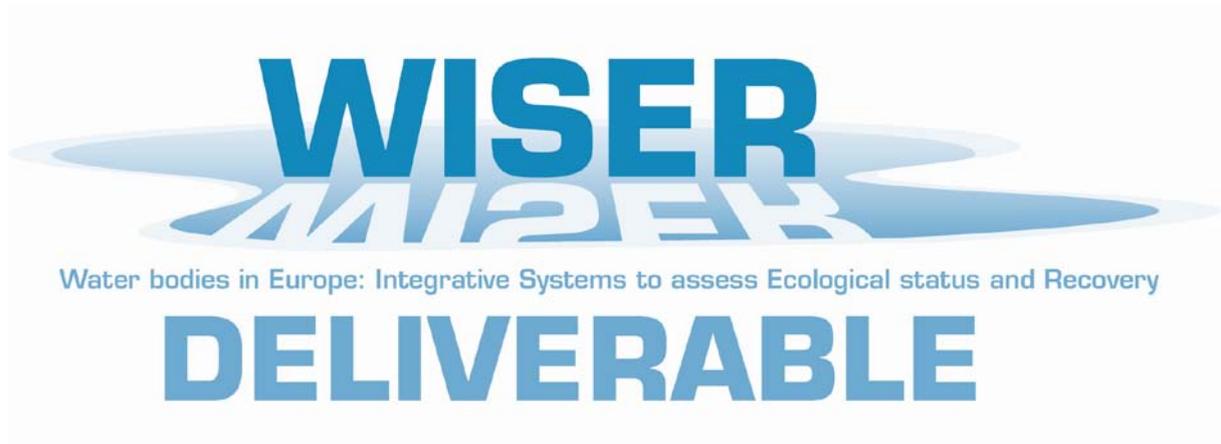


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## **Deliverable D2.2-1: Database on assessment methods for lakes, rivers, coastal and transitional waters in Europe**

Lead contractor: **University of Duisburg-Essen (UDE)**

Contributors: **Sebastian Birk**

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Dissemination Level

PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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## Non-technical summary

EU Member States are monitoring the ecological status of their surface waters by the use of biological assessment methods. These methods address various biological groups (i.e. Biological Quality Elements) such as phytoplankton, benthic flora, benthic invertebrates and fish fauna. Most Member States have developed their own assessment methods, thus many different methods currently exist to monitor the ecological status.

To provide an overview of the different methods, the WISER project has collected detailed information by means of a questionnaire-based survey. Data of more than 200 national methods were received and have been stored in the WATERVIEW2-Database. All information will be made publicly available via the project's website <http://www.wiser.eu>. This deliverable contains the detailed descriptions of 231 national assessment methods.

## Database on assessment methods for lakes, rivers, coastal and transitional waters in Europe

### Introduction

The European Water Framework Directive (WFD) requires to classify the quality status of rivers, lakes, coastal and transitional waters. The ecological status is evaluated by biological assessment methods using selected biological quality elements, i.e. phytoplankton, macrophytes and phytobenthos (lakes and rivers), angiosperms and macroalgae (coastal and transitional waters), benthic invertebrate fauna and fish fauna. The 27 European Member States are in charge of developing these methods, and the classification of good ecological status is harmonised in a Europe-wide intercalibration exercise.

Against this background there is a growing need for the exchange of information and data on biological assessment methods. Most methods have been developed only recently, and Member States are interested in improving and updating their schemes. The obligation to intercalibrate the national classification of good ecological status further requires precise descriptions of the national methods' features (European Communities 2009).

A main objective of the WISER project is thus to generate an overview of biological assessment methods for lakes, rivers, coastal and transitional waters currently in use for the implementation of the WFD. Furthermore, the project aims at providing the water managers in Europe with a concise and easily accessible summary of methods being approved and under development. This deliverable is reporting on the 231 national assessment methods collated within workpackage 2.2. All data are stored in the WATERVIEW2-Database whose online version will be launched by April 2010, accessible via the WISER webpage <http://www.wiser.eu>.

### Data collection

Data on national assessment methods were collected by means of a questionnaire circulated to the Member States via the CIS<sup>1</sup> Working Group "Ecological Status" (ECOSTAT) on October 8<sup>th</sup>, 2009. The preparation of the survey was done in a joint activity with the Intercalibration Steering Group (Joint Research Centre, Ispra). The questionnaire was divided into three sections covering the topics A - *General information*, B - *Data acquisition* and C - *Data evaluation*. The enquiry was mostly focussing on general aspects that all biological assessment methods have in common – irrespective of water category or biological quality element. However, the completion of the questionnaire required good knowledge about the respective national method, thus it was best be undertaken by persons responsible for method development or implementation. The blank of the questionnaire is attached to this deliverable.

### Contents of the WATERVIEW2-Database

By February 28<sup>th</sup>, 2010 25 countries have returned the descriptions of 231 biological assessment methods used in their WFD monitoring programmes (Table 1). Most methods (n=79) were

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<sup>1</sup> Common Implementation Strategy of the EU Water Framework Directive

provided for the river assessment (Figure 1). With 64 methods the benthic invertebrates are the most often used Biological Quality Elements in WFD monitoring programmes reported by the Member States (Figure 2). The majority of methods is intercalibrated within the Central-Baltic Geographical Intercalibration Group (Figure 3).

Table 1: Number of method descriptions provided by each country.

Country	Number of methods
Austria	7
Belgium	20
Croatia	3
Cyprus	3
Czech Republic	3
Denmark	5
Estonia	7
Finland	10
France	9
Germany	17
Greece	4
Hungary	8
Ireland	10
Italy	16
Lithuania	11
Luxembourg	1
Netherlands	12
Poland	8
Portugal	2
Romania	7
Slovakia	6
Slovenia	12
Spain	17
Sweden	18
United Kingdom	15

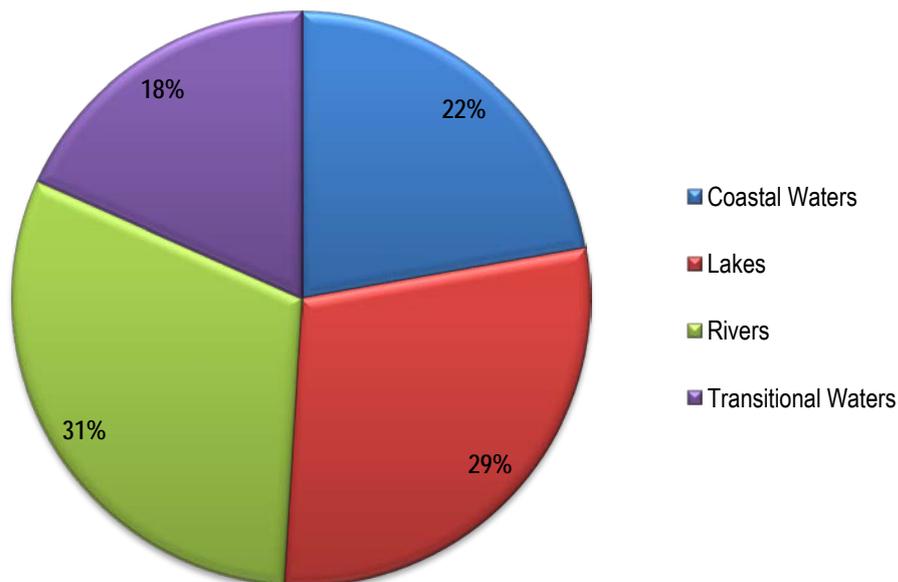


Figure 1: Share of water categories to which methods are applied.

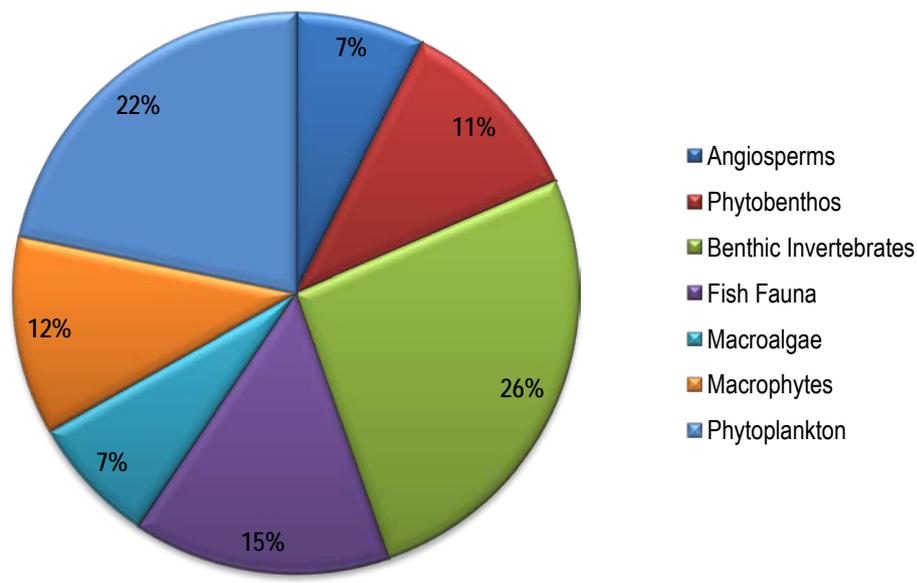


Figure 2: Share of biological quality elements addressed by the methods.

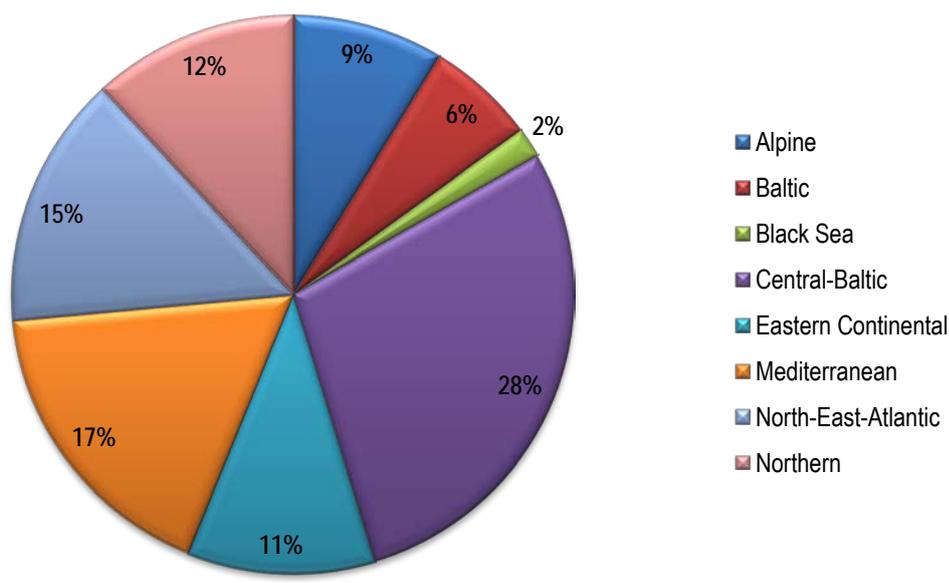


Figure 3: Share of Geographical Intercalibration Groups that national methods belong to.

A complete overview of the information stored in the database is annexed to this deliverable.

## References

European Communities (2009). Guidance document on the intercalibration process 2008-2011. Guidance Document No. 14. Implementation Strategy for the Water Framework Directive (2000/60/EC).

# Questionnaire on biological assessment methods used in national WFD monitoring programmes

## Introduction

The European Water Framework Directive (WFD) requires to classify the quality status of rivers, lakes, coastal and transitional waters. The ecological status is evaluated by biological assessment methods using selected biological quality elements, i.e. phytoplankton, benthic flora, benthic invertebrate fauna and fish fauna. The 27 European Member States are in charge of developing these methods, and the classification of good ecological status is harmonised in a Europe-wide intercalibration exercise.

## Purpose

Against this background there is a growing need for the exchange of information and data. Therefore, a joint activity was launched between the Intercalibration Steering Group and the EU research project WISER (<http://www.wiser.eu>) to collate consistent data about the national assessment methods used in WFD quality monitoring by the 27 Member States.

Information on the methods is collected by means of this questionnaire. Member States' delegates are asked to provide the requested data on screen and submit the questionnaire's content by email. The information will be collated by the University of Duisburg-Essen (Germany). By April 2010 the descriptions of the national methods can be queried from an online-database accessible via the WISER webpage.

## Relevance to the intercalibration exercise

The method descriptions will be used as part of the intercalibration reporting procedure. The Geographical Intercalibration Groups can have access to the data as soon as they are available. Information will serve as the basis for WFD compliance and IC acceptance checking according to the new Intercalibration Process Guidance.

## Content

The questionnaire is divided into three sections that cover the topics *A - General information*, *B - Data acquisition* and *C - Data evaluation*. This enquiry is mostly focussing on general aspects that all biological assessment methods have in common - irrespective of water category or biological quality element. However, the completion of the questionnaire requires good knowledge about the respective national method, thus it might best be undertaken by persons responsible for method development or implementation.

## Technical information

This questionnaire was produced using the software *Adobe LiveCycle Designer*. It can be completed using the Adobe Acrobat reader. By pressing the email-button at the end of this document the filled-in data becomes converted into xml-format and attached to an email addressed to [sebastian.birk@uni-due.de](mailto:sebastian.birk@uni-due.de). You can also send the completed pdf-file itself via email. Sebastian is responsible for content and technical issues of this task. Please contact him in case of problems and further questions.

**Please complete one questionnaire for each individual national assessment method<sup>1</sup> and send the information by December 1<sup>st</sup>, 2009.**

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<sup>1</sup> **Note 1:** Usually countries apply one assessment method per BQE and water category (like benthic invertebrate fauna in rivers), but in certain cases individual methods are using only parts of the full BQE (like angiosperms in coastal waters) or different habitat types (soft bottom benthic invertebrate fauna in coastal waters). A separate questionnaire shall be filled in for each of these.

**Note 2:** This request is also focussing on (future) methods that will be applied for the second River Basin Management Plan (and even beyond) (see A-16). Please deliver also information for these methods (if already available), this will help in intercalibration planning.

## A - General information

A-01 Name of person completing this questionnaire

*free entry*<sup>A01</sup>

*Example: Max Mustermann*

A-02 Email address of person completing this questionnaire

*free entry*<sup>A02</sup>

*Example: max.mustermann@web.de*

A-03 Institution of person completing this questionnaire

*free entry*<sup>A03</sup>

*Example: Department of Environmental Protection, University of Berlin*

A-04 Name of assessment method (original full name)

*free entry*<sup>A04</sup>

*Example: Bewertungsverfahren von Fließgewässern auf Basis des Makrozoobenthos*

A-05 Name of assessment method (translated into English)

*free entry*<sup>A05</sup>

*Example: Assessment system for rivers using macrozoobenthos*

A-06 Abbreviation of assessment method

*free entry*<sup>A06</sup>

*Example: PERLODES*

A-07 EU Member State

*free entry*<sup>A07</sup>

*Example: Germany*

A-08 Water Category

Rivers<sup>A08a</sup>

Lakes<sup>A08b</sup>

Coastal Waters<sup>A08c</sup>

Transitional Waters<sup>A08d</sup>

*Example: Rivers*

A-09 If *Transitional Waters*, please specify

Estuary<sup>A09a</sup>

Lagoon<sup>A09b</sup>

Fjord<sup>A09c</sup>

Others: *free entry*<sup>A09d</sup>

A-10 Biological Quality Element

Phytoplankton<sup>A10a</sup>

Macrophytes<sup>A10b</sup>

Phytobenthos

Diatoms<sup>A10c</sup>

Other phytobenthos<sup>A10d</sup>

Macroalgae<sup>A10e</sup>

Angiosperms<sup>A10f</sup>

Benthic invertebrate fauna<sup>A10g</sup>

Fish Fauna<sup>A10h</sup>

*Example: Benthic Invertebrate Fauna*

A-11 If *Angiosperms*, please specify

Only Seagrass<sup>A11a</sup> (specify species: *free entry*<sup>A11b</sup>)

Other Angiosperms<sup>A11c</sup> (specify groups: *free entry*<sup>A11d</sup>)

## A-12 Scope of detected pressures

Acidification<sup>A12a</sup>  
 Aquatic habitat destruction<sup>A12b</sup>  
 Catchment land use<sup>A12c</sup>  
 Eutrophication<sup>A12d</sup>  
 Flow modification<sup>A12e</sup>  
 General degradation (unspecific pressures)<sup>A12f</sup>  
 Heavy metals<sup>A12g</sup>  
 Hydromorphological degradation<sup>A12h</sup>  
 Impact of alien species<sup>A12i</sup>  
 Pollution by organic compounds (e.g. DDT, PCB)<sup>A12j</sup>  
 Pollution by organic matter<sup>A12k</sup>  
 Riparian habitat alteration<sup>A12l</sup>  
 Other: *free entry*<sup>A12m</sup>

Example: *General degradation, organic pollution, hydromorphological degradation, acidification*

## A-13 Has the pressure-impact relationship of the assessment method been tested?

Yes, with qualitative data (e.g. response at reference against impacted sites). / Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient). / No, pressure-impact the relationship has not been tested.<sup>A13a</sup>

If yes, please specify pressure and impact metrics, the amount of used, statistical significance of pressure.

*free entry*<sup>A13b</sup>

Example: *Ecological data from 39 lakes (> 50 mg l<sup>-1</sup> CaCO<sub>3</sub> alkalinity and 3-15 m mean depth) were examined to establish pressure-impact relationship between macrophyte metrics and eutrophication gradient. The relationship between four macrophyte metrics and TP (measured in spring or early summer) showed significant correlation (Spearman Correlation Coefficient ranging from 0.3 to 0.5).*

## A-14 Is the assessment method applied to water bodies in the whole country?

Yes / No <sup>A14a</sup>

If no, please specify region of application: *free entry*<sup>A14b</sup>

Example: *No, only ecoregion "Central Plains"*

## A-15 If the method has been/is intercalibrated, please specify the relevant Geographical Intercalibration Group(s) and common intercalibration type(s).

Alpine GIG (rivers and lakes)<sup>A15a</sup>  
 Central Baltic GIG (rivers and lakes)<sup>A15b</sup>  
 Eastern Continental GIG (rivers and lakes)<sup>A15c</sup>  
 Mediterranean GIG (rivers and lakes, coastal and transitional waters)<sup>A15d</sup>  
 Northern GIG (rivers and lakes)<sup>A15e</sup>  
 North East Atlantic GIG (coastal and transitional waters)<sup>A15f</sup>  
 Baltic Sea GIG (coastal and transitional waters)<sup>A15g</sup>  
 Black Sea GIG (coastal and transitional waters)<sup>A15h</sup>  
 Common intercalibration type(s): *free entry*<sup>A15i</sup>

Example: *Central Baltic GIG: Siliceous mountain brooks (R-C3)*

## A-16 Status of assessment method: Method (will be) used for ...

First RBMP<sup>2</sup> (2009)<sup>A16a</sup>  
 Second RBMP (2015)<sup>A16b</sup>  
 neither first nor second RBMP (probably later RBMP)<sup>A16c</sup>

Example: *First River Basin Management Plan (2009) and Second River Basin Management Plan (2015)*

## A-17 Web page describing national method

*free entry*<sup>A17</sup>

Example: <http://www.fliessgewaesserbewertung.de>

## A-18 Name of responsible person having developed the assessment method

*free entry*<sup>A18</sup>

Example: *Erwin Entwickler*

<sup>2</sup> River Basin Management Plan

A-19 Email address of responsible person having developed the assessment method  
*free entry*<sup>A19</sup>

Example: *erwin.entwickler@web.de*

A-20 Institution of responsible person having developed the assessment method  
*free entry*<sup>A20</sup>

Example: *Department of Environmental Protection, University of Chisinau*

A-21 Pertinent literature of mandatory character (e.g. official note, national standard)  
*free entry*<sup>A21</sup>

Example: *LAWA-AO, 2006. RaKon Monitoring Teil B. Arbeitspapier III: Untersuchungsverfahren für biologische Qualitätskomponenten. Ständiger Ausschuss "Oberflächengewässer und Küstengewässer" der Bund/Länder-Arbeitsgemeinschaft Wasser (LAWA-AO).*

A-22 Scientific literature (preferably quote references written in English)  
*free entry*<sup>A22</sup>

Example: *Hering, D., J. Böhrer, P. Haase & J. Schaumburg, 2004. New methods for assessing freshwaters in Germany. Limnologica 34: 281-282.*

A-23 Comments  
*free entry*<sup>A23</sup>

## B - Data acquisition

B-01 Which guidelines are followed for the sampling/surveying and sample processing?  
*free entry*<sup>B01</sup>

Example: *Meier, C., Haase, P., Rolauffs, P., Schindehütte, K., Schöll, F., Sundermann, A. & D. Hering, 2006. Methodisches Handbuch Fließgewässerbewertung. Handbuch zur Untersuchung und Bewertung von Fließgewässern auf der Basis des Makrozoobenthos vor dem Hintergrund der EG-Wasserrahmenrichtlinie. University of Duisburg-Essen, Essen.*

B-02 Sampling/survey device  
*Phytoplankton*

Plankton net<sup>B02a</sup>

Water sampler<sup>B02b</sup>

Multiple Opening/Closing Net and Environmental Sampling System (MOCNESS)<sup>B02c</sup>

Other: *free entry*<sup>B02d</sup>

*Macrophytes*

Rake<sup>B02e</sup>

Grapple<sup>B02f</sup>

Dredge<sup>B02g</sup>

Other: *free entry*<sup>B02h</sup>

*Phytobenthos*

Scraper<sup>B02i</sup>

Spoon<sup>B02j</sup>

Brush<sup>B02k</sup>

Other: *free entry*<sup>B02l</sup>

*Benthic macroinvertebrate fauna*

Hand net<sup>B02m</sup>

Surber or Hess sampler<sup>B02n</sup>

Corer<sup>B02o</sup>

Airlift sampler<sup>B02p</sup>

Grab<sup>B02q</sup>

Dredge<sup>B02r</sup>

Artificial substrate<sup>B02s</sup>

Other: *free entry*<sup>B02t</sup>

*Fish fauna*

Fyke net<sup>B02u</sup>  
 Gill net<sup>B02v</sup>  
 Beam trawl<sup>B02w</sup>  
 Otter trawl<sup>B02x</sup>  
 Seine netting<sup>B02y</sup>  
 Electrofishing gear<sup>B02z</sup>  
 Echo sounder (hydroacoustics)<sup>B02aa</sup>  
 Other: *free entry*<sup>B02ab</sup>

Example: *Grab*

B-03 Please specify sampling/survey device

*free entry*<sup>B03</sup>

Example: *Van Veen Grab (short arm, warp rigged)*

B-04 Minimum size of organisms sampled and processed

*free entry*<sup>B04</sup>

Example: *500 µm (mesh-size of hand net)*

B-05 Sampled/surveyed habitat

All available habitats per site (Multi-habitat) / Single habitat(s)<sup>B05</sup>

Example: *All available habitats per site (Multi-habitat)*

B-06 If *Single habitat(s)* are sampled/surveyed, please specify habitat(s)

*free entry*<sup>B06</sup>

Example: *soft bottom, hard bottom, phytal fauna (e.g. seagrass)*

B-07 Which zone is sampled/surveyed in areas with tidal influence (only coastal and transitional waters)?

Intertidal zone / Subtidal zone / Both tidal zones<sup>B07</sup>

Example: *Both tidal zones*

B-08 How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area?

*free entry*<sup>B08</sup>

Example: *One occasion per sampling season*

B-09 Sampling/survey month(s)

*free entry*<sup>B09</sup>

Example: *Brooks: February to April, Streams: May to August*

B-10 Which method is used to select the sampling/survey site or area?

Random sampling/surveying<sup>B10a</sup>  
 Stratified sampling/surveying<sup>B10b</sup>  
 Expert knowledge (e.g. sites most representative of water body)<sup>B10c</sup>  
 Other: *free entry*<sup>B10d</sup>

Example: *Expert knowledge (e.g. sites most representative of water body)*

B-11 How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area?

*free entry*<sup>B11</sup>

Example: *20 replicates (one per stream microhabitat >5% coverage)*

B-12 Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based

*free entry*<sup>B12</sup>

*Example: Sum of 20 spatial replicates à 0.0625 square-metres = 1.25 square-metres of stream bottom in total*

B-13 Short description of field sampling/survey procedure

*free entry*<sup>B13</sup>

*Example: Multi-habitat sampling designed for sampling major habitats in proportion to their presence within a sampling reach is carried out. A sample consists of 20 "sampling units" taken from all habitat types at the sampling site with a share of at least 5 % coverage. A "sampling unit" is a stationary sampling performed by positioning the net and disturbing the substrate in a quadratic area that equals the frame-size upstream of the net (0.25 x 0.25 m). Sediments must be disturbed to a depth of 15-20 cm (where possible) depending on substrate compactness.*

B-14 Sample processing

Organisms of the complete sample are identified / Sample is divided (sub-sampling) and organisms of a sub-sample are identified<sup>B14</sup>

*Example: Sample is divided (sub-sampling) and organisms of a sub-sample are identified*

B-15 If *Sub-sampling* is performed, please describe procedure

*free entry*<sup>B15</sup>

*Example: One/sixth of sampling material is separated from which 350 organisms are analysed.*

B-16 Record of biological data: Level of taxonomical identification

Species/species groups level<sup>B16a</sup>

Genus level<sup>B16b</sup>

Family level<sup>B16c</sup>

Other level<sup>B16d</sup>

*Example: Species level, Family level, Other level*

B-17 If level of taxonomical identification differs (multiple answers on B-16), please specify what groups are mainly identified to which level.

*free entry*<sup>B17</sup>

*Example: Most insecta and hirudinea to species level except for chironomids and simuliids; chironomids and simuliids to family level; oligochaets to level of order.*

B-18 Record of biological data: How is the biota's abundance within the sample/survey measured?

Individual counts<sup>B18a</sup>

Percent coverage<sup>B18b</sup>

Abundance classes (ordinal scale)<sup>B18c</sup>

Relative abundance (i.e. one species relatively to other species)<sup>B18d</sup>

Other: *free entry*<sup>B18e</sup>

*Example: Individual counts*

B-19 Record of biological data: Abundance is related to ...

Area<sup>B19a</sup>

Volume<sup>B19b</sup>

Time<sup>B19c</sup>

Other: *free entry*<sup>B19d</sup>

*Example: Area*

B-20 Please specify unit in which the biota's abundance is expressed

*free entry*<sup>B20</sup>

*Example: Number of individuals per one square-metre*

B-21 If biomass is measured, please specify how it is quantified.

Determination of chlorophyll-a concentration by spectrophotometric analysis<sup>B21a</sup>

Determination of fresh weight by microscopic counting, cell size measurement and cell volume calculation (Utermöhl technique)<sup>B21b</sup>

Other: *free entry*<sup>B21c</sup>

*Example: Determination of fresh weight by microscopic counting, cell size measurement and cell volume calculation (Utermöhl technique)*

B-22 Other records of biological data (e.g. organism length, plant growth form, shoot density)

*free entry*<sup>B22</sup>

*Example: Length of individual specimens*

B-23 Special cases, exceptions, additions  
*free entry*<sup>B23</sup>

*Example: Non-wadable rivers are sampled only at the banks, i.e. multi-habitat-sampling is confined to the river margin habitats.*

B-24 Comments  
*free entry*<sup>B24</sup>

## C - Data evaluation

C-01 Complete list of biological metric(s) used in assessment  
*free entry*<sup>C01</sup>

*Example: Relative abundance of taxa with oligosaprobic valence, Relative abundance of Ephemeroptera, Plecoptera and Trichoptera taxa, Number of Trichoptera taxa*

C-02 Data basis for metric calculation: From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time (see B-08)<sup>C02a</sup>

Aggregated data from multiple sampling/survey occasions in time (see B-08)<sup>C02b</sup>

Data from single spatial replicate (see B-11)<sup>C02c</sup>

Aggregated data from multiple spatial replicates (see B-11)<sup>C02d</sup>

Other: *free entry*<sup>C02e</sup>

*Example: Data from single sampling/survey occasion in time (see B-08), Aggregated data from multiple spatial replicates (see B-10)*

C-03 Does the selection of metrics differ between types of water bodies (e.g. different metrics to assess lowland brooks compared to mountain streams)?

Yes / No<sup>C03</sup>

*Example: Yes*

C-04 Combination rule for multi-metrics

Average metric scores<sup>C04a</sup>

Weighted average metric scores<sup>C04b</sup>

Worst metric score<sup>C04c</sup>

Mean quality class<sup>C04d</sup>

Worst quality class<sup>C04e</sup>

Other: *free entry*<sup>C04f</sup>

Not relevant<sup>C04g</sup>

*Example: Average metric scores*

C-05 Scope of reference conditions

Surface water type-specific / Site-specific / Habitat-specific<sup>C05</sup>

*Example: Surface water type-specific*

C-06 Key source(s) to derive reference conditions

Existing near-natural reference sites<sup>C06a</sup>

Modelling (extrapolating model results)<sup>C06b</sup>

Expert knowledge<sup>C06c</sup>

Historical data<sup>C06d</sup>

Least Disturbed Conditions<sup>C06e</sup>

Other: *free entry*<sup>C06f</sup>

*Example: Existing reference sites, modelling, expert knowledge*

C-07 Number of sites used to derive reference conditions

*free entry*<sup>C07</sup>

*Example: 26 Aegean sites in the Mediterranean Sea*

C-08 Geographical coverage of sites used to derive reference conditions

*free entry*<sup>C08</sup>

*Example: Only reference zones in natural parks from Corsica and Balearic Islands considered representative for the entire Mediterranean Sea.*

C-09 Location of sites used to derive reference conditions

*free entry*<sup>C09</sup>

*Example: Façade maritime du Parc Naturel Régional de Corse (France), Parc Natural de Ses Salines (Balearic Islands, Spain) and Reserva Marina del Nord de Menorca (Balearic Islands, Spain).*

## C-10 Time period (months + years) of data of sites used to derive reference conditions

*free entry*<sup>C10</sup>*Example: Historical data before 1980s covering 5 years.*

## C-11 Reference community description

*free entry*<sup>C11</sup>*Example:*

1. Macroalgal communities of high diversity should be dominated quantitatively by brown algae mainly of the order Fucales in high irradiance sites and red algal Corallinales in vertical cliffs.
2. Dense well-developed macroalgal communities thriving in the upper infralittoral zone with most characteristic species belonging to the genera *Cystoseira*, *Sargassum*, *Lithophyllum*, *Peyssonnelia*, *Corallina* and *Padina*. Other common species belong to the genera *Halopteris*, *Stypocaulon*, *Dictyota*, *Dictyopteris*, *Laurencia*, *Cladophora* and *Jania*.
3. In shadow zones (exposed steep vertical cliffs) *Lithophyllum byssoides* develops, forming important organogenic structures (trottoir). In marine caves with scarce light conditions a sciaphilic vegetation of red and green algae dominant.

## C-12 Reference sites' criteria

*free entry*<sup>C12</sup>

*Example: The absence of pressures had to be illustrated. The communities at the sites had to correspond with the description of the reference community description. Spatio-temporal variability had to be taken into account of the community's composition and abundance affected by hard substrata availability, intense and frequency of natural disturbances, e.g. hydrodynamism, grazing, by seasonal cycle of light period and intensity, and by limiting factors like nutrients.*

## C-13 Are the assessment results expressed as Ecological Quality Ratios (EQR)?

*free entry*<sup>C13a</sup>If no, please specify how the results are expressed: *free entry*<sup>C13b</sup>*Example: Yes*

## C-14 Setting of ecological status boundaries

Using discontinuities in the relationship of anthropogenic pressure and the biological response. <sup>C14a</sup>Using paired metrics that respond in different ways to the influence of the pressure (e.g. % sensitive taxa compared to % of impact taxa for benthic invertebrates in rivers and lakes). <sup>C14b</sup>High-good boundary derived from metric variability at near-natural reference sites (e.g. 5<sup>th</sup> percentile value). <sup>C14c</sup>Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2). <sup>C14d</sup>Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement). <sup>C14e</sup>Boundaries taken over from the intercalibration exercise. <sup>C14f</sup>Other: <sup>C14g</sup>

*Example: Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2), Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement)*

## C-15 Please describe the boundary setting procedure in relation to the pressure.

*free entry*<sup>C15</sup>

*Example: Macrophytes were placed into four nutrient response groups using empirical analysis (highly sensitive, sensitive, tolerant and highly tolerant). The ratio of the relative cover of these response groups was then related to the macrophyte nutrient score (LMNI) itself an index of nutrient pressure. Boundary values for HG and GM were determined from this relationship:*

*- The HG boundary was identified as the point at which all tolerant species were on average <10% of cover.*

*- The GM boundary was the point at which the lower confidence limits of the sensitive and upper confidence limit of the tolerant species intersect. At this point there is still a high probability of having >50% cover of sensitive species and no more than 50% cover of tolerant species. This would be indicative of slight change, the community could still easily recover to its original status. The highly sensitive species are still present (10-50% cover) and highly tolerant (undesirable) species would be <20% cover.*

*- The MP boundary was set where the lower confidence limit of the sensitive and upper confidence limit of the tolerant species intersect. At this point there is a low probability that sensitive species would be at 50% cover, but a high probability that tolerant species would be at 50% cover. Very sensitive species are still present, but the community has thus undergone a moderate change.*

*- The PB boundary is a point at which highly sensitive species are extinct and there are very few sensitive species. Here the community is dominated by tolerant species.*

## C-16 Good status community description

*free entry*<sup>C16</sup>

*Example: At good status stands of the sensitive taxa (large isoetids, *Littorella*, *Lobelia*, *Isoetes* in low alkalinity lakes or *Chara* spp. in high alkalinity lakes) are well developed, but significantly decreasing at good-moderate boundary ("sudden drop") and replaced by tolerant taxa.*

## C-17 Has the uncertainty of the method been quantified and is it regarded in the assessment?

Yes / No (to be done) <sup>C17</sup>*Example: No (to be done)*

## C-18 If the uncertainty has been quantified and regarded, please specify how this is done.

*free entry*<sup>C18</sup>

## C-19 Comments

*free entry*<sup>C19</sup>



# **Overview report of biological assessment methods used in national WFD monitoring programmes**

## ***Methods for rivers, lakes, coastal and transitional waters***

exported from the  
WaterView2 - Database on assessment methods for lakes, rivers, coastal and transitional waters in Europe  
WISER Workpackage 2.2 - <http://www.wiser.eu>

Sebastian Birk - University of Duisburg-Essen  
02 March 2010

ID: 86

LI-AN-TR

## 1. General information

- 1.01 GIG:** Baltic  
n.a.
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Angiosperms  
Potameids
- 1.04 Country:** Lithuania
- 1.05 Specification:** Only Curonian Lagoon (except Klaipeda strait)
- 1.06 Method name:** *Assessment system for transitional waters using angiosperms (maximum depth limit of potameids)*
- 1.07 Original name:** *Tarpinių vandenų ekologinės būklės vertinimo sistema pagal gaubtasėklius (maksimalus potameidų paplitimo gylis)*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication  
*Has the pressure-impact-relationship been tested?*  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Razinkovas, A. et al., 2006. Water quality criteria in transitional and coastal waters. Technical Report, Coastal Research and Planning Institute.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Doc. dr. Artūras Razinkovas  
art@corpi.ku.lt  
Coastal Research and Planning Institute, Klaipeda University
- 1.14 Method reported by**  
Nijole Remeikaite-Nikiene  
n.nikiene@jtc.am.lt  
Center of Marine Research
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
LST EN 14184: 2004. Water quality - Guidance standard for the surveying of aquatic macrophytes in running waters.
- 2.02 Short description**  
Observations of defined transects are made once per 3 years (according to monitoring programme) at the Curonian lagoon. Coverage (5-level scales), occurrence (%), biomass (kg/m<sup>2</sup>) and depth limit are measured/observed along transects. Samples of macrophyte organisms are taken only if organisms are not identified at field. In such case they are identified at laboratory by using identification keys.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Rake  
Frame (25 x 25 cm) 0,0625 m<sup>2</sup>
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** July-August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
2-3 transects per water body
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
The length (50-200 m from coast line) of transects depends on deep (up to 120 cm) in Curonian lagoon. Belt transect width is 1 m.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Species/species groups

- 2.15 Record of abundance:** n.a.  
Coverage (5-level scales), occurrence (%)  
in relation to Area  
Unit
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** biomass kg/m<sup>2</sup>
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments:** none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics:** n.a.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** Single note from literature  
**Geographical coverage:** Curonian lagoon  
**Location of sites:** northern and central parts of the Curonian lagoon  
**Data time period:** Historical data before 1959's (Minkevičius, Pipinis, 1959)  
**Criteria:**  
Maximum depth limit of potameids is more than 3.6 m
- 3.08 Reference community description**  
Maximum depth limit of potameids is more than 3,6 m
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
mainly expert judgement
- 3.11 Boundary setting procedure**  
Reference conditions: Maximum depth limit of potameids is more than 3.6 m. High status - depth limit 3-3.6 m (Minkevičius, Pipinis, 1959). Moderate status has been defined as recent depth limit of potameids. Other classes have been defined taking into account degradation of potameids belt in zones of active hydraulic process.
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:** none

ID: 88

LT-FI-TR

## 1. General information

- 1.01 GIG:** Baltic  
n.a.
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Lithuania
- 1.05 Specification:** Only to the central part of the Curonian Lagoon
- 1.06 Method name:** *Assessment system for transitional waters using ichthyofauna (abundance of gudgeon (*Gobio gobio*))*
- 1.07 Original name:** *Tarpinių vandenų ekologinės būklės vertinimo sistema pagal ichtiofauną (vidutinis grūžlio (*Gobio gobio*) gausumas)*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Razinkovas, A. et al., 2006. Water quality criteria in transitional and coastal waters. Technical Report, Coastal Research and Planning Institute.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Dr. Valdemaras Ziliukas, Dr. Rimantas Repecka  
ziliukas@ekoi.lt, repecka@ekoi.lt  
Institute of Ecology of Vilnius University
- 1.14 Method reported by**  
Nijole Remeikaite-Nikiene  
n.nikiene@jtc.am.lt  
Center of Marine Research
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Methodology for Fish stock research (approved by order the Ministry of Environment, 2005-10-20; No. D1-5010). Neuman, E., O. Sandström & G. Thoresson, 1997. Guidelines for coastal fish monitoring. Öregrund: National Board of Fisheries, 36 p.
- 2.02 Short description**  
Catches in the coastal zone are conducted with a beach seine. Each haul covers an area 250-300 m<sup>2</sup>
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.  
Beach seine
- 2.05 Specification:** 20 m length, 1,5 m height with a bag (3 mm bar mesh size)
- 2.06 Sampled/surveyed habitat:** n.a.  
Mostly soft (sandy) bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** May, July, September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Three occasions per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Two samples are taken at every site and combined into one joint sample.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 2,5 cm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per 100 square-metre
- 2.16 Quantification of biomass:** n.a.

- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
n.a.
- 3.02 Does the metric selection differ between types of water bodies?** n.a.
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data  
monitoring data
- 3.07 Reference site characterisation**  
**Number of sites:** 3 sites at the central part of the Curonian Lagoon  
**Geographical coverage:** Central part of the Curonian lagoon  
**Location of sites:** Sites close Nida and Vente at the central part of the Curonian lagoon  
**Data time period:** Data in years 1985-1988 and 1994-2005  
**Criteria:**  
Abundance of gudgeon (>250 ind. per 100 m<sup>2</sup>) is used to define reference conditions.
- 3.08 Reference community description**  
Abundance of gudgeon (>250 ind. per 100 m<sup>2</sup>) is used to define reference conditions.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
expert judgement. Based on comparison of data in years 1985-1988 and 1994-2005
- 3.11 Boundary setting procedure**  
Boundary setting procedure was based on expert judgement, taking into account data (eutrophication level of the lagoon and river Nemunas) on eutrophication (however, this relation has not been checked).
- 3.12 "Good status" community:** Abundance of gudgeon (100-199 ind. per 100 m<sup>2</sup>) is used to define good status.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
Classification rules are used to assess confidence level of the whole assessment system (not for separate methods).

ID: 227

MaQI

## 1. General information

- 1.01 GIG:** Mediterranean
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Angiosperms, Macroalgae  
Zostera marina, Cymodocea nodosa, Posidonia oceanica, Ruppia spp., Nanozostera noltii
- 1.04 Country:** Italy
- 1.05 Specification:**
- 1.06 Method name:** *Macrophyte Quality Index*
- 1.07 Original name:** *Macrophyte Quality Index*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, Habitat destruction, Hydromorphological degradation, Pollution by organic matter  
Turbidity and sedimentation

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Sfriso, A., Facca, C., Ghetti, P.F. (2009). Validation of the Macrophyte Quality Index (MaQI) set up to assess the ecological status of Italian marine transitional environments. *Hydrobiologia*, 617, 117-141. Ecological data from 20 stations situated in the Venice lagoon, 17 additional sampling sites of the lagoons of Lesina, Orbetello, Marano, Goro and in the Mar Piccolo at Taranto. New data will be available for Po delta lagoons.

- 1.10 Internet reference:** [http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione\\_tecnica.html](http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione_tecnica.html)
- 1.11 Pertinent literature of mandatory character:**  
National laws (Decreto del MATTM n.131/ 2008 e Decreto del MATTM n. 56/ 2009).
- 1.12 Scientific literature:**  
Sfriso, A., C. Facca & P.F. Ghetti, 2007. Rapid Quality Index, based mainly on Macrophyte Associations (R-MAQI), to assess the ecological status of the transitional environments. *Chemistry and Ecology* 23 (6): 1-11. Sfriso, A., C. Facca & P.F. Ghetti, 2009. Validation of the Macrophyte Quality Index (MaQI) set up to assess the ecological status of Italian marine transitional environments. *Hydrobiologia* 617: 117-141.
- 1.13 Method developed by** Prof. Adriano Sfriso - Università Ca' Foscari di Venezia (Ca' Foscari Venice University)  
sfrisoa@unive.it  
Università Ca' Foscari di Venezia
- 1.14 Method reported by** Paola Gennaro, Andrea Bonometto  
paola.gennaro@isprambiente.it,  
andrea.bonometto@isprambiente.it  
ISPRA
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
National Protocol (El-Pr-TW-Protocolli Monitoraggio-03.05 - Protocolli di monitoraggio degli elementi di qualità biologici)  
[http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione\\_tecnica.html](http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione_tecnica.html)
- 2.02 Short description**  
n.a.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying
- 2.04 Sampling/survey device:** Rake
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft-bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** May/June and October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
2 sampling (spring and autumn)
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3 - 6 for taxonomic identification and 10 - 20 for coverage (inside the sampling site of 15x15 m).
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Surveyed area of 15x15 m. The number of sampling site in each water body is related to the area and its habitat variability.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n.a.

- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups  
Two different version of MaQI are used: Expert MaQI is applied where the number of species is greater than 20.  
Taxonomic identification at species level- Rapid MaQI is applied where the number of species for site is less than 20.  
Taxonomic identification at species group level
- 2.15 Record of abundance:** Percent coverage, Relative abundance  
**in relation to** Area  
**Unit** coverage percentage
- 2.16 Quantification of biomass:** n.a.  
fresh weight (optional) Biomass is not required for MaQI calculation
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
E-MaQI: taxonomic identification R-MaQI: taxonomic identification, total and relative coverage
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

- 3.05 Scope of reference conditions:**
- 3.06 Key source(s) to derive reference conditions:**  
Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** 2  
**Geographical coverage:** n.a.  
**Location of sites:** One site in Lesina lagoon and one site in Venice lagoon  
**Data time period:** monthly sampling in Venice lagoon  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
Pristine coastal lagoons are considered to be dominated by extensive meadows of perennial seagrass species, since in oligotrophic waters rhizophytes take advantage of nutrient supply from sediment (Figure 1). Then, a key criterion to select reference sites is: "A lagoon with low human pressures covered with extensive angiosperm meadows" (Meeting of the WFD CIS MED-GIG MACROPHYTES - Technical report - Kavala)
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** Seaweed biomass composed by many species (15-25%), with high environmental score (species list is available in Sfriso et al., 2009) which are sensible to the environment stressors. Presence of calcified and crustose seaweeds, especially small epiphytes. During the species peak periods some Chlorophyceae (i.e. Chaetomorpha linum, some Cladophoraceae and filamentous Ulvaceae), or more rarely Rhodophyceae (Gracilaria spp., Polysiphonia spp., etc.) can show high or very high coverage (Chaetomorpha linum), but these never collapse. If present, Ruppia spp., Nanozostera noltii and/or Zostera marina beds are well organised with a coverage up to 60% of the considered area. In some cases (mostly euhaline environments) Cymodocea nodosa can be

present with reduced populations (<30% of the considered area).

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

In Sfriso et al., 2009 the assessment of the ecological status of each sampling site was proposed by a “class<sup>2</sup> binomial”. The first class corresponded to the class where the EQR value fell, according to the mean macroalgal<sup>2</sup>score, and the second one to the immediately upper or lower score-interval.

ID: 251

ISD

## 1. General information

- 1.01 GIG:** Mediterranean
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Greece
- 1.05 Specification:** No biomass data available for benthic communities in all the study sites
- 1.06 Method name:** *Index of Size Distribution*
- 1.07 Original name:** *Index of Size Distribution*
- 1.08 Status: Method is/will be used in** neither first nor second RBMP
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Benthic data from 3 lagoons were examined to test the relationship between ISD metrics and eutrophication gradient. The relationship between macroinvertebrates metrics and degree of eutrophication had a significant correlation (Spearman,  $p < 0.005$ )
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**
- 1.12 Scientific literature:**  
Reizopoulou, S. & A. Nicolaidou, 2007. Index of Size Distribution (ISD): a method of quality assessment for coastal lagoons. *Hydrobiologia* 577: 141-149.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Sofia Reizopoulou<br>sreiz@ath.hcmr.gr<br>Hellenic Centre for Marine Research | <b>1.14 Method reported by</b><br>Sofia Reizopoulou<br>sreiz@ath.hcmr.gr<br>Hellenic Centre for Marine Research |
|--|---|

### 1.15 Comments

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
n.a.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying
- 2.04 Sampling/survey device:** Grab
- 2.05 Specification:** Ponar grab
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Intertidal zone
- 2.08 Sampling/survey month(s):** Seasonal
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 0,5 mm mesh size
- 2.13 Sample treatment:**
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per one square-metre
- 2.16 Quantification of biomass:** n.a.  
Determination of dry weight
- 2.17 Other biological data:** organism length
- 2.18 Special cases, exceptions, additions:** none

## 2.19 Comments

### 3. Data evaluation

#### **Evaluation**

##### 3.01 List of biological metrics

Body-size distribution (skewness) of benthic macroinvertebrates

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: Not relevant

##### 3.04 From which biological data are the metrics calculated?

Aggregated data from multiple sampling/survey occasions in time

#### **Reference conditions**

3.05 Scope of reference conditions: Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Expert knowledge

##### 3.07 Reference site characterisation

Number of sites: One coastal lagoon in the Ionian Sea

Geographical coverage: n.a.

Location of sites: Amvrakikos Gulf, Ionian Sea, Hellas

Data time period: seasonal

Criteria:

n.a.

##### 3.08 Reference community description

n.a.

3.09 Results expressed as EQR? Yes

#### **Boundary setting**

3.10 Setting of ecological status boundaries: Using discontinuities in the relationship of anthropogenic pressure and the biological response.

##### 3.11 Boundary setting procedure

n.a.

3.12 "Good status" community: Non-impacted communities are characterized by an even distribution of their size structure

#### **Uncertainty**

3.13 Consideration of uncertainty:

3.14 Comments:

ID: 181

M-AMBI

## 1. General information

- 1.01 GIG:** Mediterranean  
n.a.
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Italy
- 1.05 Specification:** none
- 1.06 Method name:** *Multivariate-AZTI Marine Biotic Index*
- 1.07 Original name:** *Multivariate-AZTI Marine Biotic Index*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Pollution by organic matter

**Has the pressure-impact relationship been tested?**

No, pressure-impact relationship has not been tested.

- 1.10 Internet reference:** <http://www.azti.es>

**1.11 Pertinent literature of mandatory character:**

MCW - Sistema di classificazione ecologica. [http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione\\_tecnica.html](http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione_tecnica.html) DECRETO 14 aprile 2009, n. 56. Regolamento recante «Criteri tecnici per il monitoraggio dei corpi idrici e l'identificazione delle condizioni di riferimento per la modifica delle norme tecniche del decreto legislativo 3 aprile 2006, n. 152, recante Norme in materia ambientale, predisposto ai sensi dell'articolo 75, comma 3, del decreto legislativo medesimo». (GU n. 124 del 30-5-2009 - Suppl. Ordinario n.83) - Testo in vigore dal: 14-6-2009.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Angel Borja  
aborja@pas.azti.es  
AZTI-Tecnalia, Marine Research Division, Herrera Kaia,  
Portualdea s/n, 20110 Pasaia, Spain

**1.14 Method reported by**

Marina Penna  
marina.penna@isprambiente.it  
ISPRA Institute for Environmental Protection and Research

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

El-PR-TW Protocolli di monitoraggio 03.05. [http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione\\_tecnica.html](http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione_tecnica.html)

**2.02 Short description**

- In habitats which areas are less than 2.5 square kilometres: 2 sampling points - In habitats witch areas are between 2.5 – 50 square kilometres: as above plus one station each 5 square kilometres for a maximum of 10 sampling points - In habitats witch areas are > 50 square kilometres, as above plus one station each 25 square kilometres

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying

**2.04 Sampling/survey device:** Grab

**2.05 Specification:** Ekman-birge or Van Veen

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** From April to June and from September to October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Twice a year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

3 replicates

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

200 square centimetres per replicates

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 0.5/1 mm

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

in relation to Area

Unit Square centimeters

2.16 Quantification of biomass: n.a.

2.17 Other biological data: Body size and biomass are facultative

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

3.01 List of biological metrics

Richness, Shannon and Weaver Diversity and AMBI

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: n.a.

Factorial analysis

3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

#### Reference conditions

3.05 Scope of reference conditions: Surface water type-specific

3.06 Key source(s) to derive reference conditions:

Historical data, Modelling (extrapolating model results)

3.07 Reference site characterisation

Number of sites: 342 sampling point related to 10 coastal lagoons

Geographical coverage: Adriatic and Tirrenic coastal lagoons

Location of sites: Adriatic and Tirrenic coastal lagoons

Data time period: Historical data from the 90s to nowadays

Criteria:

With historical data has been calculated the function of distribution (Johnson's algorithm R software) than was taken into account the 90<sup>th</sup> percentile of the H' and Richness distribution and was calculated the average of the values of those parameters > of 90<sup>th</sup> percentile, that was considered the reference condition. For AMBI has been taken into account the 10<sup>th</sup> percentile, so the average of the values of AMBI < 10<sup>th</sup> percentile was considered a reference condition.

3.08 Reference community description

With historical data has been calculated the function of distribution (Johnson's algorithm R software) than was taken into account the 90<sup>th</sup> percentile of the H' and Richness distribution and was calculated the average of the values of those parameters > of 90<sup>th</sup> percentile, that was considered the reference condition. For AMBI has been taken into account the 10<sup>th</sup> percentile, so the average of the values of AMBI < 10<sup>th</sup> percentile was considered a reference condition.

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: n.a.

The boundaries were derived by the 90<sup>th</sup>, 60<sup>th</sup>, 30<sup>th</sup> and 10<sup>th</sup> percentile of the distribution functions (Johnson's algorithm R software) of the EQR

3.11 Boundary setting procedure

The boundaries were derived by the 90<sup>th</sup>, 60<sup>th</sup>, 30<sup>th</sup> and 10<sup>th</sup> percentile of the distribution functions (Johnson's algorithm R software) of the EQR.

3.12 "Good status" community: n.a.

#### Uncertainty

3.13 Consideration of uncertainty: n.a.

3.14 Comments:

none

ID: 180

BITS

## 1. General information

- 1.01 GIG:** Mediterranean  
n.a.
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Italy
- 1.05 Specification:** none
- 1.06 Method name:** *Benthic index based on taxonomic sufficiency*
- 1.07 Original name:** *Benthic index based on taxonomic sufficiency*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
n.a.
- 1.10 Internet reference:** <http://www.bits.unife.it/>
- 1.11 Pertinent literature of mandatory character:**  
MCW - Sistema di classificazione ecologica. [http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione\\_tecnica.html](http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione_tecnica.html)
- 1.12 Scientific literature:**  
n.a.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Michele Mistri and Cristina Munari<br>msm@unife.it<br>University of Ferrara | <b>1.14 Method reported by</b><br>Marina Penna<br>marina.penna@isprambiente.it<br>ISPRA Institute for Environmental Protection and Research |
|--|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
El-PR-TW Protocolli di monitoraggio 03.05. [http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione\\_tecnica.html](http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione_tecnica.html)
- 2.02 Short description**  
- In habitats which areas are less than 2.5 square kilometres: 2 sampling points - In habitats witch areas are between 2.5 – 50 square kilometres: as above plus one station each 5 square kilometres for a maximum of 10 sampling points - In habitats witch areas are > 50 square kilometres, as above plus one station each 25 square kilometres
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Grab
- 2.05 Specification:** Ekman-birge or Van Veen
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** From April to June and from September to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Twice a year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3 replicates
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
200 square centimetres per replicates

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 0.5/1 mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Family, Species/species groups  
Species level is mandatory but the indices works on family level.
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Square centimeters

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** Body size and biomass are facultative

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

$BITS = \log \left[ \frac{(6fI + fII)}{(fIII + 1) + 1} + \log \left[ \frac{nI}{(nII+1) + nI} / \frac{nI}{(nIII+1) + 0.5nII} / (nIII+1) + 1 \right] \right]$   
fI: sensitive frequency in percentage, fII: tollerant frequency in percentage, fIII: opportunistic frequency in percentage, nI: number of sensitive fammilies, nII: number of tollerant fammilies, nIII: number of opportunistic fammilies

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Historical data, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** 342 sampling point related to 10 coastal lagoons

**Geographical coverage:** Adriatic and Tirrenic coastal lagoons

**Location of sites:** Adriatic and Tirrenic coastal lagoons

**Data time period:** Historica data from the 90s to nowdays

**Criteria:**

With historical data has been calculated the function of distribution (Johnson's algorithm R software) than was taken into account the 90° percentile of the H' and Richness distribution and was calculated the average of the values of those parameters > of 90° percentile, that was considered the reference condition. For AMBI has been taken into account the 10° percentile, so the average of the values of AMBI < 10° was considered a reference condition.

**3.08 Reference community description**

With historical data has been calculated the function of distribution (Johnson's algorithm R software) than was taken into account the 90° percentile of the H' and Richness distribution and was calculated the average of the values of those parameters > of 90° percentile, that was considered the reference condition. For AMBI has been taken into account the 10° percentile, so the average of the values of AMBI < 10° was considered a reference condition.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** n.a.

The boundaries were derived by the 90°, 60°, 30° amd 10° percentile of the distribution funcions (Johnson's algorithm R software) of the EQR

**3.11 Boundary setting procedure**

The boundaries were derived by the 90°, 60°, 30° amd 10° percentile of the distribution funcions (Johnson's algorithm R software) of the EQR.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** n.a.

**3.14 Comments:**  
none

ID: 254

INVT

## 1. General information

**1.01 GIG:** Mediterranean

**1.02 Category:** Transitional Waters

**1.03 BQE:** Benthic Invertebrates

**1.04 Country:** Spain

**1.05 Specification:** Balearic islands and Valencia (East of Spain)

**1.06 Method name:** *Balearic islands multimetric*

**1.07 Original name:** *Multimétrico de las Islas Baleares*

**1.08 Status: Method is/will be used in** Second RBMP (2015)

**1.09 Detected pressure(s):** Eutrophication, Hydromorphological degradation, Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Ecological data from 284 points were examined to establish pressure-impact relationship between phytoplankton metrics, PCA degradation gradients (that included nutrients, alkalinity, temperature, pH, oxygen, salinity, chlorophylla-a and AFDM). The relationship were significative.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

**1.12 Scientific literature:**

PARDO, I., LUCENA, P., ABRAÍN, R., GARCÍA, L. & C. DELGADO. 2010. Implementación de la DMA en Baleares: evaluación de la calidad ambiental de las masas de agua epicontinentales utilizando indicadores e índices biológicos. Informe Final. Tomo II: Zonas Húmedas. Informe Técnico. Universidad de Vigo.

**1.13 Method developed by**

Isabel Pardo

ipardo@uvigo.es

Department of Ecology and Animal Biology, University of Vigo

**1.14 Method reported by**

Paloma Lucena Moya

plucena@uvigo.es

Department of Ecology, University of Vigo, Spain

**1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Multi-habitat sampling using hand-net (Spanish modification of the US EPA sampling protocol) Quantitative Sampling Protocol (20 kicks) based on USA Environmental protection Agency procedure (Barbour, M. T., J. WRONA, F.J., J.M. CULP, AND R.W. DAVIES. 1982. Macroinvertebrate subsampling: a simplified apparatus and approach. Canadian Journal of Fisheries and Aquatic Sciences 39: 1051-1054.

**2.02 Short description**

Multihabitat quantitative sampling protocol. 20 sampling units taken from all habitats (more than 5% coverage) present at the sampling point. A sampling unit is a sampling performed by positioning the net and disturbing the substrate in an area (0.25 x 0.50 m).

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying

**2.04 Sampling/survey device:** Hand net

**2.05 Specification:** Hand net (250 µm - 0,25 m base and equal or higher height)

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:**

**2.08 Sampling/survey month(s):** February-March and May-June

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Two samples every year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

20 replicates (one per microhabitat >5% coverage)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Sum of 20 spatial replicates (0.25\*0.5 m) is 2.5 square-meters of the coastal lagoon total.

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 250 µm

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

Only fine fractions < 1 mm are sub-sampled, all other bigger organisms are counted. Subsampling follows Wrona, F.J., Culp, J.M. y R.W. Davies (1982). Macroinvertebrate subsampling: a simplified apparatus and approach. Can.J.Fish.Aquat.Sci., 39:1051 – 1054

- 2.14 Level of taxonomical identification:** Species/species groups  
All the invertebrates were identified to genus or species except for some taxa of Diptera, which were identified to family level, and Nematoda, Oligochaeta and Acari, which remained as class and order.
- 2.15 Record of abundance:** Individual counts, Relative abundance  
**in relation to** Area  
The effort unit is the 20 kicks (2.5 square-meters)  
**Unit** Number of individuals
- 2.16 Quantification of biomass:** Chlorophyll-a concentration
- 2.17 Other biological data:**
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Oligohaline type: % Sensitive genus + Genus Richness + (% Cyprideis torosa + Polychaeta) Mesohaline type: Sensitive Genus Richness + Bray Curtis coefficient index (to level Order) + (% Amphipoda + %Gastropoda + % Isopoda) Euhaline type: Sensitive Genus Richness + % Artemia salina
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**  
**Number of sites:** 6 sites (27 samples)  
**Geographical coverage:** Balearic islands  
**Location of sites:** Balearic islands (1 site in Majorca, 3 sites in Minorca and 1 site in Formentera)  
**Data time period:** Data of years 2005-2008  
**Criteria:**  
A priori evaluation: we have distinguished two buffer zones around the selected sampling sites to evaluate the different REFCOND pressures. The first one corresponds to the area immediate to the edge (<50 m band), and the second band goes from the 50 m that limits the first band to the following 300 m. A posteriori evaluation: we selected sites consisted of checking for consistency using information on water physicochemistry and biological communities.
- 3.08 Reference community description**  
Reference community of the oligohaline type is characterized by Cloeon sp., Corixidae Gen. sp., Daphnia sp., Dasyhelea sp., Herpetocypris sp., Hydrachnidia Gen. sp., Ischnura sp., Laccophilus sp., Libellulidae Gen. sp., Megacyclops sp., Physella sp., Plea sp., Psectrocladius sp., Sarscypridopsis sp. and Sigara sp. Reference community of the mesohaline type is characterized by Cyprideis sp., Gammarus sp., Hydrobia sp., Lekanospaera sp., Loxoconcha sp. and Nereis sp. Reference community of the mesohaline type is characterized by Cletocamptus sp., Corixidae Gen. sp., Halocladius sp., Heterocypris sp., Nematelus sp. and Sigara sp.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites Using paired metrics that respond in different ways to the influence of the pressure
- 3.11 Boundary setting procedure**  
The boundary limit between High and Good ecological status were obtained calculating the percentile 25 (P25) of the EQR of the reference sites. The percentil 25 in then divided into four to extract the remaining boundaries between classes. In addition, dispersion plots between paired metrics along a pressure gradient were performed to aid in the ecological interpretation of their interactions, to adjust the remaning boundaries between the classes, according the Boundary Setting protocol (i.e. if the metrics interactions corresponded to a class centre or a class boundary, and to which classes they relate).
- 3.12 "Good status" community:** Not good status community description yet.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

ID: 225

HFI

## 1. General information

- 1.01 GIG:** Mediterranean  
Transitional waters - coastal lagoons
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Italy
- 1.05 Specification:** It has not yet been adopted as a national protocol (Italy hasn't a national protocol regarding fish in TWs)
- 1.06 Method name:** **Habitat Fish Index**
- 1.07 Original name:** *Habitat Fish Index*
- 1.08 Status: Method is/will be used in** neither first nor second RBMP
- 1.09 Detected pressure(s):** Catchment land use, General degradation, Habitat destruction, Heavy metals, Hydromorphological degradation, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter, Riparian habitat alteration

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

Franco, A., P. Torricelli & P. Franzoi, 2009. A habitat-specific fish-based approach to assess the ecological status of Mediterranean coastal lagoons. Marine Pollution Bulletin 58: 1704-1717.

**1.13 Method developed by**

Anita Franco, Piero Franzoi, Patrizia Torricelli  
afranco@unive.it, pfranzoi@unive.it, torri@unive.it  
Dept. of Environmental Sciences, University of Venice

**1.14 Method reported by**

Anita Franco  
afranco@unive.it  
Dept. Environmental Sciences, University of Venice

**1.15 Comments**

The method is under modification to adjust it to the requirements of the Directive. It is based on the separate assessment of different habitat types.

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Various Authors, 2008. Protocolli per il campionamento e la determinazione degli elementi di qualità biologica e fisico-chimica nell'ambito dei programmi di monitoraggio ex 2000/60/CE delle acque di transizione. ICRAM, Rome.

**2.02 Short description**

Sampling designed for sampling major habitats in proportion to their presence within a water body (1 site per habitat with an area < 2.5 sq-km; 2 sites per habitat with area 2.5-5 sq-km; 3 sites per habitat with area 5-10 sq-km; +1 site every additional 10 sq-km, with habitat area < 50 sq-km; +2 sites every 25 sq-km, with habitat area > 50 sq-km. A "sampling unit" is a sampling performed by trawling the seine net (by hand, by two operators in the water) over an area of about 150 sq-m.

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying

**2.04 Sampling/survey device:** Seine netting

**2.05 Specification:** seine net 20m long, 2m high, mesh size (interknot distance) 2mm, baglike type

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
dominant shallow soft bottom habitats (depth < 1.5m) distinguished in vegetated (seagrass)

**2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone

**2.08 Sampling/survey month(s):** Spring: March to June; Summer: July to September; Autumn: October to December

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

at least 2 replicates per habitat

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Sampling area per replicate = 150 square-meters (total 300 square-meters sampling area per habitat)

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 1 cm

**2.13 Sample treatment:** Organisms of the complete sample are identified.

- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** number of individuals per 100 square-meters
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** fish individual body size (total length, mm; wet weight, g)
- 2.18 Special cases, exceptions, additions:** Body size measured for all individuals if sample size <100 individuals. ☐ Body size measured for 100 individuals (randomly subsampled) if sample size >100 individuals. ☐ Possible additional measures: sex, maturity, gastric contents, parasites, individual health status (morphological anomalies, external lesions...)
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Total species richness, Presence-absence of indicator species (i.e. species associated to the habitat of conservational value), Presence-absence of alien species, Species assemblage composition, Species assemblage structure (% abundance), Species dominance (no. of species that make up 90% of total fish abundance in the sample), Species richness of Estuarine Residents, Species richness of Marine Migrants, % abundance of Estuarine Residents, % abundance of Marine Migrants, Species richness of strictly Benthivorous species, Species richness of detritivorous species (for unvegetated marsh habitat) or of species feeding on demersal-pelagic prey (hyperbenthos-zooplankton-fish) (for seagrass habitat), % abundance of strictly Benthivorous species, % abundance of detritivorous species (for unvegetated marsh habitat) or of species feeding on demersal-pelagic prey (hyperbenthos-zooplankton-fish) (for seagrass habitat).
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
Sum of metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Habitat-specific
- 3.06 Key source(s) to derive reference conditions:**  
n.a.  
Best values observed for each metric calculated on a dataset used for the index local calibration (not necessarily corresponding to a single geographic reference site); NB: reference conditions derived separately per season per habitat
- 3.07 Reference site characterisation**  
**Number of sites:** 20 sites per habitat per season in the Venice lagoon  
**Geographical coverage:** see C-06  
**Location of sites:** see C-06  
**Data time period:** April-May (Spring), July-August (Summer), October-December (Autumn) 2002  
**Criteria:**  
For each metric the reference represent the best value observed in the calibration dataset, hence a single geographic reference site is not identified (e.g. one site may have an optimal species richness, another one may have an optimal dominance value)
- 3.08 Reference community description**  
Rich assemblage (both in terms of total species and species per ecological and trophic guild), with the presence of at least one indicator species (e.g. *Aphanius fasciatus* and *Pomatoschistus canestrinii* in marsh habitats; *Syngnathus abaster*, *Hippocampus hippocampus*, and *H. guttulatus*, *Zosterisessor ophiocephalus* in seagrass) and absence of alien species, with a good balance (in terms of % abundance) among species and functional guilds.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

Possible modification of boundaries according to the results of the intercalibration exercise

**3.11 Boundary setting procedure**

Not yet performed

**3.12 "Good status" community:** n.a.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 255

FITOHMIB

## 1. General information

- 1.01 GIG:** Mediterranean
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Spain
- 1.05 Specification:** Balearic islands and Valencia (East of Spain)
- 1.06 Method name:** *Balearic islands multimetric*
- 1.07 Original name:** *Multimétrico de las Islas Baleares*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Ecological data from 284 points were examined to establish pressure-impact relationship between phytoplankton metrics, PCA degradation gradients (that included nutrients, alkalinity, temperature, pH, oxygen, salinity, chlorophylla-a and AFDM ). The relationship were significative.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

**1.12 Scientific literature:**

PARDO, I., LUCENA, P., ABRAÍN, R., GARCÍA, L. & C. DELGADO. 2010. Implementación de la DMA en Baleares: evaluación de la calidad ambiental de las masas de agua epicontinentales utilizando indicadores e índices biológicos. Informe Final. Tomo II: Zonas Húmedas. Informe Técnico. Universidad de Vigo.

**1.13 Method developed by**

Isabel Pardo  
ipardo@uvigo.es  
Department of Ecology and Animal Biology, University of Vigo

**1.14 Method reported by**

Rut Abraín Sánchez  
rutabrain@gmail.com  
Department of Ecology, University of Vigo, Spain

**1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

MEDGIG agreement 2005-IIAMA, 2005. Informe sobre los trabajos realizados para establecer las presiones, recopilar la información histórica y determinar las tipologías, los parámetros y rangos de calidad exigidos por la directiva marco del agua para las aguas costeras y de transición. Comunidad Valenciana. España. 39 pp + Anexos. Lund, J.W.G., Kipling, C. y Le Cren, E.D. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia* 11(2): 143-170. Vargo, G.A., 1978. Using the fluorescence microscope. In: SOURNIA, A. (ed.), *Phytoplankton Manual. Monographs on oceanographic methodology*. UNESCO, pp. 108-112.

**2.02 Short description**

The samples were collected from water with a depth of 0.3 m. The samples were fixed with glutaraldehyde (2%) (Sournia 1978) and stored at 4°C in dark.

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying

**2.04 Sampling/survey device:** n.a.

**2.05 Specification:** The samples were collected from water with a depth of 0.3 m. The samples were fixed with glutaraldehyde (2%) and stored at 4°C in dark. Algal counts were made by epifluorescence microscopy with a Leica DM2500

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
Water column.

**2.07 Sampled/surveyed zones in areas with tidal influence:**

**2.08 Sampling/survey month(s):** February-March and May-June

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Two samples every year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1 replicate

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

125 mL

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 0.7 µm

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

The sample analysis is performed by filtering a sample volume determined with a polycarbonate membrane pore 0.2 µm (Millipore GTPP Ø 25mm). To select the sample volume to be filtered takes into account the previously measured value of chlorophyll-a.

- 2.14 Level of taxonomical identification:** Other  
Class level.
- 2.15 Record of abundance:** Individual counts, Relative abundance  
**in relation to** Volume  
**Unit** Number of cells per liter and abundance %
- 2.16 Quantification of biomass:** Chlorophyll-a concentration
- 2.17 Other biological data:**
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Oligohaline type: Cyanobacteria (%) + Chlorophyll-a (µg/L) ☐ Mesohaline type: [ Prasinophyta (%) + Cryptophyta (%) + Diatom (%) ] + Chlorophyll-a (µg/L)
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**  
**Number of sites:** 6 sites (27 samples)  
**Geographical coverage:** Balearic islands  
**Location of sites:** Balearic islands (1 site in Majorca, 3 sites in Minorca and 1 site in Formentera)  
**Data time period:** Data of years 2005-2008  
**Criteria:**  
A priori evaluation: we have distinguished two buffer zones around the selected sampling sites to evaluate the different REFCOND pressures. The first one corresponds to the area immediate to the edge (<50 m band), and the second band goes from the 50 m that limits the first band to the following 300 m. A posteriori evaluation: we selected sites consisted of checking for consistency using information on water physicochemistry and biological communities.
- 3.08 Reference community description**  
under development
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites Using paired metrics that respond in different ways to the influence of the pressure
- 3.11 Boundary setting procedure**  
The boundary limit between High and Good ecological status were obtained calculating the percentile 25 (P25) of the EQR of the reference sites. The percentil 25 in then divided into four to extract the remaining boundaries between classes. In addition, dispersion plots between paired metrics along a pressure gradient were performed to aid in the ecological interpretation of their interactions, to adjust the remaning boundaries between the classes, according the Boundary Setting protocol (i.e. if the metrics interactions corresponded to a class centre or a class boundary, and to which classes they relate).
- 3.12 "Good status" community:** Not good status community description yet.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**



ID: 224

FR-FI-TR

## 1. General information

**1.01 GIG:** Mediterranean, North-East-Atlantic  
For estuaries : the common type exist only because each country come to one estuary to apply its own methodology. The results are to be intercalibrated after. For lagoons, common types are based on lagoon surface (> or < 2.5km<sup>2</sup>) ; the salinity regime is a

**1.03 BQE:** Fish Fauna

**1.04 Country:** France

**1.05 Specification:**

**1.06 Method name:** *French Transitional Water Fish Index*

**1.07 Original name:** *Indicateur poisson pour les eaux de transition françaises*

**1.08 Status: Method is/will be used in** Second RBMP (2015)

**1.09 Detected pressure(s):** General degradation, Heavy metals, Pollution by organic compounds (e.g. DDT, PCB)

### *Has the pressure-impact-relationship been tested?*

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

The following steps were adopted: (1) Indices, based on pollutions (for estuaries) and human activities (for lagoons), were elaborated and used as proxies to describe the anthropogenic disturbances affecting fishes. (2) The impact of these disturbances on a large panel of fish metrics describing the functioning of the ecosystem was tested via pression / impact models (fish metrics ~ indices of anthropogenic disturbances). These models take into account the metrics variability due to the sampling protocol and environmental features. (3) A methodology was developed to identify thresholds for metrics presenting significant trends with increasing pressure. (4) Redundant metrics and metrics with non-discriminant thresholds were removed. For lagoons we considered the land use pressure according to Corinne Land Cover (mining, urban areas, agriculture, industries). The fish data base contains about 350 fishing occasions (= 24 hours of a fyke net). For estuaries pressures were characterised thanks to the National Network of Observation (hold by Ifremer on behalf of the Ministry of environment). We used the median over 6 years of seasonal measures (2000 to 2005) for 5 heavy metals (Cu, Zn, Cd, Hg, Pb), PCB, HAP in oysters and mussels flesh. We used 1100 fishing occasions done in French 20 estuaries in order to find the relationship between the level of pollutant and the observed abundance of ecological guilds in the fish samples. GLM were developped to estimate the metrics' response to the computed indices of anthropogenic pressures; variability due to sampling protocol and environmental features has been taken into account in the models.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Girardin, M., M. Lepage, R. Amara, P. Boët, A. Courrat, C. Delpech, B. Durozoi, P. Laffargue, O. Le Pape, J. Lobry, E. Parlier & S. Pasquaud, 2009. Développement d'un indicateur poisson pour les eaux de transition - Fish Index development for transitional waters. Programme Liteau II 2005, 50p.

**1.12 Scientific literature:**

Delpech, C., A. Courrat, S. Pasquaud, J. Lobry, O. Le Pape, D. Nicolas, P. Boët, M. Girardin & M. Lepage, 2010. Development of a fish-based index to assess the ecological quality of transitional waters: The case of French estuaries. Marine Pollution Bulletin, doi: 10.1016/j.marpolbul.2010.01.001.

**1.13 Method developed by**

Mario Lepage  
mario.lepage@cemagref.fr  
CEMAGREF

**1.14 Method reported by**

Mario Lepage  
mario.lepage@cemagref.fr  
CEMAGREF

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Lepage, M. & M. Girardin, 2006. Inventaire Poisson dans les eaux de transition. Protocole d'échantillonnage de la façade Atlantique et Manche. Cestas, Cemagref - groupement de Bordeaux: 32p. Lepage, M. & M. Girardin, 2006. Inventaire Poisson dans les eaux de transition. Protocole d'échantillonnage pour le District Rhône Méditerranée et Corse. Cestas, Cemagref- groupement de Bordeaux: 32.

**2.02 Short description**

In estuaries, the sampling is done in a way covering well the area from the upper limit to the downstream limit of the water body. Hauls are done against the current in a depth between 1 and 14 m. The shape of the bottom should rather be flat or at least without big spike from the bottom. Sampling can be done on a variety of bottom types but not on big stones or rocky substratum. Spring tides are avoided because of the strong current preventing the sampling gear to work properly. The ground speed should be maintained between 1.5 and 2.5 knots measured with a GPS. In lagoons, the sampling is done with winged fyke nets equipped with a lead that is guiding the fish toward the trap. The fykes are set to sample the littoral area of the lagoon. The lead start from the edge and is set perpendicular to the coastline. We avoid to set the net close to a known emission point of sewage or other human disturbance. The sampling station are chosen according to the expert knowledge of the lagoon in order to have a good representation of the waterbody. The fykes are recovered every 24h for a total duration of 4 days.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge, Stratified sampling/surveying

- 2.04 Sampling/survey device:** Beam trawl, Fyke net
- 2.05 Specification:** Beam trawl 1.5m wide and 0.5m height for small estuaries (<100 km<sup>2</sup>) and oligohaline zone of big estuaries (>100 km<sup>2</sup>) ; Beam trawl 3.0m wide and 0.5m height for polyhaline and mesohaline zones of large estuaries. For lagoons, fyke net are used with a 20m long lead, wings and mesh size of 3mm
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** Survey in spring (mid-april to june) and in autumn (september to early november) for estuaries and lagoons
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Two campaigns a year (spring and autumn) for 3 years in a row/management plan for estuaries and for lagoons
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
6 to 8 replicates/salinity zone with a minimum of 12 replicates/estuary, in spring and autumn . 20 replicates/ large lagoon (on 5 sites - 4 replicates per site) and 8 replicates/small lagoon (on 2 sites - 4 replicates per site), in spring and autumn.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
For estuaries : 6 hauls X 15min / salinity zone. ex : When polyhalin, mesohalin and oligohalin areas exist, a minimum of 18 hauls X 15 min is done = 270 min for estuaries. For large lagoons 5 nets are set for 4 days (24 hours/day) and on small lagoons 2 nets are set for 4 days.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** Minimum size for fyke net >15 mm and Minimum size for beam trawl >25 mm
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
We identify every species and measure the length of a minimum of 30 individuals/species. The extra individuals are simply counted. When sub-sample is performed, the large individuals are put aside and weighted individually. We look at the sample in case one rare species would be present and when visible, the individuals are put aside and measured. For the rest of the sample, it is divided as many time as necessary in order to have no more than a few hundred individuals in the sub-sample (200-300). This sub-sample is completely identified and measured up to 30 individuals/species, the extra is only counted. The total number of individuals is then estimated by n time the sample was divided.
- 2.14 Level of taxonomical identification:** Species/species groups  
For the particular case of mugilidae and gobiidae, it happens that the identification stays at the level of family but we try to avoid this as much as possible. Sometimes we can come back on the family level data to split into the known species of that particular family in the waterbody with a proportion of occurrence. ex : Total number mugilidae = 100 (10% Liza ramada, 50% Chelon labrosus, 40% Liza aurata). This can be done for the calculation of certain metrics.
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area, Time  
For beam trawl, abundance is related to area. With the fyke nets, the abundance is related to time  
**Unit** In Estuaries the CPUE are expressed in number of individuals/ha, while in lagoon the CPUE is expressed in number of individuals/fyke net/day
- 2.16 Quantification of biomass:** n.a.  
total weight (g)/species/fishing occasion; sometimes some individual weights are also available
- 2.17 Other biological data:** Individual fork length of fish in mm
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## 3. Data evaluation

### Evaluation

- 3.01 List of biological metrics**  
Several metrics were tested : ☐- densities per functional guild (ecological, trophic and vertical distribution guilds). ☐- number of species per guild ("")☐+ two other metrics : total densities and total number of species.☐Each metric is calculated at the fishing occasion scale in order to take into account the effects of the sampling protocol.☐Metric selection has not been completed yet and new metrics may be tested in the coming months.☐For instance, the last version of the indicator includes the following metrics :☐- for estuaries : total log-density, log-density of CA, log-density of MJ, log-density of benthic fishes (see : Delpech et al., 2010)☐- for lagoons : log-density of CA, log-density of benthic fishes, log-density of zooplankton-feeder.☐All metrics are calculated per trawl haul (or per day of fyke net for lagoons)
- 3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Average metric scores

For instance, but it might change soon toward weighted average metric scores including incertitude measures.

**3.04 From which biological data are the metrics calculated?**

Data from single spatial replicate

### **Reference conditions**

**3.05 Scope of reference conditions:** Habitat-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Least Disturbed Conditions, Modelling (extrapolating model results)  
Still in progress

**3.07 Reference site characterisation**

**Number of sites:** No site in reference condition for fish in transitional water in France

**Geographical coverage:** No site in reference condition for fish in transitional water in France

**Location of sites:** No site in reference condition for fish in transitional water in France

**Data time period:** No site in reference condition for fish in transitional water in France

**Criteria:**

For estuaries: Organic contaminants value in the pressure index is set to 0 Heavy metals values in the pressure index were set to background noise based on the minimum value observed over a period of 30 years. For lagoons: We considered the land use pressure according to Corinne Land Cover (mining, urban areas, agriculture, industries). In reference site these activities do not exist.

**3.08 Reference community description**

(still in progress) Values derived from models for each fish metric. This reference metric' value is calculated per habitat i.e. salinity zone (and also season) Method 1 : Reference community corresponds to the value of each metric in the least disturbed conditions based on our pressure index. Method 2 : pressure index set to 0 and the corresponding value of each metric calculated from the models is used as reference community description.

**3.09 Results expressed as EQR?**

Yes

For the moment we have five homogenous classes but work is still in progress

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

There are two levels for boundary setting : at the metric level and at the multimetric indicator level. - at the metric level : boundaries are set using fitted results of the models for different levels of pressure (3 values - low, medium and high - of the pressure index are used) - at the indicator level, boundaries were set using the pressure index : all pressure index' values for French estuaries were plotted with the reference site index value (see C12) and expert knowledge was then used to set boundaries in relation with the pressure index.

**3.12 "Good status" community:** Metric values calculated from models.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

Protocol is standardized. Variability on fish metrics due to environmental features and residual variability from the protocol were taken into account via modelling. However, final incertitude on the classification of the water body has still to be studied.

**3.14 Comments:**

none

ID: 27

FL-AN-CO

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
TW-NEA11
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Angiosperms  
tidal marshes
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish region
- 1.06 Method name:** *Tidal marsh quality index*
- 1.07 Original name:** *Schor kwaliteitsindex*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Riparian habitat alteration
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Brys et al found a positive relationship between vegetation diversity and marsh surface ( $t_{2,33}=1,939$ ;  $P=0,045$ ), shape index ( $t_{2,33}=2,898$ ;  $P=0,007$ ); creekdensity ( $t_{2,33}=3,477$ ;  $P=0,001$ ) (Multiple regression analysis). These geomorphological characteristics are directly related to the above mentioned pressures
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. Vlaamse Milieumaatschappij, Erembodegem, Belgium.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Erika Van den Bergh  
erika.vandenbergh@inbo.be  
Research Institute for Nature and Forest
- 1.14 Method reported by**  
Erika Van den Bergh  
erika.vandenbergh@inbo.be  
Research Institute for Nature and Forest
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
A vegetation map is made of all the tidal marshes every 6 years, using aerial photographs or hyperspectral images in combination with ground truthing; vegetation relevés are made according to the Braun-Blanquet method of the permanent quadrats. Additionally sites of underrepresented vegetation types are selected according to a stratified random selection strategy and a vegetation relevé is made.
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying, Stratified sampling/surveying
- 2.04 Sampling/survey device:** n.a.  
vegetation relevé; vegetation
- 2.05 Specification:** Aerial photographs and lidar topographic data.
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Intertidal zone
- 2.08 Sampling/survey month(s):** august-september
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
1
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
5
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
all tidal marshes

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** n.a.

all plant species in vegetation relevés are identified.

**2.14 Level of taxonomical identification:** Other, Species/species groups  
species level and vegetation type

**2.15 Record of abundance:** Percent coverage, Relative abundance  
**in relation to** Area  
**Unit** % coverage

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**  
See Brys et al 2005

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.  
decision tree including several of the above mentioned methods

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time  
Data from single spatial replicate

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** n.a.

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**  
n.a.

**3.08 Reference community description**

Reference conditions are assumed to correspond to an EQR value of 1, which is associated with expert-based type-specific metric values reflecting high taxa richness, sensitivity and diversity.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
K-means clustering

**3.11 Boundary setting procedure**

EQR gradient is assumed to represent a continuous trend with general degradation.

**3.12 "Good status" community:** Is that of an estuary in GEP status.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 249

AQI

## 1. General information

- 1.01 GIG:** North-East-Atlantic
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Angiosperms, Other Angiosperms  
Seagrass, halophilus scrubs, Spartina swards, Atlantic salt meadows, etc. (Habitats of Directive 92/43/CEE; DOCE 1992)
- 1.04 Country:** Spain
- 1.05 Specification:** Cantabrian estuaries (Atlantic area)
- 1.06 Method name:** **Angiosperms Quality Index**
- 1.07 Original name:** *Índice de Calidad de Angiospermas*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Riparian habitat alteration
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).
- 1.10 Internet reference:** <http://www.indurot.uniovi.es/actividades/macro/paginas/Angiospermas.aspx>
- 1.11 Pertinent literature of mandatory character:**  
Annex I of the Habitat Directive 92/43/CEE and Interpretation Manual of European Union Habitats (European Commission, 2003)
- 1.12 Scientific literature:**  
García, P., E. Zapico & A. Colubi, 2009. An angiosperm quality index (AQI) for Cantabrian estuaries. *Ecological Indicators*, 9(5): 856-865
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Pilar García, Eva Zapico, Ana Colubi<br>eva@indurot.uniovi.es<br>Instituto de Recursos Naturales y Ordenación del Territorio<br>(Indurot - Universidad de Oviedo) | <b>1.14 Method reported by</b><br>Eva Zapico Redondo<br>eva@indurot.uniovi.es<br>Instituto de Recursos Naturales y Ordenación del Territorio<br>(Indurot - Universidad de Oviedo) |
|--|---|
- 1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Interpretation Manual of European Union Habitats (European Commission, 2003)
- 2.02 Short description**  
The method is based on a detailed habitat mapping, using a suitable topographic scale selected before field surveys. Scale depends on estuarine extension (for most Atlantic estuaries, a scale range between 1:5000 and 1:15000 could be considered optimal). The mapping is carried out during field surveys by taxonomic expert in cartographic techniques, with the aid of GPS and aerial photographs. The information provided by aerial photography is checked during field surveys, by examining boundaries between patches and also community density (coverage). Geographic Information System are used to store information on a geodatabase.
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** n.a.  
Habitat mapping and coverage
- 2.05 Specification:** Habitat mapping with the aid of GPS and aerial photographs in Geographical Information Systems
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** June to September (summer)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Once during the surveillance monitoring
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Replicates are not necessary.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
For example, an estuary of 450 ha could be done in four days (once during River Basin Management Plan).

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** Macroscopic organisms (macrophytes)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Percent coverage

**in relation to** Area

**Unit** Percent coverage in respect to surface area

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:**

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Diversity of estuarine habitats: Gini-Simpson's index. Status of estuarine habitats (relative deviations from optimal coverage) Variations in the surface area of natural tidal habitats See formulas of sub-metrics in García et al. (2009).

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

Geometric mean rate

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data

**3.07 Reference site characterisation**

**Number of sites:** 17 estuaries

**Geographical coverage:** Asturias and Cantabria (Spanish regions)

**Location of sites:** Transitional water bodies of Asturias and Cantabria

**Data time period:** 1993-1996 and 2005-2007

**Criteria:**

The reference conditions are obtained from historical data and expert knowledge. There are no estuaries in reference conditions.

**3.08 Reference community description**

The number of different natural habitats in the estuary should be high. Habitats should have coverages equal or higher than expected ones. There should be no loss of extension of natural habitats of the estuary.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

Boundary setting at 0.85 (High/Good); 0.70 (Good/Moderate); 0.50 (Moderate/Poor) and 0.25 (Poor/Bad).

**3.11 Boundary setting procedure**

see García et al. (2009)

**3.12 "Good status" community:** The number of habitats deviates slightly from the reference conditions, between 15 and 30 %. The deviations from optimal coverages are between 15 and 30 % from the reference conditions. Natural habitats occupy 70 to 85 % of the total estuary.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

ID: 62

BEQI and IOBS

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
TW-NEA11
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish region
- 1.06 Method name:** *Benthos Ecosystem Quality Index and Indice oligochètes de bioindication des sédiments*
- 1.07 Original name:** *Benthos Ecosystem Quality Index and Indice oligochètes de bioindication des sédiments*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter, Riparian habitat alteration
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** <http://www.beqi.eu/>
- 1.11 Pertinent literature of mandatory character:**  
VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. Vlaamse Milieumaatschappij, Erembodegem, Belgium.
- 1.12 Scientific literature:**  
n.a.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Erika Van den Bergh<br>erika.vandenbergh@inbo.be<br>Research Institute for Nature and Forest | <b>1.14 Method reported by</b><br>Jeroen Speybroeck<br>jeroen.speybroeck@inbo.be<br>Research Institute for Nature and Forest |
|---|--|
- 1.15 Comments**  
The use of either BEQI or IOBS depends on the national type of transitional water

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
intertidal: 1 core (4.5 cm across) per site, used as replicates for the habitat within the water body; subtidal: same but from Reineck box-corer
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying, Stratified sampling/surveying
- 2.04 Sampling/survey device:** Corer, Grab
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** september
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
1 per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
depends on sites; ca. 5 per habitat
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
depends on number of sites per habitat per water body

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** All animals retained after sieving with 500 µm (IOBS) or 1000 µm (BEQI) mesh size
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups  
For BEQI, Oligochaeta treated as 1 taxon.
- 2.15 Record of abundance:** Individual counts

in relation to Area

Unit individuals/m<sup>2</sup>

2.16 Quantification of biomass: n.a.

AFDW

2.17 Other biological data: none

2.18 Special cases, exceptions, additions: none

2.19 Comments

freshwater method remains 'best available'

### 3. Data evaluation

#### Evaluation

3.01 List of biological metrics

see Speybroeck et al. 2008

3.02 Does the metric selection differ between types of water bodies? Yes

3.03 Combination rule for multi-metrics: Average metric scores

3.04 From which biological data are the metrics calculated?

Aggregated data from multiple sampling/survey occasions in time

Aggregated data from multiple spatial replicates

Data from single sampling/survey occasion in time

#### Reference conditions

3.05 Scope of reference conditions: n.a.

3.06 Key source(s) to derive reference conditions:

Expert knowledge, Historical data, Least Disturbed Conditions

3.07 Reference site characterisation

Number of sites: n.a.

Geographical coverage: n.a.

Location of sites: n.a.

Data time period: n.a.

Criteria:

Time period, habitat type

3.08 Reference community description

Reference conditions are assumed to correspond to an EQR value of 1, which is associated with expert-based type-specific metric values reflecting high taxa richness, sensitivity and diversity. BEQI references used from Dutch historical data

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites

Remark: option 3 (High-good boundary derived from metric variability at near-natural reference sites (e.g. 5th percentile value)) refers to BEQI only

3.11 Boundary setting procedure

n.a.

3.12 "Good status" community: Is that of an estuary in GEP status.

#### Uncertainty

3.13 Consideration of uncertainty: No (to be done)

3.14 Comments:

Scope of reference conditions: Surface water type-specific (IOBS) / Habitat-specific (BEQI)

ID: 252

AeTV

## 1. General information

- 1.01 GIG:** North-East-Atlantic
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Germany
- 1.05 Specification:** NEA, TW, Elbe, Weser, Ems
- 1.06 Method name:** *Estuary-Type Method*
- 1.07 Original name:** Ästuartypieverfahren
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation, Habitat destruction

*Has the pressure-impact-relationship been tested?*

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:** <http://www.fgg-elbe.de>

**1.11 Pertinent literature of mandatory character:**  
n.a.

**1.12 Scientific literature:**  
only in German; divers publications to the subject AeTV in ARGE ELBE (2006, 2007, 2008)

**1.13 Method developed by**  
Hans-Joachim Krieg 2005-2008  
[huug.krieg@t-online.de](mailto:huug.krieg@t-online.de)  
Consultant/Senior expert; Hydrobiologische Untersuchungen  
und Gutachten - HUUG Tangstedt, Germany

**1.14 Method reported by**  
Hans-Joachim Krieg  
[huug.krieg@t-online.de](mailto:huug.krieg@t-online.de)  
Consultant, Hydrobiologische Untersuchungen und Gutachten -  
HUUG Tangstedt, FRG

**1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
SOP (in German only): Standardarbeitsanweisung (SOP) für Laboratorien des Bund/Länder-Messprogramms Nord-/Ostsee des UBA Berlin (FRG): Untersuchungen der benthischen wirbellosen Fauna in Sedimenten der Übergangsgewässer (Weichböden)
- 2.02 Short description**  
3 and 6 sediment samples are taken from 1 ecotope. Each sample is sieved separately. The samples from Van Veen-grab with 500 µm immediately; the corer-samples floated over 250 µm later in the laboratory. ) In principal residues are stored and transferred to the laboratory. Benthic species are separated and identified to the lowest taxonomic level.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Corer, Grab  
the method is based on 2 mes
- 2.05 Specification:** Van Veen-grab (0,1 m<sup>2</sup>); corer with diameter 4.5 cm and 12 cm
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** March to April until early May; End of September to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3 + 6 replicates
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Sum of 6 spatial replicates á 16 cm<sup>2</sup> (corer) = 96 cm<sup>2</sup> AND sum of 3 replicates (Van Veen-grab) á 0,1 m<sup>2</sup> = 0,3 m<sup>2</sup>; a minimum of 8 ecotopes per water body

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** mesh-size 250 µm (meiofauna, oligochaetes, small polychaetes) and 500 µm (especially MZB)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.  
If more than 200 individuals of a species are counted in a sample, the material is splitted in a round 10-chamber-plankton-divisor; sub-sample(s) of 1/10 to 5/10 are analysed.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
all to species level (inclusive oligochaetes and chironomids), but except Nemertini, Turbellaria, Nematoda

- 2.15 Record of abundance:** Abundance classes, Individual counts  
the individual counts are converted into 7 abundance classes; Fibonacci-Ranking 1, 2, 3, 5, 8, 13, 21  
**in relation to** Area  
**Unit** number of individuals per 1m<sup>2</sup>
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:**
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
(1) AeTI (Estuary-Type-Index) and biodiversity (2) Mean species number and (3) alpha-diversity according to Fisher et al. (1945)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
(1) core-metric = AeTI (estuary-type-index); two co-metrics of biodiversity in the ranking (2) mean species number and (3) alpha-diversity (syn. Fisher-index)
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** a minimum of 8 sites in each surface water body (n = 4) of the Elbe-Estuary  
**Geographical coverage:** The Elbe-Estuary, so-called Koordinierungsraum Tideelbe of the federal states Hamburg, Lower Saxony and Schleswig-Holstein  
**Location of sites:** Elbe-Estuary between km 589 (Geesthacht) and km 727 (Cuxhaven); eulittoral and sublittoral sites  
**Data time period:** Historical datas 1862, 1869-1870, 1886, 1893, 1900-1930; contemporary datas 1950-1970  
**Criteria:**  
The communities at the sites had to correspond with description of the reference community description referring to a certain habitat. Spatio-temporal variability has to be taken into account of the community composition.
- 3.08 Reference community description**  
species number, structural composition and diversity of benthic invertebrate communities, the abundance and share of sensitive species
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites
- 3.11 Boundary setting procedure**  
The boundary setting procedure is orientated at the normative descriptions of the WFD (PE-CONS 3639/00 Annex V; Rev.1: 1.2.3). The boundaries were additionally adjusted by the assessment of expert judgement (H.-J. Krieg, in ARGE ELBE 2007).
- 3.12 "Good status" community:** High portion of sensitive taxa; complex benthic community: presence of opportunists low to moderat. High species number and high diversity structure. Slightly deviations, within tolerance <=20% from high.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
The Estuary-Type Method (AeTV) is actual used as the biological assessment method (= standard-tool) for the benthic evertbrates in the Tidal Elbe (Estuary) (ARGE ELBE, KOR-TEL); apart from this the AeTV was evaluated in the estuaries of Weser and Ems (NLWKN Oldenburg, FR Germany)



ID: 250

QSB

## 1. General information

- 1.01 GIG:** North-East-Atlantic
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Spain
- 1.05 Specification:** North East Atlantic. Region of Cantabria
- 1.06 Method name:** *Quality of Soft Bottoms*
- 1.07 Original name:** *Quality of Soft Bottoms*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Heavy metals, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [www.chcantabrico.es](http://www.chcantabrico.es); [www.dmacantabria.com](http://www.dmacantabria.com)
- 1.11 Pertinent literature of mandatory character:**  
Confederación Hidrográfica del Cantábrico. 2010. Plan Hidrológico de la Demarcación Hidrográfica del Cantábrico. (River Basin Mangement Plan)
- 1.12 Scientific literature:**
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Araceli Puente Trueba<br>puentea@unican.es<br>IH Cantabria, University of Cantabria | <b>1.14 Method reported by</b><br>Araceli Puente Trueba<br>puentea@unican.es<br>IH Cantabria, University of Cantabria |
|--|---|
- 1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Standard procedures for the study of benthic macroinvertebrates. e.g. Norma UNE-EN ISO 16665. Directrices para el muestreo cuantitativo y el tratamiento de muestras de la macrofauna de los fondos blandos marinos (Guidelines for quantitative sampling and sample processing of marine soft-bottom macrofauna); Methods for the study of marine benthos. 2007. Holme & McIntery.
- 2.02 Short description**  
Intertidal: direct sampling of specify surface and 15 cm depth. Random distribution of replicates in each station. Subtidal: Random taking of replicates in each station. Each replicate is sieved through a 1 mm mesh screen and the residue preserved in 4% formalin until sorting.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Stratified sampling/surveying
- 2.04 Sampling/survey device:** Corer  
Direct sampling (for intertidal)  
Box-Corer (for subtidal)
- 2.05 Specification:** Box-Corer (for subtidal)
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Soft bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** June to august
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Not specify. Depending on spatial variability, but usually from 2-4 (intertidal) to 6-10 (subtidal) replicates in each station. From 2 to 20 stations per water body.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
0.25 m<sup>2</sup> per replicate (intertidal), most cases 0.50 m<sup>2</sup> per station ; 0.017 m<sup>2</sup> per replicate (subtidal), most cases 0.1 m<sup>2</sup> per station

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 1 mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
Biomass

in relation to Area

Unit number of individuals per one square-metre, grams of fresh weight per square-metre

**2.16 Quantification of biomass:** n.a.  
individuals weight

**2.17 Other biological data:**

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Richness, Bray-Curtis similarity index (comparing to a predefined community type), percentage of opportunistic species (Ecological groups IV and V of AMBI index) and total abundance.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores, Weighted average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Habitat-specific

**3.06 Key source(s) to derive reference conditions:**

Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** 40 (distributed by community type)

**Geographical coverage:** North Coast of Spain. NEA

**Location of sites:** Cantabria Region. North Coast of Spain. NEA

**Data time period:** from 2005 to 2008

**Criteria:**

Least disturbed sites, mainly regarding sewage discharges.

**3.08 Reference community description**

Three types of communities have been defined. Abra community is a high richness assemblage characterized by *Loripes lacteus*, *Nephtys hombergii*, *Melinna palmata*, *Cerastoderma edule*, *Nassarius reticulatus*, *Abra alba*, etc. *Scrobicularia* (called *Scrobicularia* II) is a low richness and high dominance community, dominated by *Hediste diversicolor*, *Scrobicularia plana*, *Cyathura carinata* and *Carcinus maenas*. A third community is described (called *Scrobicularia* I), with an intermediate richness, in which are present the dominant species of *Scrobicularia* II but other species are also important (*Abra tenuis*, *Cerastoderma edule*, *Ruditapes decussatus*).

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** Richness, structure and composition of the community are similar to those of reference habitats (in other words, many of the species characteristics of each community type are present). Opportunistic species are absent or rare, total abundance is not very high (as in organic polluted sites) and not very low (as in very degraded sites).

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

ID: 53

Z-EBI

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
TW-NEA11
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish Region
- 1.06 Method name:** *Zone-specific Estuarine index of Biotic Integrity*
- 1.07 Original name:** *Zone-specifieke Estuariene index voor Biotische Integriteit*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation, Riparian habitat alteration

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

oxygen (Maes et al., 2007, 2008) on diadromous species. Breine 2009: oxygen on species distribution within the estuary

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. Vlaamse Milieumaatschappij, Erembodegem, Belgium.

**1.12 Scientific literature:**

Breine, J., 2009. Fish assemblages as ecological indicator in estuaries: the Zeeschelde (Belgium). PhD thesis. Katholieke Universiteit Leuven and Research Institute for Nature and Forest. INBO.T.2009.1. 263 pp.

**1.13 Method developed by**

Jan Breine

jan.breine@inbo.be

Research Institute for Nature and Forest

**1.14 Method reported by**

Jan Breine

jan.breine@inbo.be

Research Institute for Nature and Forest

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

n.a.

**2.02 Short description**

At each site, one or two double fyke nets are positioned at low tide and emptied daily for a 48 hours period. All fish caught are identified to species level on site. Each survey per site is standardized as number of fish per fyke per day. These CPUE data are grouped per salinity zone (mesohaline, oligohaline and freshwater) and pooled per year.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Fyke net, Seine netting

**2.05 Specification:** Doel Nuclear Power Station: cooling water survey (monthly 3 hours)

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones

**2.08 Sampling/survey month(s):** March - November

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

3 per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

3

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

48 hours with fykes

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 1 mm but all fish are processed (weighed and measured)

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

in relation to Area, Time, Volume

(Volume in case of Doel catches)

Unit CPUE

- 2.16 Quantification of biomass:** n.a.  
balance
- 2.17 Other biological data:** macro-invertebrates are recorded
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
method is WFD-proof

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
see Breine, 2009
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** 1850 combined with 1995-2008  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
is that of an estuary in MEP status: EQR = 1
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** Is that of an estuary in GEP status: EQR = 0.8.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 162

FAT-TW

## 1. General information

**1.01 GIG:** North-East-Atlantic  
n.a.

**1.02 Category:** Transitional Waters

**1.03 BQE:** Fish Fauna

**1.04 Country:** Germany

**1.05 Specification:** none

**1.06 Method name:** *Fish-Based Assessment Tool - Transitional Waterbodies*

**1.07 Original name:** *Fischbasiertes Bewertungswerkzeug für Übergangsgewässer der norddeutschen Ästuare*

**1.08 Status: Method is/will be used in** First RBMP (2009)

**1.09 Detected pressure(s):** General degradation, Habitat destruction, Hydromorphological degradation

*Has the pressure-impact-relationship been tested?*

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:** <http://www.arge-elbe.de/wge/Download/Berichte/FischBewertungT1.pdf>

**1.11 Pertinent literature of mandatory character:**

REFCOND 2.3: Leitfaden zur Ableitung von Referenzbedingungen und zur Festlegung von Grenzen zwischen ökologischen Zustandsklassen für oberirdische Binnengewässer. CIS-Arbeitsgruppe 2.3 - Referenzbedingungen für oberirdische Binnengewässer. REFCOND 2.4: Leitlinien zur Typologie, zu Referenzbedingungen und Klassifikationssystemen für Übergangs- und Küstengewässer. CIS-Arbeitsgruppe 2.4 (Coast).

**1.12 Scientific literature:**

Elliot, M. & F. Dewailly, 1995. The structure and components of European estuarine fish assemblages. *Netherlands Journal of Aquatic Ecology* 29 (3-4): 397-417. Elliot, M. & K.L. Hemingway (eds), 2002. *Fishes in Estuaries*. Blackwell Science 656 pp.

**1.13 Method developed by**

Jörg Scholle & Oliver Lichte, BioConsult  
scholle@bioconsult.de  
BioConsult Schuchardt & Scholle GbR

**1.14 Method reported by**

Eva Christine Mosch; Jörg Scholle  
eva-christine.mosch@laves.niedersachsen.de; scholle@bioconsult.de  
Lower Saxony State Office for Consumer Protection & Food Safety (LAVES) - Dep. Inland Fisheries; BioConsult Schuchardt & Scholle GbR

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Scholle, J., B. Schuchard & D. Kraft, 2006. *Fischbasiertes Bewertungswerkzeug für Übergangsgewässer der norddeutschen Ästuare*. Bioconsult GbR, Bremen. <http://www.arge-elbe.de/wge/Download/Berichte/FischBewertungT1.pdf>

**2.02 Short description**

in each salinity zone (oligo-, meso- and polyhalin) one fixed sample site; one survey for each fishing site and catch date over one entire tidal phase (1 low-tide and 1 hightide catch in each case), the low-tide and high-tide catches should be evaluated separately

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Beam trawl  
stow net fishery

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone

**2.08 Sampling/survey month(s):** two seasons: May and September / October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Two occasions per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

three sampling sites (one per salinity zone) over entire tidal phase; data from spring and autumn,

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Exposition time per catch (in min., from letting out to hauling in net)& data standardization to h-1\* 80m-2 & additionally filtered water volume measurement per catch (in m<sup>3</sup>)

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 6 - 12 mm at the cod end

**2.13 Sample treatment:** Organisms of the complete sample are identified.

The sampling procedure and the size of subsamples for abundance and biomass differ according to specific species and

catch so that a general specification in % cannot be generally defined with regard to a minimum size of the subsample. However, it must be

- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to Area, Volume  
Unit: Individuals / hour / 80m<sup>2</sup>
- 2.16 Quantification of biomass:** n.a.  
for information only: g\*h<sup>-1</sup>\*80 m<sup>-2</sup>, not relevant for assessment
- 2.17 Other biological data:** 1cm below, individual level/species // in g, total catch weight/species (for high catch numbers suitable subsample)
- 2.18 Special cases, exceptions, additions:** Differentiation of size categories for twaite shad – *Alosa fallax*, smelt – *Osmerus eperlanus* as well as indication of the optimal catch time in each case and the catch site for all quantitatively relevant species (hering, flounder, striped seesnail, plaice, eelpout, ruffe). Classification of the age groups slightly modified according to LAVES - Dep. Inland Fisheries
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

Qualitative metrics: number of species per guild (a) marin (marin, marine-juvenile, marine-seasonal); b) estuarine; c) diadromous (diadromous, diadromous-estuarine); d) limnic (indifferent, rheophilic, stagnant) Quantitative metrics: abundance (ind.\*h<sup>-1</sup>\*80 m<sup>-2</sup>) of indicator species, 1. Species, 2. Age group classification according to size (cm), 3. Catch season relevant for the assessment, 4. Catch site relevant for the assessment (oligo-, meso-, polyhalin), 5. Abundance: 1. *Alosa fallax* 0+, 2. <11, 3. autumn abundances, 4. meso and poly, 5. mean value (mv) (spatial); 1. *Alosa fallax* subadult, 2. 11-23, 3. spring abundances, 4. meso and poly, 5. mean value (spatial); 1. *Alosa fallax* adult, 2. >23, 3. spring abundances, 4. oligo, meso and poly, 5. mean value (spatial); 1. *Osmerus eperlanus* 0+, 2. <7, 3. autumn ab. (possibly also spring), 4. meso and poly, 5. mean value (spatial); 1. *Osmerus eperlanus* subadult, 2. 7-10, 3. no differentiation, 4. oligo, meso and poly, 5. mean value (spatial + time); 1. *Osmerus eperlanus* adult, 2. >10, 3. spring abundances, 4. oligo, meso and poly, 5. mean value (spatial); 1. *Plathichthys flesus*, 2. no diff., 3. no diff., 4. oligo, meso and poly, 5. mean value (spatial + time); 1. *Liparis liparis*\*, 2. no diff., 3. spring or autumn abundances, 4. meso and poly, 5. mean value (spatial); 1. *Zoarces viviparus*\*\*, 2. no differentiation, 3. autumn abundances, 4. meso and poly, 5. mean value (spatial); 1. *Clupea harengus*, 2. no differentiation, 3. no differentiation, 4. meso and poly, 5. mean value (spatial + time) \*not for Ems and Eider; \*\*only for Ems (fishing method: beam trawl, data from the Dutch Demersal Fish Survey (DFS) Programme)

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

##### 3.04 From which biological data are the metrics calculated?

Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Expert knowledge, Historical data  
recent catch data

##### 3.07 Reference site characterisation

**Number of sites:** historical and recent data from 3 transitional water bodies  
**Geographical coverage:** rivers flowing to the german part of the North Sea  
**Location of sites:** estuaries of Ems, Weser, Elbe and Eider (Germany)  
**Data time period:** reference time: end of 19. century; eldest fish data from ~1880; eldest maps ~1830  
**Criteria:**  
There are no reference sites in Germany.

##### 3.08 Reference community description

the assessment tool includes a tab labelled 'References' with a 'historical frequency category' for each species (1 rare – 6 very frequent, on a massive scale). This provides indications of how frequently the species was found in the estuaries at the reference time.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites

#### **3.11 Boundary setting procedure**

The boundary setting procedure is orientated at the normative descriptions of the WFD (Annex V; 1.2.3). The boundary setting results interpretive based on the normative terms. Because of the high variability of the fish community there are little differences in comparison to the REFCOND suggestions for class boundaries. a) normative description b) assessment/similarity to reference condition c) EQR d) ecological status e) 1a) ...completely or nearly..., barely differences, b)  $\geq 90\%$  agreement of all variables (average), c)  $\geq 0.9$ , d) high (5) e) 2a) ...marginal differences..., indications for anthropogenic disturbances, b) at least 60% agreement of all variables (average), c)  $0.7 < 0.9$ , d) good (4) e) 3a) ...moderate differences, major indications for anthropogenic disturbances, b) at least 40% agreement of all variables (average), c)  $0.5 < 0.7$ , d) moderate (3) e) 4a) ...significant differences, b) at least 20% agreement of all variables (average), c)  $0.25 < 0.5$ , d) poor (2) e) 5a) ...absence of large parts of the community, b)  $< 20\%$  agreement of all variables (average), c)  $< 0.25$ , d) bad (1)

**3.12 "Good status" community:** The lower limit of the 'good status' is reached, if 1 metric is equivalent to the reference condition and 8 further metrics reach a similarity of at least 60 %.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 134

KRW-maatlatten

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
TW-NEA11
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Netherlands
- 1.05 Specification:** only the large estuaries Ems-Dollard and Western Scheldt
- 1.06 Method name:** *WFD-metrics for natural watertypes*
- 1.07 Original name:** *KRW-maatlatten voor natuurlijke watertypen*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter  
fishery
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [http://themas.stowa.nl/thema/ecologische\\_beoordeling/krw-maatlatten.aspx?mid=7213&rid=817](http://themas.stowa.nl/thema/ecologische_beoordeling/krw-maatlatten.aspx?mid=7213&rid=817)
- 1.11 Pertinent literature of mandatory character:**  
Besluit Kwaliteitseisen en Monitoring Water, 2009. Ministry of Housing, Spatial Planning and the Environment (presently under public consultation).
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
development by national expert group commissioned by  
STOWA, Bas van der Wal & RWS Waterdienst, Diederik van der Molen  
b.van.der.wal@stowa.nl  
STOWA Foundation for Applied Water Management Research & Rijkswaterstaat Waterdienst
- 1.14 Method reported by**  
Roel Knobben  
r.knobben@royalhaskoning.com  
Rijkswaterstaat Waterdienst
- 1.15 Comments**  
selection of the indicator species is based on detecting effects of anthropogenic pressures of fish species and fish composition in estuary. The intention is that all indicators combined give an assessment for all anthropogenic pressures.  
  
further readin

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) Quality Handbook Hydrobiology (in prep). 2009. STOWA.
- 2.02 Short description**  
fishing with swing net/stow net with standardized surface area and standardized fish time frame in order to get an broad overview of fish species present
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** n.a.  
swing net/stow net (in dutch:
- 2.05 Specification:**  
swing net/stow net : conical net held open by one or more horizontal beams below an anchored boat
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
pelagic
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** spring: may; autumn: september/october
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
two sampling occasions per year; but classification preferably averaged over three years.
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
several zones along salinity gradient are sampled (oligohaline to euhaline)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
sampling duration: one tidal cycle(so low tide and high tide)

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** > 10 mm (mesh size of net)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
# individuals per 80m<sup>2</sup> per hour  
**in relation to** Area, Time  
**Unit** # individuals per 80m<sup>2</sup> per hour
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** for smelt and twaite shad: length
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

#### **3.01 List of biological metrics**

2 metrics as part of the assessment: species composition: 4 fish groups are counted: # diadromous species; # resident transitional species; # marine seasonal species; # marine juvenile species. A score for each group is determined with a table in the metric. The 4 scores are averaged to get the EQR for species composition metric. abundance: abundance of several species: European smelt: 3 age groups; Twaite shad: 3 age groups; Viviparous eelpout; Flounder; juvenile European plaice; juvenile Atlantic herring; Ruffe. A score for each group is determined with a table in the metric. For twaite shad and smelt a score is determined for each age group and the lowest score is taken into account for the assessment. The 7 scores are averaged to get the EQR for abundance metric

#### **3.02 Does the metric selection differ between types of water bodies?** No

#### **3.03 Combination rule for multi-metrics:** n.a.

final assessment: scores for species composition and abundance are averaged. If the score results in a good or high status, but with one of the indicators (4 for species composition; 7 for abundance) is < 0,4 EQR then the total assessment score for fish i

#### **3.04 From which biological data are the metrics calculated?**

n.a.

### **Reference conditions**

#### **3.05 Scope of reference conditions:** Surface water type-specific

#### **3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data

#### **3.07 Reference site characterisation**

**Number of sites:** There are no undisturbed reference areas in the Netherlands

**Geographical coverage:** Netherlands, Germany

**Location of sites:** species composition: Ems-Dollard, Westerschelde and historical data from the Zuiderzee (nowadays: Lake IJsselmeer). Abundance: Weser, Elbe estuaries

**Data time period:** abundance: 1900 and present day data; species composition: 1850-1900

#### **Criteria:**

Dutch sites were tested against reference criteria by Wasson (2006) and all rejected.

#### **3.08 Reference community description**

estuary fish fauna characterized by strong seasonal dynamics, both in abundance and in composition. Vulnerable species to anthropogenic pressures are present in the system. The estuary is populated by resident species, diadromous species, marine seasonal species, marine juvenile species. Furthermore a general description is given (in Dutch) in: STOWA (2009) Referenties en maatlaten voor natuurlijke watertypen. report 2007-32

#### **3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Using discontinuities in the relationship of anthropogenic pressure and the biological response.  
expert judgement;

### 3.11 Boundary setting procedure

Assumption for species composition: skewed linear relation between quality of the ecosystem and the species composition. I.e. there is resilience against pressures, but once species start disappearing more will follow, leaving only the hardest species to remain in the ecosystem. Abundance: merging of available historical catch data. 20%-percentile values are the class boundaries.

**3.12 "Good status" community:** Slight degradation compared to the reference condition. Most sensitive species might have disappeared. Furthermore a general description is given (in Dutch) in: STOWA (2009) Referenties en maatlaten voor natuurlijke watertypen. report 2007-32

### Uncertainty

**3.13 Consideration of uncertainty:** Yes

Precision and uncertainty is regarded in Van Herpen, van Tongeren, Knobben, Baggelaar, van Loon (2009) Quick scan precision and confidence of KRW assessment (in Dutch). This study resulted in a statistical method to assess the level of precision and confidence monitoring results and status classifications (including identifying outliers and estimates for missing values). The confidence of a status classification is expressed as the probability of exceeding a chemical limit value or the biological status classification moderate/good. Recommendations from this study are incorporated in the Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) (see question B.0).

**3.14 Comments:**

none

ID: 173

AFI

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Spain
- 1.05 Specification:** NEA coastal regions (Basque Country)
- 1.06 Method name:** *AZTI's Fish Index*
- 1.07 Original name:** *AZTI's Fish Index*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Heavy metals, Hydromorphological degradation, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter  
Dredging
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).
- 1.10 Internet reference:** <http://www.azti.es>; [http://www.uragentzia.euskadi.net/u81-0003/es/contenidos/informacion/calidad\\_aguas/es\\_doc/calidad\\_aguas\\_superficiales\\_transicion\\_costeras\\_in.html](http://www.uragentzia.euskadi.net/u81-0003/es/contenidos/informacion/calidad_aguas/es_doc/calidad_aguas_superficiales_transicion_costeras_in.html)
- 1.11 Pertinent literature of mandatory character:**  
ORDEN ARM/2656/2008 por la que se aprueba la instrucción de la planificación hidrológica. BOE229, 22 de septiembre de 2008.
- 1.12 Scientific literature:**  
Borja, A., J. Bald, J. Franco, J. Larreta, I. Muxika, M. Revilla, J.G. Rodríguez, O. Solaun, A. Uriarte & V. Valencia, 2009. Using multiple ecosystem components, in assessing ecological status in Spanish (Basque Country) Atlantic marine waters. *Marine Pollution Bulletin* 59 (1-3): 54-64. Borja, A., J. Franco, V. Valencia, J. Bald, I. Muxika, M. Jesus Belzunce & O. Solaun, 2004. Implementation of the European water framework directive from the Basque country (northern Spain): a methodological approach. *Marine Pollution Bulletin* 48 (3-4): 209-218. Nicolas, D., J. Lobry, M. Lepage, B. Sautour, O. Le Pape, H. Cabral, A. Uriarte & P. Boët, 2010. Fish under influence: A macroecological analysis of relations between fish species richness and environmental gradients among European tidal estuaries. *Estuarine, Coastal and Shelf Science*, In Press, Uncorrected Proof. Uriarte, A. & A. Borja, 2009. Assessing fish quality in transitional waters, within the European Water Framework Directive: setting boundary classes and responding to anthropogenic pressures. *Journal of Estuarine, Coastal and Shelf Science* 82 (2): 214-224.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Angel Borja<br>aborja@azti.es<br>AZTI-Tecnalia | <b>1.14 Method reported by</b><br>Angel Borja<br>aborja@azti.es<br>AZTI-Tecnalia |
|---|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Elliott, M. & K.L. Hemingway, 2002. *Fishes in estuaries*. Elliott, M. & K.L. Hemingway (eds), NN. Blackwell Editorial Ltd. Blackwell Publishing Ltd. Oxford. 658 pp.
- 2.02 Short description**  
The demersal fish and epibenthic invertebrate communities were sampled using a 40 mm mesh beam trawl with 8 mm mesh cod end. The trawl has 1.5 m beam length. Site locations were initially determined by the suitability of the sea-bed for trawling sampling as well as by the requirement to cover the range of water quality and sediment conditions present. The speed average: ~ 1.5 Kn; Time: 10 minutes, but sometimes the trawl period differs.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Beam trawl
- 2.05 Specification:** 1.5 m beam length with one tickler chain
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Soft-bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** Autumn
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Once per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3 replicates per station (3-4 station per water body)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
30 minutes (10 min. per replicate)

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 10 mm mesh size and 8 mm mesh size cod end
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups  
Samples were identified and counted on-board, immediately. Species which could not be identified were fixed in a solution of 4% formalin, then examined in the laboratory; but some groups can be identified at higher taxonomic levels (i.e. Mugilidae)
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per one square-metre
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** Organism length
- 2.18 Special cases, exceptions, additions:** In general, Basque estuaries are small, containing only a small number of 'estuarine resident' fish species. Thus, in the case of small river-dominated estuaries and estuaries with extensive intertidal flats, it is necessary to incorporate crustaceans as a characteristic demersal component of the estuaries. In the case of estuaries with extensive subtidal areas the method only includes fishes.
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
1) richness (number of species); 2) indicator and introduced species (percentage of individuals); 3) fish health (percentage affected); 4) trophic composition (percentage of omnivorous and piscivorous); 5) resident estuarine species (number and percentage of individuals).
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time  
AFI was calculated for each trawl line (after pooling 3 replicates); the total AFI for the water body can be calculated directly by weighting by that area
- Reference conditions**
- 3.05 Scope of reference conditions:** Habitat-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, n.a.  
Historical data, expert knowledge
- 3.07 Reference site characterisation**  
**Number of sites:** No specific number  
**Geographical coverage:** Northern Spain  
**Location of sites:** Basque Country  
**Data time period:** 1989-2009  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
See: Arregi, L., E. Puente, P. Lucio, Y. Sagarmínaga, R. Castro y A. Uriarte. 2004. Coastal Fisheries and Demersal Estuarine Fauna. En: Oceanography and Marine Environment of the Basque Country. A. Borja y M. Collins (Ed.). Elsevier Oceanography Series. Amsterdam. 493-513 pp. San Vicente, Carlos. 1988. Estudio de las rías guipuzcoanas : I. Primeros datos sobre el estudio de la ría del Oria. Nº 11. 179-199pp. San Vicente, C., A. Miner y M. Ibáñez, 1988. Estudio de las rías guipuzcoanas: estudio de las comunidades de peces y macroinvertebrados + memoria-resumen. INSUB. Donostia - San Sebastián. Miner, A., M. Ibañez & C. San Vicente. 1990. Estudio de la fauna demersal de las rías de Guipuzcoa, País Vasco. Bentos 6: 439-454
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** n.a.  
Applying oxygen saturation standards (high/good: 100% of oxygen saturation; good/moderate: 80%, quality standards for some uses of marine waters (shellfishing and aquaculture); moderate/poor: 60% minimum value to be reached at any time and anywhere in the

**3.11 Boundary setting procedure**

See: Uriarte, A. y A. Borja, 2009. Assessing fish quality in transitional waters, within the European Water Framework Directive: setting boundary classes and responding to anthropogenic pressures. *Journal of Estuarine, Coastal and Shelf Science*, 82 (2):214-224.

**3.12 "Good status" community:** See reference above.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 114

TFCI

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** United Kingdom
- 1.05 Specification:** none
- 1.06 Method name:** *Transitional Fish Classification Index (TFCI)*
- 1.07 Original name:** *Transitional Fish Classification Index (TFCI)*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** General degradation
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/](http://www.wfduk.org/bio_assessment/)
- 1.11 Pertinent literature of mandatory character:**  
[http://www.wfduk.org/bio\\_assessment/](http://www.wfduk.org/bio_assessment/)
- 1.12 Scientific literature:**  
Coates, S., A. Waugh, A. Anwar & M. Robson, 2007. Efficacy of a multimetric fish index as an analysis tool for the transitional fish component of the Water Framework Directive. Marine Pollution Bulletin 55: 225-240.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Steve Coates<br>steve.coates@environment-agency.gov.uk<br>Environment Agency (England & Wales) | <b>1.14 Method reported by</b><br>Steve Coates<br>steve.coates@environment-agency.gov.uk<br>Environment Agency (England & Wales) |
|---|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Environment Agency multiple method bi-annual sampling guidelines developed for TFCI.
- 2.02 Short description**  
depends on typology but a combination of:-<sup>1</sup> 44m seine net (2 replicates).<sup>2</sup> 1.5m 200m tow.<sup>3</sup> Otter trawl or 2m beam trawl 15' tow
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Beam trawl, Fyke net, Otter trawl, Seine netting
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** Spring (April, May, June) & Autumn (Sept, Oct, Nov)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
depends on water body size
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
depends on water body size
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
depends on water body size

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 5mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to n.a.  
'relative abundance' i.e. within the catch  
Unit 'relative abundance' = % of catch.
- 2.16 Quantification of biomass:** n.a.

- 2.17 Other biological data:** length
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments:** none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
see:- [http://www.wfduk.org/bio\\_assessment/](http://www.wfduk.org/bio_assessment/)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
ratio to reference on a 1 to 5 scale
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** depends upon typology  
**Geographical coverage:** UK & ROI  
**Location of sites:** UK & ROI  
**Data time period:** Historical data to circa 1800; ecotype sample data 1973 to date & WFD monitoring data  
**Criteria:**  
meet hydromorph criteria
- 3.08 Reference community description**  
For each of the UK TW typologies reference conditions are calculated for the 10 metrics used by the TFCI. These metrics reflect the WFD normative for TW Fish - species composition, abundance & disturbance sensitive taxa.
- 3.09 Results expressed as EQR?** No metric score, the sum of 10 metrics is then converted to an EQR

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
TFCI developed to reflect general disturbance
- 3.12 "Good status" community:** Depends on typology.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:** none

ID: 65

FL-PP-TR

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
TW-NEA11
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish region
- 1.06 Method name:** *Flemish phytoplankton assessment method for transitional waters*
- 1.07 Original name:** *Vlaamse fytoplankton beoordelingsmethode voor overgangswateren*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. Vlaamse Milieumaatschappij, Erembodegem, Belgium.
- 1.12 Scientific literature:**  
n.a.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Jeroen Van Wichelen<br>jeroen.vanwichelen@UGent.be<br>Ghent University | <b>1.14 Method reported by</b><br>Jeroen Van Wichelen<br>jeroen.vanwichelen@UGent.be<br>Ghent University |
|---|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
The phytoplankton is sampled with so-called non-concentrated, hence non-filtered samples. A surface sample is taken in a large container from which the necessary subsamples can be taken.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** Surface water sample taken with a bucket
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Surface water
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** April-september
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
at least one occasion per month during the growing season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Total volume sampled (prior to subsampling) is (bucket volume) x (1 sample per occasion) x (6 months) x (number of monthly samples; at least one)

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** All cells in the sample, including picocyanobacteria
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Subsamples are taken from a thoroughly homogenised sample
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
To the species level where possible, otherwise genus
- 2.15 Record of abundance:** n.a.  
counts of individuals or, where applicable, colonies

in relation to Volume

Unit biomass per volume

**2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Biomass (chlorophyll a); percentage diatoms in total biomass

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Worst metric score

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time  
Data from single spatial replicate

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** n.a.

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**

n.a.

**3.08 Reference community description**

Reference conditions are characterised by a relatively low biomass per volume, and a significant relative proportion of diatoms in the total biomass

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** n.a.

Expert judgement

**3.11 Boundary setting procedure**

EQR gradient is assumed to represent a continuous trend with general degradation.

**3.12 "Good status" community:** The EQR values at good status are characterised by metric values that are only slightly lower than at (expert-based) reference state, hence a slightly increased biomass per volume, and a slightly decreased relative proportion of diatoms are possible.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 226

Spanish Phytoplankton Tool (NEA TW- Cantabrian estuaries)

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
Transitional Waters
- 1.02 Category:** Transitional Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Spain
- 1.05 Specification:** North Spanish regions (Basque Country, Cantabria and Asturias)
- 1.06 Method name:** *Spanish Phytoplankton Tool for North East Atlantic Transitional Waters. Part 1-Cantabrian estuaries (Bay of Biscay)*
- 1.07 Original name:** *Spanish Phytoplankton Tool for North East Atlantic Transitional Waters. Part 1-Cantabrian estuaries (Bay of Biscay)*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

Phytoplankton data from 12 estuaries (32 sampling stations) in the Basque coast were analysed for a recent 6-year period (2003-2008). The eutrophication risk was evaluated by expert judgment and historical data analysis. The risk assessment took into account the anthropogenic pressure in terms of sewage discharges and the hydrographical and physico-chemical conditions that could influence importantly on the phytoplankton responses (i. e., river flow, tidal exchange, turbidity and nutrient levels). The method resulted effective at discriminating water bodies at different eutrophication risk levels.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

Revilla, M., A. Borja, J. Bald, J. Franco & V. Valencia, 2008. A method based on chlorophyll- a concentration for the assessment of phytoplankton status in coastal and transitional waters. XI International Symposium on Oceanography of the Bay of Biscay. Revista de Investigación Marina 3: 219–220. [www.azti.es](http://www.azti.es)

Revilla, M., A. Borja, P. García, X. Guinda, J.A. Juanes, A. Puente & E. Zapico, 2009. Description of National Methods: Spanish Phytoplankton Tool for North East Atlantic Transitional Waters (NEA TW). Part 1- Cantabrian estuaries (Bay of Biscay). November 23, 2009. Technical Report.

Revilla, M., M. Garmendia, J. Franco & A. Borja (submitted). Comparison of methods for phytoplankton quality assessment in the Basque estuaries (North Spain). Revista de Investigación Marina. <http://www.azti.es>.

**1.13 Method developed by**

Several: M. Revilla (coordinator), Borja A., García P., Guinda X., Juanes, J.A., Puente A., Zapico, E.  
mrevilla@pas.azti.es

Several institutions covering the north of Spain: AZTI-Tecnalia (coordination), INDUROT-Universidad de Oviedo, IH Cantabria-Universidad de Cantabria

**1.14 Method reported by**

Marta Revilla

mrevilla@pas.azti.es

AZTI-Tecnalia; Marine Research Division

**1.15 Comments**

The tool was agreed among three regional governments in the north of Spain (País Vasco, Cantabria and Asturias) for the purpose of the European intercalibration (Revilla et al., 2009).

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Several regional governments and their corresponding laboratories are involved in the water monitoring of the Spanish coast and, therefore, sampling strategy and analytical techniques can present some regional variation. In the Basque Country, standard protocols are used for sampling and laboratory analysis: Lorenzen, C.J. & S.W. Jeffrey, 1980. Determination of chlorophyll in seawater. UNESCO Technical Papers in Marine Science, 35. Utermöhl, H., 1958. Zur vervollkommung der quantitativen Phytoplankton-Methodik. Mitteilungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 9: 1-38.

**2.02 Short description**

CTD vertical profiles (fluorescence, salinity, oxygen, temperature and PAR) are conducted along the whole water column in the deep estuaries (>30 m). Simultaneously, water samples are collected in surface (0-1 m) for phytoplankton counts, chlorophyll-a and additional physico-chemical variables (such as, nutrients, suspended solids and turbidity). Physico-chemical variables only to be used as complementary information; they are not involved in the classification of the phytoplankton element.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler  
Also, CTD fluorescence is used

**2.05 Specification:** Niskin bottle or clean bucket

- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Surface waters (0-1 m depth)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** For chlorophyll-a, a minimum of four months that represent all seasons (winter, spring, summer and fall). For phytoplankton counts, a minimum of two months (once in spring and once in summer)...
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
For chlorophyll, two occasions (high and low tide) per sampling season. For phytoplankton, one occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Usually, 1 replicate per sampling station and several stations per water body
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
For chlorophyll-a: about 0.2-2 L per sampling station; for phytoplankton counts: about 10-50 mL per sampling station.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** For chlorophyll-a, a single measurement (CTD/filter) is allowed. For phytoplankton (Utermöhl), at least 2 cm are counted with 400 x. This usually implies 50-100 units from the dominant taxa.
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
When chlorophyll-a is determined by spectrophotometry, a water sample of about 10 L is collected in the field and, subsequently, a subsample of about 0.2-2 L is filtered in the laboratory (the exact volumen depends on the particulate matter); the remaining water is used for several physico-chemical analysis. For phytoplankton counts, samples of 125-250 ml are collected and fixed with Lugol or glutaraldehyde. Then, the volumen of water used in sedimentation chambers for phytoplankton counting by the Utermöhl technique is about 10 or 50 mL.
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
Most diatoms and armoured dinoflagellated are identified at the genus or species level. However, broader groups are also used when it is not possible to identify at the higher levels. The taxa usually grouped are the naked dinoflagellates, euglenophytes, small flagellates, small coccoids, chlorophytes and cryptophytes.
- 2.15 Record of abundance:** Individual counts  
in relation to Volume  
Unit Cells/L
- 2.16 Quantification of biomass:** Chlorophyll-a concentration  
Also, determination of chlorophyll-a concentration by CTD fluorescence (deep estuaries), regularly calibrated by spectrophotometry
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
Chl-a is extracted in cold acetone.

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Sub-metric1 (biomass indicator): 90th percentile of chlorophyll-a with all data recorded at a sampling area during a 6-year period. Two salinity ranges are used in order to apply different reference conditions and class boundaries (euhaline waters and oligo/meso/polyhaline waters). Sub-metric2 (bloom indicator): percentage of samples, at a sampling area during a 6-year period, where any single taxa exceeds a threshold. The threshold is 750,000 cells/L. No salinity ranges are used for the bloom indicator.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
In some Spanish regions the metrics are calculated also with aggregated data from multiple spatial replicates (several sampling stations within a water body).

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data, Least Disturbed Conditions

Risk Assessment

### 3.07 Reference site characterisation

**Number of sites:** 1-2 sites per estuary type

**Geographical coverage:** Basque Country

**Location of sites:** Basque Country

**Data time period:** 1995-2008

**Criteria:**

Sites at least disturbed conditions presented low values in chl-a concentration and bloom frequency. Also, the risk of eutrophication in these water bodies was considered to be low, taking into account the wastewater treatment in the water basin, nutrients concentrations, hydrodynamics (river and tide influence) and turbidity (Revilla et al., 2009; submitted). See comments in C-19 section for more information.

### 3.08 Reference community description

Composition-metrics were not developed for phytoplankton quality assessment. ☐Chlorophyll-metric reference: 2.67 ug/L (euhaline waters) and 5.33 ug/L (oligo/meso/polyhaline waters) ☐Bloom-metric reference: 16.7%

**3.09 Results expressed as EQR?** Yes

## Boundary setting

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites

### 3.11 Boundary setting procedure

For euhaline TW the EQR values at the class boundaries were intentionally similar to those established for CW (see Questionary for Spanish NEA CW). The increments allowed among the status classes for the oligo/meso/polyhaline TW were in some cases relatively lower when compared to those established for the euhaline TW. It resulted in different EQR values at the Good/Moderate and Moderate/Poor boundaries. It was taken into account that in the oligo-, meso- or polyhaline stretches of the estuaries, lower increments in Chl-a above the reference could have stronger effects on the ecosystems as these salinity zones are usually under more stressing conditions (e. g., lower oxygen saturation, higher and more frequent variations in salinity, turbidity, etc.).

**3.12 "Good status" community:** Composition-metrics were not developed for phytoplankton quality assessment. ☐Good status for the chlorophyll-metric: 4.0-8.0 ug/L (euhaline waters) and 8.0-12.0 ug/L (oligo/meso/polyhaline waters). ☐Good status for the bloom-metric: 20-40%.

## Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

### 3.14 Comments:

In the Basque estuaries it is not possible to find sampling stations at reference conditions (i. e., with no, or only very minor anthropogenic disturbance) because all of the estuaries have been historically impacted by human activities. Moreover, the Basque Country has no pre-industrial historical data. Therefore, in order to set reference conditions and class boundaries for the phytoplankton-based metrics, data analysis and expert judgement (physico-chemical and phytoplankton variables measured from 1995 to 2008) were used. The chlorophyll reference condition set for euhaline TW was only slightly higher than for CW. This decision was made assuming that, under no anthropogenic pressure, physico-chemical conditions and phytoplankton communities should be very similar in the Basque CW and euhaline TW, as these estuaries are generally subject to a strong tidal exchange at their outer reaches. In contrast, for the oligo-, meso- or polyhaline TW a much higher reference condition was established to allow for the natural nutrient loads in these waters that could result in a higher phytoplankton biomass. For phytoplankton blooms, in TW a similar tool than for CW is used, as it resulted useful for classifying sampling stations along eutrophication gradients in these estuaries.

ID: 184

DE-AN-TR

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Rivers, Transitional Waters
- 1.03 BQE:** Macrophytes  
submerged and emerged higher plants
- 1.04 Country:** Germany
- 1.05 Specification:** Tideelbe, Eider, parts of Weser and tidal influenced main tributaries of Elbe and Weser
- 1.06 Method name:** **Assessment system for estuaries including tidal influenced freshwater sections based on macrophytes and angiosperms**
- 1.07 Original name:** *Bewertungsverfahren QK Makrophyten und Angiospermen in Übergangsgewässern und tidebeeinflussten Gewässern*
- 1.08 Status: Method is/was used in:** RBMP (2009)
- 1.09 Detected pressure(s):** Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
data from 40 tidal influenced river and estuary sections were examined to test relationship between pressure-impacts and macrophyte-degeneration
- 1.10 Internet reference:** [www.arge-elbe.de](http://www.arge-elbe.de) - only river Tideelbe
- 1.11 Pertinent literature of mandatory character:**  
Instruction protokol is in preparation for authorities in Schleswig-Holstein (LLUR) and Niedersachsen (NLWKN).
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Gabriele Stiller - Entwicklerin  
Gabriele.Stiller@t-online.de  
Biologische Kartierungen und Gutachten, Hamburg - freelancer
- 1.14 Method reported by**  
Gabriele Stiller  
Gabriele.Stiller@t-online.de  
Biologische Kartierungen und Gutachten, Hamburg - freelancer
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Stiller, G., 2005. Bewertungsverfahren für die Qualitätskomponenten Makrophyten und Angiospermen in der Tideelbe gemäß EU-Wasserrahmenrichtlinie. Gutachten i. A. der ARGE ELBE, Wassergütestelle Elbe, Hamburg. Based on Landesamt für Umwelt, Naturschutz und Geologie Mecklenburg- Vorpommern, 2002. Verfahrensanleitung zur ökologischen Bewertung von Fließgewässern in Mecklenburg-Vorpommern mittels Standorttypindex. - Schriftenreihe Nr. 02, Güstrow.
- 2.02 Short description**  
The mapping of the macrophyte vegetation is carried out in the main vegetation period (July/August). As investigation areas, sections of 100 m length are selected. Within this sections the registration of the vegetation takes place by on-site inspection between the mean high tide water line and the vegetation lower limit during low tide ( $\pm$  2-3 hours). In addition to species composition and abundance characteristic features of the population structure of the tidal reeds are recorded as there are the spatial spread, the number of vegetation zones and the vitality of the vegetation.
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** n.a.  
on-site inspection during low ti
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** n.a.
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** spring (April/May) and main vegetation period (July/August)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
two occasions due to the seasonal rhythm of the tidal reeds
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
one - as usually for macrophyte surveys
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Abundance classes  
in relation to Area  
Unit ordinal scale from 1 to 5 according to KOHLER (1978)
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** registration of the vegetation takes place by on-site inspection between the mean high tide water line and the lower vegetation limit only during low tide ( $\pm$  2-3 hours)
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
species and their abundances according to KOHLER (1978); characteristic features of the population structure of the tidal reeds and the saltplant communities are recorded as there are the spatial spread, the number of vegetation zones and the vitality of the vegetation.
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** approximately 40 sites  
**Geographical coverage:** n.a.  
**Location of sites:** sites in Tideelbe, Eider, Pinnau, Krückau and Stör (Schleswig-Holstein)  
**Data time period:** historical data before 1960  
**Criteria:**  
No actual reference sites in German estuaries and tidal influenced rivers.
- 3.08 Reference community description**  
Characteristic macrophytes are emerged fresh and brackish water reeds as well as saltplant communities in the polyhalin segment of the transitional water body. There used to be submerged macrophytes in the tidal rivers and most parts of the transitional waters as well.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites
- 3.11 Boundary setting procedure**  
Macrophytes are placed into four ecological categories (such as highly sensitive, sensitive, tolerant and highly tolerant). The ratio of the relative cover of these response groups was then related to the grade of naturalness of the site. Good status is reached when species from all categories are present and (well) balanced. At the same time the vegetation structure (spatial spread, zonation and vitality) shows no significant negative effects.
- 3.12 "Good status" community:** At good status tidal reeds and saltplant communities consist of characteristic species composition with species out of the four ecological categories. The vegetation structure shows sufficient spatial spread, four vegetation zones and a good vitality.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

In preparation is a simple method of indicating confidence such as "high", "medium" and "low" - referring to the site of the EQR in relation to the boundaries (e.g. low confidence for results close to status boundaries).

**3.14 Comments:**

none

ID: 42

FIA

## 1. General information

- 1.01 GIG:** Alpine  
common intercalibration types are not used
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Austria
- 1.05 Specification:** none
- 1.06 Method name:** *Fish Index Austria*
- 1.07 Original name:** *Fisch Index Austria*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Acidification, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Riparian habitat alteration  
temperature alterations
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).
- 1.10 Internet reference:** <http://www.baw-igf.at/cms/index.php>
- 1.11 Pertinent literature of mandatory character:**  
Leitfaden für die Erhebung der biologischen Qualitätselemente A1 Fische (BMLFUW) EN 14757 (CEN 2005) EN 14692 (CEN 2004).
- 1.12 Scientific literature:**  
n.a.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Reinhard Haunschmid<br>reinhard.haunschmid@baw.at<br>Federal Agency for Water Management | <b>1.14 Method reported by</b><br>Haimo Prinz<br>haimo.prinz@baw.at<br>Federal Agency for Water Management |
|---|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Leitfaden für die Erhebung der biologischen Qualitätselemente A1 Fische (BMLFUW) EN 14757 (CEN 2005) EN 14692 (CEN 2004).
- 2.02 Short description**  
depends on river size → electrofishing according to literature cited (see B-01) → large rivers with additional methods
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Beam trawl, Echo sounder, Electrofishing gear, Fyke net, Gill net, Otter trawl, Seine netting  
long lines, snorkeling
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** depends on water temperature, generally June until trout spawning season
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Epirhithron: at least 3, calculated via CV (coefficient of variation); rivers with > autochthonous species: 1; large rivers (strip fishing): 3 per habitat, 25 strips
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
rivers up to 2 autochthonous species: between 60m x wetted width and 150m x wetted width (m<sup>2</sup>); rivers > 2 autochthonous species: <5m wetted width: 100m x wetted width; 5-15m wetted width: 100-150m x wetted width; >15m wetted width: at least 2250m<sup>2</sup>

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 0+ fish
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts

in relation to Area

Unit Individuals per hectare

**2.16 Quantification of biomass:** n.a.

weight measured with digital scales with a precision of 1g for fish >10cm; result given as kg/ha

**2.17 Other biological data:** total length of fish (precision of 0,5cm)

**2.18 Special cases, exceptions, additions:** large rivers require additional field sampling methods; only habitats with water depth <2m sampled by electrofishing

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

1. fish biomass (kg/ha), as a metric for the trophic level and of special importance in systems with low diversity (epirhithron, metarhithron f.i.)  
2. percentage of dominant species  
3. percentage of subdominant species  
4. percentage of rare species  
5. presence of habitat guilds  
6. deviation from the index of fish region (SCHMUTZ et al. 2000)  
7. presence of reproduction guilds  
8. expert judgement on length frequency-distribution of dominant species  
9. expert judgement on length frequency-distribution of subdominant species  
The metrics are combined using the following formula:  $FIA = (ZKART * 2 + ZKFRI + ZKAS * 3) / 6$   
ZKART status class – fish species assemblage (decimal from 1 to 5)  
ZKFRI status class – fish region index (whole numbers from 1 to 5)  
ZKAS status class – age structure (decimal from 1 to 5)

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Weighted average metric scores

##### 3.04 From which biological data are the metrics calculated?

Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Expert knowledge, Historical data  
Comparison actual and historical fish data

##### 3.07 Reference site characterisation

**Number of sites:** more than 50

**Geographical coverage:** all over Austria

**Location of sites:** n.a.

**Data time period:** Historical data: appr. 1850-1900; existing near natural reference sites: fish stock census: 1995-2002

##### Criteria:

Reference sites were selected using expert judgement.

##### 3.08 Reference community description

A catalogue of reference fish communities ("leitbild") has been established for all defined biocoenotic regions in the different bioregions.

**3.09 Results expressed as EQR?** No a value between 1 and 5 representing 5 status classes 1-5 (high-good-moderate-poor-bad)

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites

##### 3.11 Boundary setting procedure

Preclassification of sites (status 1-5) by expert judgement - statistical difference testing among the preclassification values for each metric - weighing of the metrics.

**3.12 "Good status" community:** Minor deviation of the different metrics from the reference condition. More than 50% and <99% of the origin dominant fish species occur (>50<75% subdominant fish species; >20<50% rare species); <1 guild missing compared to reference condition, all age classes occur, juveniles are minor performed, higher amount of adult individuals.

#### Uncertainty

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**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

Specific metrics were combined to new fish survey data and pressures.

ID: 218

Phylib

## 1. General information

- 1.01 GIG:** Alpine, Central-Baltic  
R-A1, R-C1, R-C3, R-C4, R-C5
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms, Macrophytes, Other Phytobenthos
- 1.04 Country:** Germany
- 1.05 Specification:**
- 1.06 Method name:** *German Assessment system for Macrophytes & Phytobenthos according to the EU WFD*
- 1.07 Original name:** *Deutsches Bewertungsverfahren für Makrophyten & Phytobenthos nach EG-WRRL*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Acidification, Eutrophication, General degradation, Habitat destruction  
Flow modification, hydromorphological degradation but without quantification

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

The included trophic and saprobic assessment systems for diatoms (Rott et al.) are calibrated at chemo-physical data.

- 1.10 Internet reference:** [http://www.lfu.bayern.de/wasser/forschung\\_und\\_projekte/phylib\\_deutsch/index.htm](http://www.lfu.bayern.de/wasser/forschung_und_projekte/phylib_deutsch/index.htm)

**1.11 Pertinent literature of mandatory character:**

LAWA- AO, 2006. RaKon Monitoring Teil B. Arbeitspapier III: Untersuchungsverfahren für biologische Qualitätskomponenten. Ständiger Ausschuss "Oberflächengewässer und Küstengewässer" der Bund/ Länder-Arbeitsgemeinschaft Wasser (LAWA-AO).

**1.12 Scientific literature:**

Schaumburg, J., U. Schmedtje, C. Schranz, B. Köpf, S. Schneider, P. Meilinger, D. Stelzer, G. Hofmann, A. Gutowski & J. Foerster, 2004. Erarbeitung eines ökologischen Bewertungsverfahrens für Fließgewässer und Seen im Teilbereich Makrophyten und Phytobenthos zur Umsetzung der EU-Wasserrahmenrichtlinie. Bayerisches Landesamt für Wasserwirtschaft, Abschlussbericht an das Bundesministerium für Bildung und Forschung (FKZ 0330033) und die Länderarbeitsgemeinschaft Wasser (Projekt Nr. O 11.03), 635. p., München.

Schaumburg, J., U. Schmedtje, C. Schranz, B. Köpf, S. Schneider, P. Meilinger, D. Stelzer, G. Hofmann, A. Gutowski & J. Foerster, 2005. Bewertungsverfahren Makrophyten & Phytobenthos, Fließgewässer- und Seenbewertung in Deutschland nach EGWRRL. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft, Heft 1 (05): 245 p. München.

Schaumburg, J., C. Schranz, G. Hofmann, D. Stelzer, S. Schneider & U. Schmedtje, 2004. Macrophytes and phytobenthos as indicators of ecological status in German lakes - a contribution to the implementation of the Water Framework Directive. *Limnologia* 34: 302-314.

Schaumburg, J., C. Schranz, P. Meilinger, D. Stelzer, G. Hofmann, J. Foerster, S. Schneider, B. Köpf & U. Schmedtje, 2005. Makrophyten und Phytobenthos in Flüssen und Seen. Das deutsche Bewertungsverfahren: Entwicklung, Praxistest und Ausblick. In Feld, C. & M. Sommerhäuser (eds), Typologie, Bewertung, Management von Oberflächengewässern, Stand der Forschung zur Umsetzung der EG-Wasserrahmenrichtlinie. - *Limnologie aktuell*: Band 11: 63-75.

Stelzer, D., S. Schneider & A. Melzer, 2005. Macrophyte based assessment of lakes - a contribution to the implementation of the European Water Framework Directive in Germany. *Int. Rev. Hydrobiol.* 9 (2): 223 – 237.

**1.13 Method developed by**

Jochen Schaumburg, Christine Schranz, Petra Meilinger, Doris Stelzer, Gabriele Hofmann, Antje Gutowski, Julia Foerster  
christine.schranz@lfu.bayern.de  
Bavarian Environment Agency LfU

**1.14 Method reported by**

Christine Schranz  
christine.schranz@lfu.bayern.de  
Bavarian Environment Agency LfU

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Schaumburg, J., C. Schranz, D. Stelzer, G. Hofmann, A. Gutowski & J. Foerster, 2006. Instruction Protocol for the ecological Assessment of Running Waters for Implementation of the EC Water Framework Directive: Macrophytes and Phytobenthos.

**2.02 Short description**

all macrophytes of one site (whole riverbed, length minimum about 100m) are registered, determined at species-level and calculated the abundance of each taxon. a minimum of five cobbles are taken all over the river profile. The biofilm is taken from those cobbles with a spoon. all phytobenthos taxa (without diatoms) of one site (whole riverbed, length about 50m) are registered, determined at species-level and calculated the abundance of each taxon. Also samples are taken for microscopical determination

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Brush, Spoon  
diving or rake and aquascope

**2.05 Specification:** macrophytes: a rake with a telescopic stick. Phytobenthos: spoon, sharpened on one side or

toothbrush, cleaned solid after each sample.

**2.06 Sampled/surveyed habitat:** n.a.

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** summer, July until middle of August

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

specified above

### **Sample processing**

**2.12 Minimum size of organisms sampled and processed:** ca. 2µm length

**2.13 Sample treatment:** n.a.

macrophytes: specified above; diatoms: after chemical oxidation of the material 400 objects of diatoms are determined and enumerated; phytobenthos without diatoms: specified above

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Abundance classes, Individual counts

**in relation to** Area

**Unit** abundance-class after Kohler 1987 and number of individuals

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

Sampled habitats: Macrophytes: complete riverbed, length minimum about 100 m, Phytobenthos without diatoms: complete riverbed, length about 50 m, diatoms: minimum five cobbles evenly distributed at the whole river-profile

## **3. Data evaluation**

### **Evaluation**

**3.01 List of biological metrics**

Referenzindex:  $((\sum Q_{Ai} - \sum Q_{Ci}) / (\sum Q_{gi})) * 100$  RI = Referenzindex Q<sub>Ai</sub> = Quantität des i-ten Taxons aus Gruppe A Q<sub>Ci</sub> = Quantität des i-ten Taxons aus Gruppe C Q<sub>gi</sub> = Quantität des i-ten Taxons aller Gruppen n<sub>A</sub> = Gesamtzahl der Taxa aus Gruppe A n<sub>C</sub> = Gesamtzahl der Taxa aus Gruppe C n<sub>g</sub> = Gesamtzahl der Taxa aller Gruppen Total Quantity of several taxa/depth of macrophyte-expansion Total quantity of macrophytes total abundance of aerophile benthic diatom-taxa Trophie-Index (Hofmann 1999) Trophieindex Schönfelder et al. Referenzartenquotient

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Average metric scores, Mean quality class

average for assess one site, mean quality class for assessing the waterbody

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

### **Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Historical data, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** typespecific all undisturbed sites in which were available

**Geographical coverage:** typespecific all undisturbed sites in which were available

**Location of sites:** typespecific all undisturbed sites in which were available

**Data time period:** summer and autumn, all data from reference.sites since 1990

**Criteria:**

The appropriate experts had to deliver reference conditions for the sites, in addition the chemical, physical and structural parameters had to show an undisturbed situation, also the environs of the sites.

**3.08 Reference community description**

The reference community should be dominated by the type specific defined species group "reference-species" A (macrophytes and phytobenthos). E.g. macrophytes in alpine rivers with cobbles and rocks as a dominating sediment: mostly oligotrophic mosses, some characeae, only a few potamogeton-species and some others are in species group A.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
High-good boundary derived from metric variability at near-natural reference sites

#### **3.11 Boundary setting procedure**

The boundaries were set at the zones of distinct changings of the biocoenosis (macrophytes and phytobenthos), and depending on indicator species lists derived from nutrient dependent TI (diatoms).

**3.12 "Good status" community:** Typespecific reference species and tolerant species are still dominant, pressure indicators are rare. = slightly deviation from high status (normative definitions)

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 246

fiBS (Version 8.0.6)

## 1. General information

- 1.01 GIG:** Alpine, Central-Baltic
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Germany
- 1.05 Specification:**
- 1.06 Method name:** *Fish-based Assessment System*
- 1.07 Original name:** *Fischbasiertes Bewertungssystem*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Acidification, Eutrophication, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter  
Connectivity
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [http://www.landwirtschaft-bw.info/servlet/PB/menu/1190131\\_11/index1241097210642.html?showOnlyChilds=true&showChildsFor=1041089](http://www.landwirtschaft-bw.info/servlet/PB/menu/1190131_11/index1241097210642.html?showOnlyChilds=true&showChildsFor=1041089)
- 1.11 Pertinent literature of mandatory character:**  
Dußling, U., 2009. Handbuch zu fiBS. – Schriftenreihe des Verbandes Deutscher Fischereiverwaltungsbeamter und Fischereiwissenschaftler e.V., Heft 15.
- 1.12 Scientific literature:**  
Dußling, U., 2009. Handbuch zu fiBS. – Schriftenreihe des Verbandes Deutscher Fischereiverwaltungsbeamter und Fischereiwissenschaftler e.V., Heft 15. Dußling, U., R. Berg, H. Klinger & C. Wolter, 2004. Assessing the Ecological Status of River Systems Using Fish Assemblages. Handbuch Angewandte Limnologie 20. Erg.Lfg. 12/04: 1-84.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>U. Dußling, A. Bischoff, R. Haberbosch, A. Hoffmann, H. Klinger, C. Wolter, K. Wysujack & R. Berg<br>UDussling@aol.com<br>Several institutions in the framework of a joint-project, headed and coordinated by: Fisheries Research Station of Baden-Württemberg | <b>1.14 Method reported by</b><br>Uwe Dußling<br><br>UDussling@aol.com<br>Büro Gewässer & Fisch |
|---|---|
- 1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Dußling, U. (2009): Handbuch zu fiBS. – Schriftenreihe des Verbandes Deutscher Fischereiverwaltungsbeamter und Fischereiwissenschaftler e.V., Heft 15
- 2.02 Short description**  
n.a.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Electrofishing gear
- 2.05 Specification:**
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:**
- 2.08 Sampling/survey month(s):** July to October (highly recommended)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
3 samples within same site per one assessment period of 6 years
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
no spatial replicates
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
General minimum stretch to be sampled per fishing occasion is 100 m. Moreover, rules for minimum stretches to be sampled (referring to cumulated stretches of all fishing occasions per site) are given, dependant on river size and depth: - wadable rivers: 40-fold of the average river width; - rivers to be sampled by boat: 100-fold of the average river width along river banks; maximum: 10 km along banks in rivers of > 100 m width.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** No standard for minimum size. Detectable minimum size is limited technically by method and equipment.
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** n.a.  
 proportion of total catch; area-related abundance is an additional metric to be used for down-grading the assessment result (if too low by expert judgement) in rivers with a reference fish community of < 10 species.  
**Unit** percentage (proportion of total catch)
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** length-classes; individuals of age class 0+ to be counted separately during sampling procedure.
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
 If available, additional data about species not detected in the framework of samplings, but known from other sources (like e.g. fish ladder counts) can be used as "dummies", if these data are referring to the targeted river section and time span. However, each "dummy" is to be used as a detection of the referring species exclusively and thus, can be solely considered with 1 adult individual.

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
 (1) no. of "Type Specific Species" (2) no. of "Accompanying Species" (3) no. of anadromous and potamodromous species (4) species "far from reference" (5) no. of habitat guilds (6) habitat guilds "far from reference" (7) no. of reproductive guilds (8) reproductive guilds "far from reference" (9) no. of trophic guilds (10) trophic guilds "far from reference" (11) abundance of "Guiding Species" → Guiding Species A → Guiding Species B ... (max. 10 species) (12) perch/roach abundance (13) distribution of ecological guilds → Guild A → Guild B ... (max. 8 guilds) (14) percentage of 0+ age class of each "Guiding Species" → Guiding Species A → Guiding Species B ... (max. 10 species) (15) Migration Index MI (16) Total Index of Fish Regions IFR tot (17) Guiding Species Index GSI (18) Community Dominance Index CDI  
 Additionally: metrics (4), (6), (8) and (10) are only applied in river sections with a reference fish community of < 10 species. metric (18) is only applied in river sections with a reference fish community of ≥ 10 species. metric (15) is not applied if the reference-value of MI = 1.00.
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores
- 3.04 From which biological data are the metrics calculated?**  
 Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
 Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions  
 Marked key sources are used for quantitative reconstruction of reference fish communities on base of expert knowledge .
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
 n.a.
- 3.08 Reference community description**  
 Complete list of species with linked percentage per species to be expected under unimpaired conditions, taking into consideration: river type- river system (catchment)/zoogeographical aspects- natural longitudinal river zonation- known local distribution patterns of species
- 3.09 Results expressed as EQR?** Yes (additionally to original values ranging from 1.00 to 5.00)

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** n.a.

Unequal division, mathematically derived from definitions of metric-scoring

#### **3.11 Boundary setting procedure**

Possible scores for each metric are: 5 - if the metric according to defined criteria reflects the high status; 3 - if the metric according to defined criteria reflects the good status; 1 - if the metric according to defined criteria reflects a moderate or worse status. Total assessment result is expressed as a value from 1.00 (worst) to 5.00 (best) derived as a weighted average of all metric-scores. Based on the definitions for scoring, the boundary settings are as follows: good/moderate boundary = 2.50 (EQR 0.38): As values > 2.50 are to be mathematically rounded to 3 (which as a metric-score is reflecting the good status). Accordingly, 2.50 as well is the highest possible total assessment result for the moderate status. high/good boundary = 3.75 (EQR 0.69): The boundary was obtained by dividing the range of possible values above ("better" than) the good/moderate boundary (> 2.50 – 5.00) into 2 equidistant sub-ranges. moderate/poor boundary = 2.00 (EQR 0.25) poor/bad boundary = 1.50 (EQR 0.13): The boundaries were obtained by dividing the range of possible values below ("worse" than) the good/moderate boundary (1.00 – 2.50) into 3 equidistant sub-ranges.

**3.12 "Good status" community:** n.a.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

ID: 168

AT-PB-RI

## 1. General information

- 1.01 GIG:** Alpine, Central-Baltic, Eastern Continental  
R-C3, R-A1, R-A2, R-E4
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms, Other Phytobenthos
- 1.04 Country:** Austria
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment of the biological quality elements - part phytobenthos*
- 1.07 Original name:** *Leitfaden zur Erhebung der biologischen Qualitätselemente - Teil A3 - Phytobenthos*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Only partly tested (in trophic module/metric). Ecological data from 1221 datasets (from river sites of all Austrian aquatic bioregions) were examined to establish pressure-impact relationships between phytobenthos trophic metric (all algal groups) and eutrophication gradient. The relationship between trophic metric (index) and TP (spot measures) showed significant correlation (Pearson's Correlation Coefficient for In-transformed TP-values is 0,77).

- 1.10 Internet reference:** <http://wisa.lebensministerium.at/article/articleview/74897/1/27032/>

**1.11 Pertinent literature of mandatory character:**

BMLFUW, 2009. Leitfaden zur Erhebung der biologischen Qualitätselemente.

**1.12 Scientific literature:**

Rott, E., G. Hofmann, K. Pall, P. Pfister & E. Pipp, 1997. Indikationslisten für Aufwuchsalgen. Teil 1: Saprobielle Indikation. Publ. Wasserwirtschaftskataster, BMFLF: 1-73. Rott, E., H. Van Dam, P. Pfister, E. Pipp, K. Pall, N. Binder & K. Ortler, 1999. Indikationslisten für Aufwuchsalgen. Teil 2: Trophieindikation, geochemische Reaktion, toxikologische und taxonomische Anmerkungen. Publ. Wasserwirtschaftskataster, BMFLF: 1-248.

**1.13 Method developed by**

Peter Pfister & Eveline Pipp  
peter.pfister@limnologie.at  
ARGE Limnologie GesmbH, Innsbruck

**1.14 Method reported by**

Peter Pfister  
peter.pfister@limnologie.at  
ARGE Limnologie GmbH, Innsbruck

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

In close accordance to the following CEN standards: Europäische Norm EN 13946, 2003. Wasserbeschaffenheit - Leitfaden zur Probenentnahme und Probenaufbereitung von benthischen Kieselalgen in Fließgewässern: 1-18. Europäische Norm EN 14407, 2004. Wasserbeschaffenheit - Anleitung zur Bestimmung, Zählung und Interpretation von benthischen Kieselalgen in Fließgewässern: 1-13. Europäische Norm EN 15708 dt, 2007. Wasserbeschaffenheit - Anleitung zur Beobachtung, Probenahme und Laboranalyse von Phytobenthos in flachen Fließgewässern.

**2.02 Short description**

'Non-diatoms': The whole sampling reach (within the bankfull line) is scanned with the aqua scope and all found macroscopic algal plant growth forms have to be sampled (and described in detail (texture, colour, thickness, degree of cover, preferred microhabitat...) in a field record). Samples can be whole stones or you have to remove the different algal plant growth forms and put it into different vials. Diatoms: 5-10 stones (with diatom film) are removed from the river bed and have to be brushed. The resulting diatom suspension is filled into vials for subsequent examination in the laboratory.

- 2.03 Method to select the sampling/survey site or area:** Expert knowledge

- 2.04 Sampling/survey device:** Brush

- 2.05 Specification:** No special device for sampling but an Aqua-scope (or bucket with clear Perspex base) for scanning the river bottom

- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

- 2.08 Sampling/survey month(s):** All seasons possible (optimal at the end of low discharge season: in alpine flow regimes end of winter, in all other autumn).

- 2.09 Number of sampling/survey occasions (in time) to classify site or area**

In most cases one occasion per sampling season

- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

No replicates in this method.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Total surveyed area depending on heterogeneity of phytobenthos community - stretch length normally 4-5 x river width (20m as a minimum).

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:** All sizes

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Relative abundance

**in relation to** n.a.

Area which is covered by algae

**Unit** relative abundance in % of 100 (for diatoms and also for 'non-diatoms')

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** Description of any algal plant growth form (texture, colour, thickness, degree of cover, preferred microhabitat...) has to be recorded.

**2.18 Special cases, exceptions, additions:** Non-wadable rivers are sampled only at the banks. No survey allowed under turbid conditions. No survey allowed if a flood (>HQ1) occurred within the last month before sampling.

**2.19 Comments**  
none

**3. Data evaluation****Evaluation****3.01 List of biological metrics**

A) Module/metric trophic status - based on trophic index (TI) acc. to ROTT et al. 1999.

$TI = (\text{Sum of (Indicator Taxa Abundance * Indicator trophic value * Indicator weighting score)}) / \text{Sum of (Indicator Taxa Abundance * Indicator weighting score)}$ .

B) Module/metric saprobic status - based on saprobic index (SI) acc. to ROTT et al. 1997.

$SI = (\text{Sum of (Indicator Taxa Abundance * Indicator saprobic value * Indicator weighting score)}) / \text{Sum of (Indicator Taxa Abundance * Indicator weighting score)}$ . C) Module/metric reference species portion (portion of defined reference and bioregion- specific species in total abundance and species number).

Reference-index-abundance (RIabund) = (Sum of relative abundances of reference species) / (200 - Sum of relative abundances of spp.-taxa).

Reference-index-taxanumber (RI<sub>taxa</sub>) = (Sum of reference species taxa) / (total taxa - Sum of spp.-taxa). Reference species

Index (RI) = (RIabund + RI<sub>taxa</sub>) / 2.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Worst quality class

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

**Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge

**3.07 Reference site characterisation**

**Number of sites:** 1.800 sites (3.250 datasets)

**Geographical coverage:** Basically whole area of Austria (all aquatic bioregions)

**Location of sites:** No special locations

**Data time period:** n.a.

**Criteria:**

No indication of substantial chemical impairment. No intensive agricultural and urban land use within catchment area. No relevant deficiency indicated by other biota (esp. macroinvertebrates). No relevant deficiency in algal indices (TI, SI). No 'veto' from the competent limnologists of the different federal governments in Austria.

**3.08 Reference community description**

See Table 18.5 in 'Leitfaden zur Erhebung der biologischen Qualitätselemente - Teil A3 - Phytobenthos', where reference

species associations of all Austrian bioregions are listed.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

#### **3.11 Boundary setting procedure**

H/G: 10th percentile of high class TI / SI values (all values lying within the defined type specific trophic / saprobic reference class based on TI / SI classes according to ROTT's trophic / saprobic indication system) – recalculated to EQR G/M: Upper TI boundary of next worse trophic class (following the type specific trophic reference class) - recalculated to EQR... And so on

**3.12 "Good status" community:** For good status defined common reference species and/or rivertype-specific species (see Leitfaden zur Erhebung der biologischen Qualitätselemente - Teil A3 - Phytobenthos, Tab. 18.5) must obtain a certain percentage of all occurring algae (percentage varying in different bioregions).

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

#### **3.14 Comments:**

none

ID: 49

MMI ("Detaillierte MZB-Methode")

## 1. General information

- 1.01 GIG:** Alpine, Central-Baltic, Eastern Continental  
R-A1, R-A2, R-C3, R-E4
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Austria
- 1.05 Specification:** only for water bodies with catchment size > 10km<sup>2</sup>
- 1.06 Method name:** *Assessment of the biological quality elements - part benthic invertebrates*
- 1.07 Original name:** *Erhebung der biologischen Qualitätselemente - Teil Makrozoobenthos ("Detaillierte MZB-Methode")*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Acidification, Catchment land use, Eutrophication, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration  
Acidification only for parts of Austria and only for specific monitoring programmes
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).
- 1.10 Internet reference:** <http://wisa.lebensministerium.at>
- 1.11 Pertinent literature of mandatory character:**  
Ofenböck, T., O. Moog, A. Hartmann & I. Stubauer, 2008. Leitfaden zur Erhebung der biologischen Qualitätselemente, Teil A2 - Makrozoobenthos. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, 214 p.
- 1.12 Scientific literature:**  
Moog, O., T. Ofenböck, I. Stubauer & A. Hartmann, 2007. Grundlagen der Bewertung des guten Zustandes nach WRG - Qualitätselement Makrozoobenthos (MZB). Wiener Mitteilungen 201: 87-132.
- 1.13 Method developed by**  
BOKU- Institut für Hydrobiologie und Gewässermanagement,  
Arbeitsgruppe Benthosökologie und Gewässerbewertung  
ilse.stubauer@boku.ac.at, patrick.leitner@boku.ac.at  
as above
- 1.14 Method reported by**  
Ilse Stubauer and Astrid Schmidt-Kloiber  
  
ilse.stubauer@boku.ac.at  
BOKU - Inst. f. Hydrobiology and Aquatic Ecosystem Management
- 1.15 Comments**  
several background literature existing

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Ofenböck, T., O. Moog, A. Hartmann & I. Stubauer, 2008. Leitfaden zur Erhebung der biologischen Qualitätselemente, Teil A2 - Makrozoobenthos. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, 214 p.
- 2.02 Short description**  
Multi-habitat sampling designed for sampling major habitats in proportion to their presence within a sampling reach is carried out. A sample consists of 20 "sampling units" taken from all habitat types at the sampling site with a share of at least 5 % coverage. A "sampling unit" is a stationary sampling performed by positioning the net and disturbing the substrate in a quadratic area that equals the frame-size upstream of the net (0.25 x 0.25 m). Sediments must be disturbed to a depth of 15-20 cm (where possible) depending on substrate compactness.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net, Surber or Hess sampler  
mostly standardised handnet;
- 2.05 Specification:** Handnet 25 x 25 cm, mesh size 500 µm, length of net minimum 1m
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** pre-requisite low water conditions and representative benthic invert. community; Rhithral: spring ahead of regular spring floods (acc. to regime type); Potamal: early summer / summer during low flow conditions
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one (for Austrian monitoring), probably more acc. to aim of research within other projects
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1 MHS consisting of 20 sampling units, one of each 5 % habitat coverage of river bottom
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
1.25 m<sup>2</sup>

## **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 500 µm mesh-size of net but also smaller animals are sampled because meshes are "clogged" and animals do not slip directly through the mesh
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
preferably whole sample is sorted; if much material, sub-sampling is possible; first: pre-picking - second: sub sample (area based, with Caton pan, 5 cells or minimum 700 Individuals), third: post-sorting
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Mollusca (excl. Sphaeriidae) species-level  
Oligochaeta species-(genus-)level  
Hirudinea species-(genus-)level  
Crustacea (Amphipoda, Decapoda, Isopoda) species-level  
Ephemeroptera species-(genus-)level  
Plecoptera genus-(species-)level  
Trichoptera (excl. Limnephilidae) species-(genus-)level  
Coleoptera species-(genus-)level  
Odonata species-(genus-)level  
Heteroptera genus-(species-)level  
Megaloptera genus-(species-)level  
Chironomidae species-(genus-)level  
Simuliidae species-level  
Blephariceridae species-level  
Limoniidae genus-(species-)level  
other Diptera genus-(family-)level  
Bryozoa species-(genus-)level
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** number of individuals per m2
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** non-wadeable rivers: for routine monitoring only river banks are sampled with MHS; for other studies in non-wadeable rivers, airlift samples are preferred, but no national method so far;
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Saprobic Index, Degradation-Index, Rhithron-Feeding-Type-Index, Ratio of the abundance of functional feeding types, Total number of taxa, Number of EPT-taxa, Ratio of EPT-Taxa, Share of littoral-preferring taxa, Ratio of Oligochaeta and Diptera taxa, Longitudinal Zonation Index, Margalef diversity
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge
- 3.07 Reference site characterisation**  
**Number of sites:** 198  
**Geographical coverage:** existing sites in whole Austrian territory  
**Location of sites:** distributed over all Austrian bioregions

**Data time period:** only sites with MHS samples; 1999 -2004

**Criteria:**

- no to very minor eco-morphological impairment (class 1 or 1-2 acc. to different classification systems used in Austria, e.g. Werth 1987 or Spiegler 1989); main focus was put on parameter "river bottom", as this is the most important part of the river for benthic invertebrates
- no residual flow or upsurge/ down surge influence
- no disruption of longitudinal continuum with direct (local) influence (remark: not true for whole river/catchment)
- no intensive land use at investigation site
- no punctual sewage water disposal/discharge directly above or at sampling site
- no organic pollution (information on existing river quality assessment data were consulted)

**3.08 Reference community description**

expressed by metrics for each river type; table existing, no verbal description

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** Expressed by metric for each river types, no verbal description.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 157

IBD 2006

## 1. General information

- 1.01 GIG:** Alpine, Central-Baltic, Mediterranean  
R-A1, R-A2, R-M1, R-M2, R-M4, R-C1, R-C2, R-C3, R-C4, R-C6
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** France
- 1.05 Specification:** none
- 1.06 Method name:** *Biological Diatom Index 2006*
- 1.07 Original name:** *Indice Biologique Diatomées 2006*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Correlation with NH<sub>4</sub> and PO<sub>4</sub> has been studied through 2556 samples. The relationships between IBD2006 and those 2 parameters were significant: R<sup>2</sup> (IBD2006/NH<sub>4</sub>) = 0.45 and R<sup>2</sup> (IBD2006/PO<sub>4</sub>) = 0.46.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Norme AFNOR NF T90-354, December 2007. Qualité de l'eau - Détermination de l'Indice Biologique Diatomées (IBD).
- 1.12 Scientific literature:**  
Coste, M., S. Boutry, J. Tison-Rosebery & F. Delmas, 2009. Improvements of the Biological Diatom Index (BDI): Description and efficiency of the new version (BDI-2006). Ecological Indicator 9 (4): 621-650.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Michel COSTE, Sébastien BOUTRY, Juliette ROSEBERY, François DELMAS<br>michel.coste@cemagref.fr<br>CEMAGREF groupement Bordeaux | <b>1.14 Method reported by</b><br>Juliette ROSEBERY<br>juliette.rosebery@cemagref.fr<br>CEMAGREF groupement de Bordeaux |
|---|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Norme AFNOR NF T90-354, December 2007. Qualité de l'eau - Détermination de l'Indice Biologique Diatomées (IBD).
- 2.02 Short description**  
Samples are collected on stones (100cm<sup>2</sup>, >= 5 stones) on a sunny and running site of the river, thanks to a brush or a scraper.  
The biofilm collected is fixed with a 10% formaldehyde solution.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Brush, Scraper
- 2.05 Specification:** scraper or brush
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
stones
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** summer low flow period
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one / year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
one
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
100 cm<sup>2</sup> (>= 5 stones)

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** about 100 cm<sup>2</sup> biofilm sampled on stones
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups  
The level of taxonomical identification is the species, or the variety when existing.
- 2.15 Record of abundance:** Individual counts, Relative abundance

in relation to n.a.

relative abundance is calculated for a total of 400 individuals counted / slide minimum

Unit per thousand

2.16 Quantification of biomass: n.a.

2.17 Other biological data: none

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

Relative abundance of key taxa ( $A_x$ ), with pollution sensitivity ( $P_{xi}$ ) and valence values ( $V_x$ ) ( $=F(i)=\sum B = 1x F(1) + 2x F(2) + 3x F(3) + 4x F(4) + 5x F(5) + 6x F(6) + 7x F(7)$ ) is transform into a /20 note.

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: Not relevant

##### 3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

#### Reference conditions

3.05 Scope of reference conditions: Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions

##### 3.07 Reference site characterisation

Number of sites: 234

Geographical coverage: Whole French hydrosystem

Location of sites: Whole French hydrosystem

Data time period: From 1977 to 2007

##### Criteria:

The national dataset has been analysed with an unsupervised neural network, the self-organizing-map, a well accepted method for community ordination. 11 different communities were identified, 5 corresponding to non-impacted or slightly impacted conditions and representing the diatom natural variability of our dataset. These 5 natural communities corresponded to 5 different types of hydro-ecoregions, i.e. 5 river types with similar geological context and range in altitude.

☐

All the stations corresponding to those 5 reference community types were checked according to REFCOND criteria (land use criteria and physico-chemical parameters values) or from expert knowledge when chemical values were not available (samples from the national reference stations network).

##### 3.08 Reference community description

According to the river type, 5 different reference communities have been described. See: TISON, J., Y. S. PARK, M. COSTE, J.G. WASSON, L. ECTOR, F. RIMET, F. DELMAS (2005) – Diatom community variability and hydro-ecoregions: a French assessment. Water Research, 39: 3177-3188.

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: High-good boundary derived from metric variability at near-natural reference sites

##### 3.11 Boundary setting procedure

The good/moderate boundary was calculated using a two step procedures (this procedure based on diatom-derived biotypes to define the provisional threshold values of the good ecological status of French river (ministerial circular DE/MAGE/BEMA 05 n°14 of the 28th July 2005):

☐ 1: For each type, the remaining range below the H/G boundary and the IBD minimum value was split into 4 equal classes to derive a preliminary G/M boundary, following a procedure proposed in the REFCOND guidance.

☐ 2: This preliminary boundary was then increased by 1 point on the IBD scale for all national types.

☐ This procedure of boundaries calculation was chosen to be congruent with the French macroinvertebrates approach.

☐ Then the IBD values obtained were checked to verify their compliance with normative definitions: the graph below shows the percentage of sensitive species ('oligotraphent' + 'mesotraphent' species: van Dam et al., 1994) in reference conditions and along the ecological status gradient.

☐ This graph shows (impossible to paste a graph here):

☐ - no significant difference in sensitive species % between reference conditions and high status;

☐ - a very slight but significant decrease of sensitive species between

high and good status;<sup>2</sup>- a drop in the percentage of sensitive species between good and moderate status.

**3.12 "Good status" community:** n.a.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

First results of uncertainty will be available at the end 2010.

ID: 147

IBGN

## 1. General information

- 1.01 GIG:** Alpine, Central-Baltic, Mediterranean  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** France
- 1.05 Specification:** none
- 1.06 Method name:** *Global biological normalized index*
- 1.07 Original name:** *Indice Biologique Global Normalisé*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, General degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Large-scale model (PLS regression) linking IBGN index to river basin and riparian land cover were developed using national monitoring networks (3662 sites and 12682 samples covering the period 1992-2002). Land cover explained 18% of IBGN index variability. This model showed significant negative effect of urbanization and agriculture.
- 1.10 Internet reference:** <http://starwp3.eu-star.at/detail.php?id=29>
- 1.11 Pertinent literature of mandatory character:**  
Norme AFNOR NF T 90 350 (1992;2004) and circular MEDD/DE 05 n° 14 (july 05).
- 1.12 Scientific literature:**  
Terrasson, I., 2004. The IBGN- its history, sampling and future. Technical synthese. ENGREF Centre de Montpellier.
- 1.13 Method developed by**  
Jean Verneaux  
Laboratoire de biologie Faculté des Sciences de Besançon
- 1.14 Method reported by**  
André Chandesris & Virginie Archaimbault  
andre.chandesris@cemagref.fr virginie.archaimbault@cemagref.fr  
CEMAGREF
- 1.15 Comments**  
IBGN is the french historical assesment method applied since 1992 in our national monitoring network. This methodology was not entirely compliant with the WFD requirements therefore we are improving it by modifying the sampling design and by creating a

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Until 2005 Norme AFNOR NF T 90 350 (1992; 2004) since 2005 Circulaire DE / MAGE / BEMA 04 / n° 18 ( 23 décembre 2004) modified by Circulaire DE / MAGE / BEMA 07 / n° 4 ( 11 avril 2007) (Environment Ministry) ; now normalized Norme AFNOR XP T 90 333 (sept 2009).
- 2.02 Short description**  
Before 2005 : multi-habitat sampling designed for sampling the 8 most biogenous habitats is carried out After 2005 : multi-habitat sampling designed is carried out for sampling : 1/ minor habitats (<= 5%) according to their habitability 2/ major habitats (> 5%) in proportion to their presence within a sampling reach. 3 A sample consists of 12 "sampling units" taken from all habitat types at the sampling site according to above rules. 4 A "sampling unit" is a stationary sampling performed by positioning the net and disturbing the substrate in an area that equals the frame-size upstream of the net (0.20 x 0.25 m).  
Sediments must be disturbed to a depth of 5 cm (where possible).
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Stratified sampling/surveying
- 2.04 Sampling/survey device:** Hand net, Surber or Hess sampler
- 2.05 Specification:** surber sampler 1/20 m2
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** during low water level (june to september in lowlands, februar to march in high mountains)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
8 replicates (according to their habitability) until 2005 , 12 replicates (4 for substrates < = 5% coverage and 8 for substrates > 5% coverage in proportion to their presence ) since 2005
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
8/20 = 0.4 square meter until 2005 ; 12/20 = 0.6 square meter since 2005

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 500 µm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Family, Genus, Other  
before 2005 Family or other levels; after 2005: Plecoptera, Ephemeroptera, Trichoptera (without Limnephilidae), Coleoptera (without Dytiscidae, Hydrophilidae et Curculionidae), Megaloptera, Planipennia, Odonata (without Coenagrionidae), Hymenoptera, Crustacea (without Asellidae), Bivalvia, Gastropoda (without Planorbidae) : Genus; Diptera, Heteroptera (without Corixinae), Lepidoptera, Hirudinea et Branchiobdellida, Turbellaria : Family; (Hydracarina), Oligochaeta, Bryozoa, Nematoda, Gordiacea, Hydrozoa, Porifera ; Nemertea : Groups
- 2.15 Record of abundance:** Abundance classes, Individual counts  
before 2005 : abundance classes after 2005 : individual counts  
**in relation to** Area  
**Unit** number of individuals per effort sampling
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### **3. Data evaluation**

#### **Evaluation**

- 3.01 List of biological metrics**  
richness, sensitive taxa, abundance classes ; in progress, see comments A-23
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates

#### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge
- 3.07 Reference site characterisation**  
**Number of sites:** 305  
**Geographical coverage:** all over the territory of France, but more sites on the medium and small streams  
**Location of sites:** all over the territory  
**Data time period:** historical datas since 1992 until 2003  
**Criteria:**  
1/ qualitative criteria's list at the basin, reach, site scale evaluated by local experts; 2/ GIS criterias based on Corine Land Cover's datas (artificial, intensive agriculture, agriculture in the watershed)
- 3.08 Reference community description**  
not available with the IBGN protocol, in progress with the new one
- 3.09 Results expressed as EQR?** Yes

#### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

#### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**

uncertainty results expected for the end of 2012

ID: 215

MacrOper

## 1. General information

- 1.01 GIG:** Alpine, Central-Baltic, Mediterranean  
R-A1, R-A2, R-C(1), R-M1, R-M2, R-M4, R-M5
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Italy
- 1.05 Specification:**
- 1.06 Method name:** *MacrOper, based on STAR\_ICM index calculation*
- 1.07 Original name:** *MacrOper, basato sul calcolo dell'indice STAR\_ICM*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Catchment land use, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration

### **Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Relationship between STAR\_ICMi (the index at the basis of the MacrOper system) and pressures were studied in many river types and many publications on the topic are available in literature. See the below section 'Scientific literature'. Good relationships were found between STAR\_ICMi and single chemical parameters, indicators of morphological alteration (e.g. HMS index), indicators of habitat diversification (e.g. HQA index), land use indices and combined pressures. These relationships were tested within different river types in Italy (e.g. small and medium Mediterranean rivers, Temporary rivers, Alpine rivers, Lowland streams) but also in European contexts. Spearman correlation coefficients are usually not lower than 0.4, even if sometimes lower coefficient can be found in particular river types like temporary rivers and in relation to specific parameters indicating pressures.

- 1.10 Internet reference:** <http://www.irsa.cnr.it/Notiziario/>

### **1.11 Pertinent literature of mandatory character:**

CNR-IRSA, 2007. Macroinvertebrati acquatici e direttiva 2000/60/EC (WFD). IRSA-CNR Notiziario dei Metodi Analitici, Marzo 2007 (1): 118 pp.

CNR-IRSA, 2008. Direttiva 2000/60/EC (WFD). Condizioni di Riferimento per fiumi e laghi. Classificazione dei fiumi sulla base dei macroinvertebrati acquatici. IRSA-CNR Notiziario dei Metodi Analitici, Numero Speciale 2008: 88pp. Official note on classification systems from Ministry of Environment (Decreto Ministeriale 2010).

### **1.12 Scientific literature:**

Buffagni, A., D.G. Armanini & S. Erba, 2009. Does the lentic-lotic character of rivers affect invertebrate metrics used in the assessment of ecological quality? *Journal of Limnology* 68 (1): 92-105.

Buffagni, A., S. Erba, M. Cazzola, J. Murray-Bligh, H. Soszka & P. Genoni, 2006. The STAR common metrics approach to the WFD intercalibration process: Full application for small, lowland rivers in three European countries. *Hydrobiologia* 566: 379-399.

Buffagni, A., S. Erba & M.T. Furse, 2007. A simple procedure to harmonize class boundaries of assessment systems at the pan-European scale. *Environ. Sci. Policy*, 10: 709-724.

Buffagni, A., S. Erba & R. Pagnotta, 2008. Definizione dello Stato ecologico dei fiumi sulla base dei macroinvertebrati bentonici per la 2000/60/EC (WFD): Il sistema di classificazione MacrOper per il monitoraggio operativo. IRSA-CNR Notiziario dei Metodi Analitici, Numero Speciale 2008: 25-41.

Buffagni, A., S. Erba, S. Birk, M. Cazzola, C. Feld, T. Ofenböck, J. Murray-Bligh, M.T. Furse, R. Clarke, D. Hering, H. Soszka & W. Van den Bund, 2005. Towards European Inter-calibration for the Water Framework Directive: Procedures and examples for different river types from the E.C. project STAR. 11th STAR deliverable. STAR Contract No: EVK1-CT 2001-00089. Rome (Italy), Quad. Ist. Ric. Acque 123, IRSA, 468 pp.

Erba, S., A. Buffagni, N. Holmes, M. O'Hare, P. Scarlett & A. Stenico, 2006. Testing River Habitat Survey features for the aims of the WFD hydro-morphological assessment: an overview from the STAR Project. *Hydrobiologia* 566: 281-296.

Erba, S., M.T. Furse, R. Balestrini, A. Christodoulides, T. Ofenböck, W. van de Bund, J.-G. Wasson & A. Buffagni, 2009. The validation of common European class boundaries for river benthic macroinvertebrates to facilitate the intercalibration process of the Water Framework Directive. *Hydrobiologia* 633:17-31.

### **1.13 Method developed by**

Andrea Buffagni  
buffagni@irsa.cnr.it  
CNR-IRSA, Water Research Institute

### **1.14 Method reported by**

Stefania Erba  
erba@irsa.cnr.it  
CNR-IRSA, Water Research Institute

### **1.15 Comments**

The classification method adopted in Italy is, in terms of calculation formula and class boundaries, the STAR\_ICMi, formally intercalibrated by Italy during the WFD IC process. The name MacrOper refers to the whole assessment system, that bases on the STAR\_ICMi, which is in turn directly related to the official river typology, quantitative sampling methods, mesohabitat selection etc. All these factors, concurrently verified and interconnected, make the MacrOper system WFD-compliant.

## 2. Data acquisition

### **Field sampling/surveying**

#### **2.01 Sampling/Survey guidelines**

CNR-IRSA, 2007. Macroinvertebrati acquatici e direttiva 2000/60/EC (WFD). IRSA-CNR Notiziario dei Metodi Analitici, Marzo 2007 (1): 118 pp.

#### **2.02 Short description**

The method for the macroinvertebrates collection is a 'multi habitat, proportional sampling procedure as was developed and tested within the EU research projects AQEM and STAR, with some adaptations for South European rivers. A reach representative of the site and including a Pool/Riffle sequence is selected and sampled. The method is based on the sampling

of the most representative habitats, in relation to their occurrence, separately considering the pool and the riffle areas. The standard Italian approach requires that 10 sampling units are allocated in a riffle or in a pool area (for operational monitoring), depending on river type. There are also cases in which it is not possible to recognize the pool/riffle sequence and thus the 10 replicates are collected referring to a generic sample. For surveillance monitoring, samples are collected from both areas. The 10 replicates are pooled in order to have the sample that will be used for classification. For each replicate, substrate and flow type are recorded. The sampling is performed by positioning the net and disturbing the substrate, according to AQEM procedures.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Artificial substrate, Hand net, Surber or Hess sampler

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** Depending on river type and Hydro-Ecoregion

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

A classification can be provided for each sample i.e. sampling occasion. To derive the overall site classification (ecological

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area:** In general terms, a minimum of 6 samples should be used to derive the overall site classification.

10 sample units (proportionally located at river site according to microhabitat occurrence) are merged to derive the overall sample, to be used for classification. (see also answer B-08)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Depending on the river type, the total sampled area (sum of the 10 sample units) corresponds to 0.5 or 1 square-meter. When surveillance monitoring is planned, 2 matched samples are collected, and the area thus results double.

### **Sample processing**

**2.12 Minimum size of organisms sampled and processed:** 500 µm (mesh-size of hand net)

**2.13 Sample treatment:** Organisms of the complete sample are identified.

Sub-sampling is allowed but not officially required and, therefore, an official procedure to do is not fixed on a National scale. Most abundant taxa (e.g. Chironomidae, Baetidae) are usually sub-sampled based on techniques adapted to the prevalent kind of substrate sampled (e.g. macrophytes, sand) and only a portion of the specimens effectively present is brought to the lab for identification.

**2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups

In the case of classification for non wadable rivers the taxonomical identification is Family for all the taxa excluding Ephemeroptera that have to be identified at Operational Unit (OU) level. The OU level corresponds in most cases to genus but for Baetidae, Caenis, Rhithrogena and Ecdyonurus it is necessary to go in a further detail.

**2.15 Record of abundance:** Individual counts

**in relation to** Area

**Unit** Number of individuals per one square-metre

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** Non-wadable rivers are sampled using multiple-plate, artificial substrates suspended in the water column. Integrative samples are collected from macrophytes and bank areas for surveillance monitoring

**2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

#### **3.01 List of biological metrics**

Six are the metrics composing the STAR\_ICMi: ASPT; Log 10 (sum of Heptageniidae, Ephemeridae, Leptophlebiidae, Brachycentridae, Goeridae, Polycentropodidae, Limnephilidae, Odontoceridae, Dolichopodidae, Stratyomidae, Dixidae, Empididae, Athericidae e Nemouridae +1); 1 - ( relative abundance of Gatropoda, Oligochaeta and Diptera); Total number of families; Number of EPT families and Shannon - Wiener Diversity Index. 1. Each metric has to be converted in a EQR value by dividing each metric value by the median value of the metric in reference samples of the considered river type. 2. Prior converting ASPT metric in EQR it is necessary to subtract 2 to the metric value. This is considered necessary because it is uncommon that ASPT reaches values lower than 2. 3. calculation of the weighted average of the 6 metrics values (expressed as EQRs), according to the weights. 4. Successively, the values obtained have to be also normalized. The normalization of the

STAR\_ICMi is necessary in order to combine the data from different stream types and geographical areas. Weights of the single metrics: ASPT: 0.333; Log 10 (Sel\_EPTD + 1): 0.266; 1- GOLD: 0.067; TotFam: 0.167; EPT Fam: 0.083; Shannon: 0.083.

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Weighted average metric scores

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

### **Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** About 50 reference sites covering a wide geographical gradient along Italy, from the Alps to Mediterranean islands.

**Geographical coverage:** Alps, Northern Central and Southern Italy, lowlands, Mediterranean region, Sardinia.

**Location of sites:** Alps: sites located in Piedmont, Lombardy and Alto-Adige; Northern Italy, lowland: sites located in Lombardy and Piedmont; Mediterranean: sites located in Emilia-Romagna, Tuscany, Lazio, Umbria, Campania, Calabria, Puglia, Sardinia

**Data time period:** Historical data were not available. So data from fixed sampling period were used. In general for each site at least 2 samples are available (usually 3). In a few cases only 1 sample is available for a site.

**Criteria:**

Reference criteria were derived on the basis of REFCOND guidance document, and the work done within EU projects (AQEM, STAR) and GIGs. The criteria are specified in CNR-IRSA, 2008. DIRETTIVA 2000/60/EC (WFD). CONDIZIONI DI RIFERIMENTO PER FIUMI E LAGHI. CLASSIFICAZIONE DEI FIUMI SULLA BASE DEI MACROINVERTEBRATI ACQUATICI. IRSA-CNR Notiziario dei Metodi Analitici, Numero Speciale 2008: 88pp. These criteria list a series of pressures that have to be quantified before selecting a site as a reference site, for which fixed pressure levels must not be exceeded.

**3.08 Reference community description**

Reference values for the six metrics composing the STAR\_ICMi and the STAR\_ICMi itself are provided for each river type present in Italy. See CNR-IRSA, 2008. DIRETTIVA 2000/60/EC (WFD). CONDIZIONI DI RIFERIMENTO PER FIUMI E LAGHI. CLASSIFICAZIONE DEI FIUMI SULLA BASE DEI MACROINVERTEBRATI ACQUATICI. IRSA-CNR Notiziario dei Metodi Analitici, Numero Speciale 2008: 88pp and CNR-IRSA, 2009. IRSA-CNR Notiziario dei Metodi Analitici, Novembre 2009. The overall lists comprised the values for about 500 river types in Italy.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites  
High-Good boundary set at the 25%ile of reference site samples.

**3.11 Boundary setting procedure**

Boundaries were not put according to pressures criteria, but a fixed percentile of reference samples distribution (i.e. the 25th percentile) was selected as the boundary between H and G, in terms of STAR\_ICMi values. This was considered to be a minimal and simple approach in line with WFD requirements (agreed within the MedGIG). A second potential value for the boundary is calculated after testing the STAR\_ICMi against an independent, benchmark dataset, the AQEM/STAR Benchmark dataset (as described in Buffagni et al., 2005; 2006; Erba et al., 2009). The value obtained according to this approach should guarantee the similarity to scientifically set (and thus ecologically sound) boundaries. The G/M boundary is then set to correspond to the H/G boundary (see above) multiplied by 0.75. I.e., the range covered by STAR\_ICMi values comprised between 0 and the 25th percentile of STAR\_ICMi observed at reference sites was partitioned into 4 equally spaced classes, Good status being the highest in terms of STAR\_ICMi. A 25% deviation from reference sites value is assumed to be, in general terms, a slight deviation. In relation to this procedure the community experienced a decreasing presence of the sensitive taxa (expressed in terms of ASPT, EPT taxa, Log sel EPTD) with decreasing ecological quality and an increasing in tolerant taxa (e.g. 1-GOLD). Meanwhile, community richness is decreasing especially going down to moderate status (and lower).

**3.12 "Good status" community:** The community changes in relation to the type. We can in general say that the sensitive taxa (e.g. Ephemeroidea, Heptageniidae, Leptophlebiidae, Brachycentridae, Goeridae, Polycentropodidae, Limnephilidae, Odontoceridae, Dolichopodidae, Stratyomidae, Dixidae, Empididae, Athericidae) are well developed, but significantly decreasing at good-moderate boundary ("sudden drop") and replaced by more tolerant taxa (e.g. Oligochaeta, Gastropoda).

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

The information provided above includes work done even after the first phase of the formal Intercalibration exercise was concluded. In particular, the definitive protocol to collect invertebrates in a WFD compliant way was approved during 2008. New data from Environment Agencies are expected by the end of 2010, so not in time to be included in the on-going, second stage of intercalibration. The system proposed is considered valid for the classification of ecological status as required by the WFD.

ID: 150

IPR

## 1. General information

- 1.01 GIG:** Alpine, Central-Baltic, Mediterranean  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** France
- 1.05 Specification:** none
- 1.06 Method name:** *Fish Biotic Index*
- 1.07 Original name:** *Indice Poisson Rivière*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter

### **Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

1/ Based on an independent data set (from calibration) : difference between 88 disturbed and 88 undisturbed sites (t test,  $p < 0.001$ ) (Oberdorff et al 2001).  
2/ Considering response to pressure gradient (unpublished)- FAME project pressure (national data set of 759 sites) -> ANOVA for 4 pressure groups rated into 4 categories : Hydrology ( $p < 0.01$ ), Morphology ( $p < 0.01$ ), Toxic ( $p < 0.01$ ), Organic/Nutrient ( $p < 0.01$ ) ; strongest responses (R2) for Organic/Nutrient input and Morphological alterations.

- 1.10 Internet reference:** [http://www.onema.fr/IMG/pdf/IPR\\_Onema-2.pdf](http://www.onema.fr/IMG/pdf/IPR_Onema-2.pdf)
- 1.11 Pertinent literature of mandatory character:**  
AFNOR, 2004. Qualité de l'eau - Détermination de l'Indice Poisson Rivière (IPR) Normes Françaises NF T90-344: pp. 16.  
National technical document ts: CSP (2006) L'indice poissons rivière (IPR).  
Belliard, J. & Roset, N. (eds), 2006. Conseil Supérieur de la Pêche, Fontenay-sous-Bois; Avril.
- 1.12 Scientific literature:**  
Oberdorff, T., D. Pont, B. Huguény & D. Chessel, 2001. A probabilistic model characterizing fish assemblages of French rivers: a framework for environmental assessment. *Freshwater Biology* 46: 399-415.  
Oberdorff, T., D. Pont, B. Huguény & J.P. Porcher, 2002. Development and validation of a fish-based index for the assessment of "river health" in France. *Freshwater Biology* 47: 1720-1734.  
Oberdorff, T., D. Pont, B. Huguény, P. Boët, J.P. Porcher & D. Chessel, 2001. Adaptation à l'ensemble du réseau hydrographique national d'un indice de qualité écologique fondé sur les peuplements de poissons : résultats actuels et perspectives. In Lemoalle, J., F. Bergot & M. Robert (eds), Etat de santé des écosystèmes aquatiques. De nouveaux indicateurs biologiques. Cemagref, Antony.

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|--|--|
| <b>1.13 Method developed by</b><br>Thierry Oberdorff<br>yorick.reyjol@onema.fr<br>Conseil Supérieur de la Pêche (CSP, now ONEMA) | <b>1.14 Method reported by</b><br>Nicolas Roset<br>nicolas.rosset@onema.fr<br>Office National de l'Eau et des Milieux Aquatiques (ONEMA) |
|--|--|

- 1.15 Comments**  
none

## 2. Data acquisition

### **Field sampling/surveying**

- 2.01 Sampling/Survey guidelines**  
Sampling methods for the design of FBI: Porcher, J.P. : Réseau Hydrobiologique et Piscicole (RHP) 1998 – Cahier des charges, Conseil Supérieur de la Pêche.
- 2.02 Short description**  
Complete sampling : electrofishing is run by wading upstream through the whole sampling site using 1 anode per 5m river width. All the electroshocked fish are collected with handnets.  
Partial sampling: depending on river type, 75 (10m < river width < 50m) or 100 (river width > 50m) point abundance samples, regularly distributed along the study site, sampled with 1 anode. All the electroshocked fish are collected by handnets. Sampling is achieved by wading and/or using a boat.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Electrofishing gear
- 2.05 Specification:** "Heron" type electric device
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** General rule = low flow period, therefore depending on hydrological regime of the studied river (varied from March to November)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
1 sampling per year every two years are carried out for the surveillance program, but no decision about the temporal
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

- For complete sampling: sampled surface = 20 times the river width - For partial sampling: 75 or 100 point abundance samples depending on river type (see B-13), assuming a mean area of 12m<sup>2</sup> per sample.

**Sample processing****2.12 Minimum size of organisms sampled and processed:** No minimal size**2.13 Sample treatment:** Organisms of the complete sample are identified.

Both complete and partial sampling are performed, depending on river width (see B-13).

**2.14 Level of taxonomical identification:** Species/species groups**2.15 Record of abundance:** Individual counts**in relation to** Area**Unit** Number of individuals per 100m<sup>2</sup>**2.16 Quantification of biomass:** n.a.

Large individuals are weighted individually. Small individuals are processed by groups.

**2.17 Other biological data:** Length is measured for large specimens. For small specimens, a sub-sample is used when numerous individuals are caught.**2.18 Special cases, exceptions, additions:** For rivers only sampled by boat, and for rivers where the main channel is not wadable (security reason or fishing efficiency): the bank is the only habitat sampled.**2.19 Comments**  
none**3. Data evaluation****Evaluation****3.01 List of biological metrics**

Total number of species, Number of lithophilic species (excluding tolerant species), Number of rheophilic species (excluding tolerant species), Density of tolerant species individuals, Density of omnivorous species individuals, Density of invertivorous species individuals (excluding tolerant species), Total density of individuals.

**3.02 Does the metric selection differ between types of water bodies?** No**3.03 Combination rule for multi-metrics:** Average metric scores**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time  
Data from single spatial replicate

**Reference conditions****3.05 Scope of reference conditions:** Site-specific**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Modelling (extrapolating model results)

**3.07 Reference site characterisation****Number of sites:** 650 sites**Geographical coverage:** Metropolitan France (excluding Corsica and ultramarine territories)**Location of sites:** No geographical selection criteria**Data time period:** Mainly between 1985 and 1998**Criteria:**

Expert were asked to select regionally the sites with no significant pressure considering water quality alterations and modification of hydrology and morphology. Criteria used for selection of reference sites were: (1) sites should belong to the water quality classes 'Excellent' or 'Good' as defined by the Water Quality Index developed by the French Water Agency (5 classes); (2) sites should suffer from minimal habitat perturbations, as measured by the following factors : amount of stream channel modification, channel morphology, substrate character and condition. The general representativeness of the site was also taken into account. Reference sites were not pristine or totally undisturbed but were those considered as least impacted within a particular biogeographical region and/or river type.

**3.08 Reference community description**

High total number of species  
High number of lithophilic species (excluding tolerant species)  
High number of rheophilic species (excluding tolerant species)  
High density of invertivorous individuals (excluding tolerant species)  
High total density of individuals  
Low density of tolerant species individuals  
Low density of omnivorous species individuals

<b>3.09 Results expressed as EQR?</b>	No	The FBI scores varies from 0 (=undisturbed) to infinity (practically rarely more than 80, exceptionally more than 100). It seems that it could be transformed into EQR assuming a virtual maximum value for the most degraded situations. 47 is currently studied (to be validated) as the maximum value for intercalibration (justified by the distribution of FBI values on a representative national dataset and the homogenisation of quality class range => every values initially higher than 47 would be forced to be scored 47). Then the median value for a national reference data set should be close to 0.8.
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### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.

#### **3.11 Boundary setting procedure**

The thresholds have been computed through the use of two independent subsets: a subset of 88 reference sites (RS88) and a subset of 88 degraded sites (DS88). Using the distribution of the percentage of unimpaired sites for RS88 and DS88 as a function of index score values, the index value for the optimal cut-off for impaired sites was defined and rated the index into five classes (Unimpaired=Excellent or Good, and Impaired=Moderate, Poor or Bad). 74% of the sites for RS88 and 77% of the sites for DS88 were correctly classified respectively as reference and disturbed sites.

**3.12 "Good status" community:** Not available at the moment.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

The statistical design of the FBI leads to a risk of about 20% of misclassification of ecological status, with a fairly balanced risk for misclassifying reference and disturbed sites.

**3.14 Comments:**

none

ID: 37

SI-PB-RI

## 1. General information

- 1.01 GIG:** Alpine, Eastern Continental, Mediterranean  
Alpine: R-A1; Eastern Continental: R-E4 ,R-EX5, R-EX6, Mediterranean: R-M1 , R-M2, R-M5
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Slovenia
- 1.05 Specification:** none
- 1.06 Method name:** *Ecological status assessment system for rivers using phytobenthos*
- 1.07 Original name:** *Vrednotenje ekološkega stanja rek s fitobentosom*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [http://www.mop.gov.si/si/delovna\\_podrocja/direktorat\\_za\\_okolje/sektor\\_za\\_vode/ekolosko\\_stanje\\_povrsinskih\\_vod\\_a/](http://www.mop.gov.si/si/delovna_podrocja/direktorat_za_okolje/sektor_za_vode/ekolosko_stanje_povrsinskih_vod_a/)
- 1.11 Pertinent literature of mandatory character:**  
Uradni list Republike Slovenije stran (pp) 832, št. (no) 10, 9.2.2009.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Gorazd Kosi  
gorazd.kosi@nib.si  
National Institute of Biology
- 1.14 Method reported by**  
Gorazd Kosi  
gorazd.kosi@nib.si  
National Institute of Biology
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Kosi, G., M. Šiško, N. Smolar-Žvanut, D. Vrhovšek, A. Krivograd-Klemenčič, 2005. Priprava metodologije vzorčenja ter laboratorijske obdelave vzorcev alg (fitobentosa) za določanje ekološkega stanja vodotokov v Sloveniji in obdelava 45 vzorcev alg. Nacionalni inštitut za biologijo, 72 str.
- 2.02 Short description**  
Brushing and splashing of different substrates collected from different habitats. Organisms from all substrates represent a sample.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Brush
- 2.05 Specification:** Brush
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** small-large rivers: June-September, very large rivers: December-February
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
several
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
500 valves per sample are counted.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Relative abundance  
in relation to n.a.

**Unit** Number of individuals of 500 counted valves.

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Saprobic index (SI = Sum of (Indicator Taxa Abundance \* Saprobic value \* Indicator weight) / Indicator Taxa Abundance \* Indicator weight), Trophic index (TI = Sum of (Indicator Taxa Abundance \* Trophic value \* Indicator weight) / Indicator Taxa Abundance \* Indicator weight)

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Worst metric score

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** Differ among types (up to 10)

**Geographical coverage:** Alps and Dinarids and Pannonian lowland

**Location of sites:** n.a.

**Data time period:** 1998-2007

**Criteria:**

The criteria for the selection of the potential reference sites in the rivers include hydromorphological and physico-chemical conditions of the site, riparian vegetation, floodplain and land use properties, saprobic index values, and some pressures' presence. Potential reference sites were defined without considering the criteria of biotic pressures that includes allochthonous species and fishery management.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

We tested the influence of number of samples on variability of EQR values at individual sampling sites.

**3.14 Comments:**

none

ID: 39

SI-BI-RI

## 1. General information

- 1.01 GIG:** Alpine, Eastern Continental, Mediterranean  
Alpine: R-A1; Eastern Continental: R-E4 ,R-EX5, R-EX6, Mediterranean: R-M1 , R-M2, R-M5
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Slovenia
- 1.05 Specification:** none
- 1.06 Method name:** *Ecological status assessment system for rivers using benthic invertebrates*
- 1.07 Original name:** *Vrednotenje ekološkega stanja rek z bentoškimi nevretenčarji*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation, Hydromorphological degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Pressure impact relationship was tested for hydromorphological pressure only and for each type separately. As hydromorphological pressure variable was used a hydromorphological quality and modification (HQM) index.
- 1.10 Internet reference:** [http://www.mop.gov.si/si/delovna\\_podrocja/direktorat\\_za\\_okolje/sektor\\_za\\_vode/ekolosko\\_stanje\\_povrsinskih\\_vod\\_a/](http://www.mop.gov.si/si/delovna_podrocja/direktorat_za_okolje/sektor_za_vode/ekolosko_stanje_povrsinskih_vod_a/)
- 1.11 Pertinent literature of mandatory character:**  
Uradni list Republike Slovenije stran (pp) 832, št. (no) 10, 9.2.2009.
- 1.12 Scientific literature:**  
n.a.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Gorazd Urbanič<br>gorazd.urbanic@izvrs.si<br>Institute for water of the Republic of Slovenia | <b>1.14 Method reported by</b><br>Gorazd Urbanič<br>gorazd.urbanic@izvrs.si<br>Institute for water of the Republic of Slovenia |
|---|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Urbanič, G., B. Tavzes, M.J. Toman, Š. Ambrožič, V. Hodnik, K. Zdešar & M. Sever, 2005. Priprava metodologij vzorčenja ter laboratorijske obdelave vzorcev bentoških nevretenčarjev (zoobentosa) nabranih v vodotokih in obdelava 70 vzorcev bentoških nevretenčarjev. Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za biologijo, 36 str.
- 2.02 Short description**  
Multi-habitat sampling designed for sampling major habitats in proportion to their presence within a sampling reach is carried out. A sample consists of 20 "sampling units" taken from all habitat types at the sampling site with a share of at least 5 % coverage. A "sampling unit" is a stationary sampling performed by positioning the net and disturbing the substrate in a quadratic area that equals the frame-size upstream of the net (0.25 x 0.25 m). Sediments must be disturbed to a depth of 15-20 cm (where possible) depending on substrate compactness.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net, Surber or Hess sampler
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** small-medium-sized rivers: June-September, large rivers: December-February
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
20 replicates (one per stream microhabitat >5% coverage)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Sum of 20 spatial replicates à 0.0625 square-metres = 1.25 square-metres of stream bottom in total

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 500 µm mesh-size of hand net
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

One-fourth of sampling material is separated and analysed.

**2.14 Level of taxonomical identification:** Family, Genus, Species/species groups  
Mostly species/genus, Chironomidae (subfamily), Tubificidae, some Brachycera (family)

**2.15 Record of abundance:** Individual counts  
**in relation to** Area

**Unit** Number of individuals per 0,3125 square meter

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** Non-wadable rivers are sampled only at the banks, i.e. multi-habitat-sampling is confined to the river margin habitats.

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Saprobic index (SI = Sum of (Indicator Taxa Abundance \* Saprobic value\* Indicator weight) / Indicator Taxa Abundance\* Indicator weight), Slovenian multimetric index for hydromorphological alteration/general degradation (SMEIH = Weighted average of three or four metrics - depends on river type)

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Weighted average metric scores

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** Differ among types (up to 10)

**Geographical coverage:** Alps and Dinarids and Pannonian lowland

**Location of sites:** n.a.

**Data time period:** 1995-2008

**Criteria:**

The criteria for the selection of the potential reference sites in the rivers include hydromorphological and physico-chemical condition of the site, riparian vegetation, floodplain and land use properties, saprobic index values, and some pressures presence. Potential reference sites were defined without considering the criteria of biotic pressures that includes allochthonous species and fishery management.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites  
Using paired metrics that respond in different ways to the influence of the pressure

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

We tested the influence of number of samples on variability of EQR values at individual sampling sites.

**3.14 Comments:**

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none

ID: 10

IPS

## 1. General information

- 1.01 GIG:** Alpine, Mediterranean  
R- A2, R-M1, R-M2, R-M4, R-M5
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Spain
- 1.05 Specification:** none
- 1.06 Method name:** *Pollution Sensitivity Index*
- 1.07 Original name:** *Indice de Polluosensibilité Spécifique*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication  
Nutrient enrichment
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).
- 1.10 Internet reference:** [http://www.mma.es/portal/secciones/fondo\\_docu\\_descargas/publi\\_manuales/pdf/Protocolos\\_muestreo\\_biologico\\_con\\_portada.pdf](http://www.mma.es/portal/secciones/fondo_docu_descargas/publi_manuales/pdf/Protocolos_muestreo_biologico_con_portada.pdf)
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
0  
CEMAGREF
- 1.14 Method reported by**  
Carmen Coletto Fiaño  
ccoletto@mma.es  
Subdirección General de Gestión Integrada del Dominio Público  
Hidráulico - Ministerio de Medio ambiente, Medio Rural y Marino
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Metodología para el establecimiento del Estado Ecológico según la Directiva Marco del Agua: Protocolo de muestreo y análisis para Fitobentos.
- 2.02 Short description**  
n.a.
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying
- 2.04 Sampling/survey device:** Brush
- 2.05 Specification:** Normal brush
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Hard bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** Spring and summer-autum
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Two samples every year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
10-5 replicates
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
100 cm<sup>2</sup>

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
400 valves
- 2.14 Level of taxonomical identification:** Genus, Species/species groups
- 2.15 Record of abundance:** Individual counts, Relative abundance  
in relation to Area

100 cm<sup>2</sup>

Unit Number of valves

2.16 Quantification of biomass: n.a.

2.17 Other biological data: none

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

3.01 List of biological metrics

IPS

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: Not relevant

3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

#### Reference conditions

3.05 Scope of reference conditions: Surface water type-specific

3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Expert knowledge, Modelling (extrapolating model results)

3.07 Reference site characterisation

Number of sites: 270

Geographical coverage: Whole country. Only some water body types could be sampled to obtain reference conditions

Location of sites: n.a.

Data time period: Historical data from 2005

Criteria:

Refcond Guidance + GIGs criteria

3.08 Reference community description

No reference community description yet.

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites

3.11 Boundary setting procedure

n.a.

3.12 "Good status" community: n.a.

#### Uncertainty

3.13 Consideration of uncertainty: No (to be done)

3.14 Comments:

none

ID: 9

IBMWP

## 1. General information

- 1.01 GIG:** Alpine, Mediterranean  
R-A2, R-M1, R-M4, R-M5
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Spain
- 1.05 Specification:** none
- 1.06 Method name:** **Iberian Biological Monitoring Working Party**
- 1.07 Original name:** *Iberian Biological Monitoring Working Party*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).
- 1.10 Internet reference:** [http://www.mma.es/portal/secciones/fondo\\_docu\\_descargas/publi\\_manuales/pdf/Protocolos\\_muestreo\\_biologico\\_con\\_portada.pdf](http://www.mma.es/portal/secciones/fondo_docu_descargas/publi_manuales/pdf/Protocolos_muestreo_biologico_con_portada.pdf)
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Alba-Tercedor, J., 1996. Macroinvertebrados acuáticos y calidad de las aguas de los ríos. IV Simposio del Agua en Andalucía (SIAGA). Alme 2: 230-130.  
  
Alba-Tercedor, J. & A. Sánchez-Ortega, 1988. Un método rápido y simple para evaluar la calidad biológica de las aguas corrientes basado en el de Hellawell. Limnetica 4: 51-56.  
  
Alba-Tercedor, J., P. Jáimez-Cuellar, M. Álvarez, J. Avilés, N. Bonada, J. Casas, A. Mellado, J. Alba-Tercedor & A. Sánchez-Ortega, 1988. Un método rápido y simple para evaluar la calidad biológica de las aguas corrientes basado en el de Hellawell. Limnetica 4: 51-56.  
  
Alba-Tercedor, J., P. Jáimez-Cuellar, M. Álvarez, J. Avilés, N. Bonada, J. Casas, A. Mellado, M. Ortega, I. Pardo, N. Prat, M. Rieradevall, S. Robles, C.E. Sáinz-Cantero, A. Sánchez-Ortega, M.L. Suárez, M. Toro, M.R. Vidal-Abarc, S. Vivas & C. Zamora-Muñoz, 2004. Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP<sup>+</sup>). Limnetica 21 (3-4) : 175-185.
- 1.13 Method developed by**  
Javier Alba-Tercedor  
  
Granada University
- 1.14 Method reported by**  
Carmen Coleto Fiaño  
ccoletto@mma.es  
Subdirección General de Gestión Integrada del Dominio Público  
Hidráulico - Ministerio de Medio ambiente, Medio Rural y Marino
- 1.15 Comments**  
Data acquisition protocol will be changed in the near future to: Quantitative Sampling Protocol (20 kicks) based on USA Environmental protection Agency procedure (Barbour, M. T., J. Gerritsen, B. D. Snyder, & J. B. Stribling. 1999.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
n.a.
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** Hand net (500 µm - 0,25 m base and equal or higher height)
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** Spring and summer-autum
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Two samples every year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
As much as necessary. Sampling until no more families are identified
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 500 µm sampled
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
First 200 organisms are identified
- 2.14 Level of taxonomical identification:** Family
- 2.15 Record of abundance:** Abundance classes, Relative abundance  
**in relation to** n.a.  
Only relative abundance is recorded  
**Unit** Abundance classes as percentage
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
IBMWP: Family level scores
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** 270  
**Geographical coverage:** Whole country. Only some water body types could be sampled to obtain reference conditions  
**Location of sites:** n.a.  
**Data time period:** Historical data from 2005  
**Criteria:**  
Refcond Guidance + GIGs criteria
- 3.08 Reference community description**  
No reference community description yet.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.  
Equidistant division of EQR for transformed metric. In the original metric boundaries based on discontinuities
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** n.a.

**3.14 Comments:**

none

ID: 127

PISIAD

## 1. General information

- 1.01 GIG:** Central-Baltic  
RC1, RC4
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish Region
- 1.06 Method name:** *Proportions of Impact-Sensitive and Impact-Associated Diatoms*
- 1.07 Original name:** *Procentuele abundantie van impact-sensitieve en impact-geassocieerde diatomeeën*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Acidification, Eutrophication, Flow modification, General degradation, Heavy metals, Hydromorphological degradation, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter  
salinity change

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
see Hendrickx & Denys (2005) for significant relations to nutrients, BOD, EC, chloride in 49 brooks

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. September 2009. Vlaamse Milieumaatschappij, Erembodegem, Belgium.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Luc Denys  
luc.denys@inbo.be  
Research Institute for Nature and Forest

**1.14 Method reported by**

Wim Gabriels  
w.gabriels@vmm.be  
Flemish Environment Agency

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

EN 13946:2003.

**2.02 Short description**

The order of preference for the substrate to be sampled is as follows: (1) stones: five different stones that were found spread throughout the location are sampled. These stones are lifted from the water and are sampled to the flow side (upstream side). With a (pocket) knife or sharpened spoon the epilithon is removed from the stones and stored in a container (60 – 100 ml) with a wide screw cap and extra closing lid. (2) non-wooden artificial structures (e.g. bridge pillars): in some cases no (suitable) stones will be present within the stretch. In this case bridge pillars can alternatively be sampled. This is done in the same way as with stones, only this will be carried out under water. (3) living reed: If no stones or bridge pillars are present, one can sample living reeds. These are cut with scissors. Only the zone about 10 cm below the water surface is collected. (4) other similar, living helophytes (monocotyls such as cattail (Typha), rushes (Scirpus, Juncus),...) are used in absence of reed; (5) artificial substrates are used in absence of all the above: preferably permanent, vandal-resistant constructions are chosen of inert material on which a biofilm can develop undisturbed during the whole year.

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying

**2.04 Sampling/survey device:** Scraper, Spoon

**2.05 Specification:** Knife

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
epilithon, or when this is not available, epiphyton

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** june-september

**2.09 Number of sampling/survey occasions (in time) to classify site or area**  
at least 1

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1 per site (3 per water body)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

about 10 cm<sup>2</sup> epilithon or epiphyton

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** All valves observed in the microscope
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
The sample is cleaned using oxidizing agents and homogenised and part of the sample is embedded in naphrax for identification with microscope. 500 valves are identified and counted.
- 2.14 Level of taxonomical identification:** Other, Species/species groups  
including subspecific taxa
- 2.15 Record of abundance:** Relative abundance  
**in relation to** n.a.  
number of valves  
**Unit** percentage, proportion
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** Flemish river type 'Mlz' (tidal rivers) is not addressed using diatoms
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Percentage of impact-associated diatoms (IAD); percentage of impact-sensitive diatoms (ISD)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
If IAD exceeds a predefined threshold, EQR gets a value between 0-0,60 based on a transformation of IAD; otherwise EQR gets a value between 0,60-1 based on a transformation of ISD.
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Least Disturbed Conditions  
foreign sites
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
Reference conditions are characterised by a relatively low relative abundance of impact-associated diatoms and a relatively high relative abundance of impact-sensitive diatoms
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** n.a.  
Class boundaries based on IAD and ISD threshold values are based on expert judgement and comparison to foreign reference sites; they are transformed in such a way that equidistant division of the EQR gradient (boundaries at 0,8; 0,6; 0,4 and 0,2) is obtained

**3.11 Boundary setting procedure**

EQR gradient is assumed to represent a continuous trend with general degradation

**3.12 "Good status" community:** The EQR values at good status are characterised by a relatively low IAD and a ISD that is slightly reduced in comparison to reference.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 14

IPS

## 1. General information

- 1.01 GIG:** Central-Baltic  
RC1, RC4, RC5, RC6, RC3
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Belgium (Wallonia)
- 1.05 Specification:** none
- 1.06 Method name:** *Pollution Sensitivity Index*
- 1.07 Original name:** *Indice de polluosensibilité*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, Pollution by organic compounds (e.g. DDT, PCB)

**Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

This relationship is showed in "Pirene-project " Fauville et al. 2004 - SPW- Belgium

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

CEMAGREF, 1982. Etude des méthodes biologiques d'appréciation quantitative de la qualité des eaux. Rapport QE Lyon Bassin Rhône-Méditerranée-Corse. AFNOR norm NF T 90-354, 2000.

**1.13 Method developed by**

Descy Jean-Pierre (1979) based on Zelinka & Marvan (1961) and Coste ( in Cemagref 1982)

jean-pierre.descy@fundp.ac.be

"Facultés Universitaires Notre Dame de la Paix à Namur- Belgique" in application of CEMAGREF 1982

**1.14 Method reported by**

Christine KEULEN

C.Keulen@spw.wallonie.be

Service Public de Wallonie - DEMNA - 5030 Gembloux - Belgium

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

AFNOR norm NF T 90-354 (2000).

**2.02 Short description**

A sample = a lot of stones etc. selected in lotic (oxygenous) parts of the stream (height of water < 20 cm) to reach the minimum size of 100 cm<sup>2</sup>.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Brush

**2.05 Specification:** tooth brush

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
hard bottom

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** first : avril to june ; second : september to october

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

two occasions per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

more than 1 to reach the minimum area size

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

minimum size of survey = 100 cm<sup>2</sup>

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 5 μ

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
sub-sampling is selected to be relevant of the complete sample and to reach a minimum of 400 organisms

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Relative abundance

**in relation to** Area, Volume  
organisms density  
**Unit** number of organisms /total observed

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Relative abundance of taxa with oligosaprobic valences (5 levels ). Formula of Zelinka & Marvan (1961) modified by Descy 1979

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** 15 sites of high status

**Geographical coverage:** all wallonia

**Location of sites:** especially in the river basins : Meuse, Rhin and Seine

**Data time period:** 1999(05/06/09/10) & 2000(05/06/09/10)

**Criteria:**

The reference sites were selected on basis of lower anthropic pressures; the physico-chemical quality of water is also taken in account. The sites must be in high biological status for the specific indicator.

**3.08 Reference community description**

The reference communities were described following national typology for diatoms. The reference communities include very sensitive organisms.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

A typology of the wallon streams based on diatoms composition has been settled. The presence of very sensitive organisms has been taken in account to define high status.

**3.12 "Good status" community:** A good community is relevant of the local typology and the presence of sensitive organisms or families is taken in account.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 111

FÜBE

## 1. General information

- 1.01 GIG:** Central-Baltic  
IC decision for IPS for types RC4, RC5, RC6, suitable for large rivers also
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Estonia
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment system for rivers using phytobenthos in Estonia*
- 1.07 Original name:** *Eesti pinnavee seisundi hindamise metoodika bioloogiliste kvaliteedinäitajate järgi. Jõed. Fütobentos (eelnoõ)*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Ecological data from 139 river reaches were examined to establish pressure-impact relationship between diatom indices and eutrophication gradient. The relationship between three indices (IPS, TGI and Watanabe index) and TP (measured in summer during low water period) showed significant correlation (Spearman correlation ranging from 0.28 to 0.55;  $p < 0.001$ ).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Methods are in practical use, currently not mandatory in legislation, will be mandatory in 2012.

**1.12 Scientific literature:**

Kahlert, M., R.-L. Albert, E.-L. Anttila, R. Bengtsson, C. Bigler, T. Eskola, V. Gälman, S. Gottschalk, E. Herlitz, A. Jarlman, J. Kasperoviciene, M. Kokocinski, H. Luup, J. Miettinen, I. Paunksnyte, K. Piirsoo, I. Quintana, J. Raunio, B. Sandell, H. Simola, I. Sunberg, S. Vilbaste & J. Weckström, 2009. Harmonization is more important than experience - results of the first Nordic-Baltic diatom intercalibration exercise 2007 (stream monitoring). *Journal of Applied Phycology* 21: 471-482. Kelly, M., C. Bennett, M. Coste, C. Delgado, F. Delmas, L. Denys, L. Ector, C. Fauville, M. Ferreol, M. Golub, A. Jarlman, M. Kahlert, J. Lucey, B. Ni Chathain, I. Pardo, P. Pfister, J. Picinska-Falynowicz, J. Rosebery, C. Schranz, J. Schaumburg, H. Van Dam & S. Vilbaste, 2009. A comparison of national approaches to setting ecological status boundaries in phytobenthos assessment for the European Water Framework Directive: results of an intercalibration exercise. *Hydrobiologia* 621: 169-182. Vilbaste, S., J. Truu, Ü. Leisk & A. Iital, 2007. Species composition and diatom indices in relation to environmental parameters in Estonian streams. *Archiv für Hydrobiologie - Supplement* 161: 307-326.

**1.13 Method developed by**

Sirje Vilbaste  
sirje.vilbaste@emu.ee  
Estonian University of Life Sciences, Centre for Limnology

**1.14 Method reported by**

Sirje Vilbaste  
sirje.vilbaste@emu.ee  
Estonian University of Life Sciences

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

CEN 2003, 2004.

**2.02 Short description**

Cobbles were gathered along a transect across the river. At deeper sites samples were taken only to a depth of 0.5 m. Diatom film was separated from the cobbles with a stiff toothbrush. The algal suspension from all gathered cobbles was mixed to obtain a bulky sample.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Brush

**2.05 Specification:** toothbrush

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
hard bottom

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** late summer (july-august) low water season

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

one occasion per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

5 cobbles or boulders (d. 7-15 cm)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

n.a.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 5 micrometre
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
One/third of sampling material were cleaned by hot acid combustion. Diatom slides were mounted into NAPHRAX
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** n.a.  
Relative abundance  
**Unit** %
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Three diatom indices: IPS, TDI, Watanabe Index calculated by means of the software OMNIDIA
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** 8  
**Geographical coverage:** Estonia  
**Location of sites:** Estonia  
**Data time period:** Single samples started from 2003  
**Criteria:**  
TP<0.05 mg/L TN<1.50 mg/L NH<sub>4</sub>< 0.02 mg/l
- 3.08 Reference community description**  
Dominating by Achnantheidium minutissimum
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
Equidistant division of the EQR gradient (boundary setting at 0.9, 0.7, 0.45, 0.2 of median reference sites)
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 159

KRW-maatlatten

## 1. General information

**1.01 GIG:** Central-Baltic  
R-C1 and R-C4

**1.02 Category:** Rivers

**1.03 BQE:** Benthic Diatoms

**1.04 Country:** Netherlands

**1.05 Specification:** none

**1.06 Method name:** *WFD-metrics for natural watertypes*

**1.07 Original name:** *KRW-maatlatten voor natuurlijke watertypen*

**1.08 Status: Method is/will be used in** First RBMP (2009)

**1.09 Detected pressure(s):** Acidification, Eutrophication, General degradation, Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

The scores with the metric have very significant negative correlations with total phosphorous (n = 259) and total nitrogen (n=165) correlations, but these are too weak to allow confident predictions of phytobenthos quality from nutrient concentrations. Correlations as Pearson correlations between logarithmically transformed nutrient concentrations and EQR.  $p \leq 0,001$ , but correlation is less clear in the larger river types. Reference: H. van Dam (2007). Een herziene KRW-maatlat voor het fyto­benthos in stromende wateren (A revised WFD-metric for river phytobenthos in The Netherlands). In opdracht van (commissioned by): Rijkswaterstaat RIZA. Herman van Dam, Adviseur Water en Natuur. Amsterdam. 47p.

**1.10 Internet reference:** [http://themas.stowa.nl/thema/ecologische\\_beoordeling/krw-maatlatten.aspx?mld=7213&rid=817](http://themas.stowa.nl/thema/ecologische_beoordeling/krw-maatlatten.aspx?mld=7213&rid=817)

**1.11 Pertinent literature of mandatory character:**

Besluit Kwaliteitseisen en Monitoring Water, 2009. Ministry of Housing, Spatial Planning and the Environment (presently under public consultation).

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

development by national expert group commissioned by STOWA, Bas van der Wal & RWS Waterdienst, Diederik van der Molen

b.van.der.wal@stowa.nl, herman.vandam@waternatuur.nl  
STOWA Foundation for Applied Water Management Research & Rijkswaterstaat Waterdienst

**1.14 Method reported by**

Roel Knobben

r.knobben@royalhaskoning.com  
Rijkswaterstaat Waterdienst

**1.15 Comments**

Description of KRW-maatlatten in Dutch. Method and metrics derived from intercalibration for phytobenthos.

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) Quality Handbook Hydrobiology (in prep). 2009. STOWA.

**2.02 Short description**

living reed: gather reed stems (4-8 per replicate) by cutting at 15-20 cm below water level (paying attention to recent water level changes). Place reed stem in container for transfer to lab. artificial substrate (consisting of dead reed stems): place 10 stems (attached to a floater) completely in the water. Remove after 7 weeks of incubation. Extraction of phytobenthos in the lab by preferably chemical extraction.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Brush, Scraper  
scissors to collect pieces of ree

**2.05 Specification:** scissors to cut pieces of reed stems. If not possible use scraper (pocket knife or ice scraper) or a brush (tooth brush)

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)  
reed (*Phragmites australis*); if not available, artificial substrate made from reed is placed on

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** April

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

minimum one occasion per year, but classification preferably averaged over three years.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

at least one sampling location; several replicates from within 50m from the sampling location.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

reed: one location, several replicates. When using artificial substrate: incubation 7 weeks

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified. suspension of extracted phytoplankton is used to prepare a microscope slide. The diatoms on this slide are identified and counted
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Relative abundance  
count strategy is aimed to determine the relative abundance of most abundant species. Up to 200 individuals are identified and counted. Other species present beyond count of 200 scales are noted.  
**in relation to** n.a.  
expressed as relative abundance in the total counted sample size (this is most often a rounded number, e.g. 200).  
**Unit** % (relative abundance) or # per sample size (e.g. # / 200 individuals)
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
Biological WFD monitoring is performed by 26 regional water boards (local/regional water systems) and 1 national water board (large rivers, large lakes, estuaries and coastal waters) . Small differences may occur in sampling strategies etc.

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
metric is adapted from the IPS method (Indice de Polluosensitivité Spécifique).  $IPS = (4,75 * (\sum (a*s*v)) / \sum (a*v)) - 3,75$   
 $a$  = (relative) abundance of species  $i$   $s$  = sensitivity of species  $i$   $v$  = indicator value for species  $i$   $IPS$  values are in the range of 1-20 and can be converted to EQR.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions  
no actual existing natural sites in rivers in Netherlands. Only one least disturbed site; spatial references from foreign countries
- 3.07 Reference site characterisation**  
**Number of sites:** 58  
**Geographical coverage:** Germany, Belgium, France, Sweden, Estonia, United Kingdom  
**Location of sites:** Hierdense Beek, NL; Rotbach and Furlbach, Germany; 56 sites from CB-GIG Intercalibration database  
**Data time period:** NL: May 2006; Germany: September 2006; Belgium, France, Sweden, Estonia, United Kingdom: t.b.a.  
**Criteria:**  
Hierdense Beek is assigned as reference but this assignment is doubtful due to pressure from agricultural land use in the upstream part of the brook. Water in the lower stretch is cleaner due to tributaries coming from natural areas with very clean water. Rotbach and Furlbach : selected by dutch hydrobiologists as reference for the original natural situation for dutch rivers regarding landscape, hydromorphology and physico-chemical characteristics (with minor human pressure). Other reference sites are coming from the CB-GIG database with reference locations.
- 3.08 Reference community description**  
benthic diatoms are abundant on most of the available substrate. Areas with low current velocity are dominated by epipellic taxa, epiphytic taxa are abundant on macrophytes, branches and tree trunks. Only epiphytic diatoms are collected for the phytoplankton metric, no species from other taxonomic groups. Furthermore a general description is given (in Dutch) in:

☐

STOWA (2009) Referenties en maatlatten voor natuurlijke watertypen. report 2007-32

**3.09 Results expressed as EQR?** No ISP; ISP can be converted to EQR using a table presented in the metrics

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise

#### **3.11 Boundary setting procedure**

By using similarities in geographic conditions the score on the IPS-scale in accordance with the reference condition is deduced for the Dutch situation. Next the scores of ten variants of an IPS-based metric were calculated for samples of CB-GIG type R-C1 and R-C4. For each of the ten variants the boundary values H/G and G/M at the intercalibration metric and several other performance characteristics were calculated, including the 95% confidence intervals of the boundary values. Finally a metric with a reference value has been chosen with boundary values which deviates less than the required 0.05 units from the mean values of all member states.

**3.12 "Good status" community:** The Good-moderate boundary is based on the Intercalibration Metric.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Precision and uncertainty is regarded in Van Herpen, van Tongeren, Knoben, Baggelaar, van Loon (2009). Quick scan precision and confidence of KRW assessment (in Dutch). This study resulted in a statistical method to assess the level of precision and confidence monitoring results and status classifications (including identifying outliers and estimates for missing values). The confidence of a status classification is expressed as the probability of exceeding a chemical limit value or the biological status classification moderate/good. Recommendations from this study are incorporated in the Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) (see question B.0). The new metric is validated by calculating the scores in the samples of the 56 foreign reference sites and by comparing the resulting quality classes with those which were originally inferred by the member state concerned.

#### **3.14 Comments:**

the metric phytobenthos (as part of macrophytes) is a good indicator for acidification and eutrophication. Because phytoplankton is a good indicator as well for the eutrophication in lakes the multi-metric for phytobenthos is not used in lakes.

ID: 236

Diatom index IO

## 1. General information

1.01 GIG: Central-Baltic

1.02 Category: Rivers

1.03 BQE: Benthic Diatoms

1.04 Country: Poland

1.05 Specification:

1.06 Method name: *Assessment system for rivers using diatom phytobenthos*

1.07 Original name: *Ocena stanu ekologicznego rzek w oparciu o fitobentos okrzemkowy (Indeks Okrzemkowy IO)*

1.08 Status: Method is/will be used in Second RBMP (2015)

1.09 Detected pressure(s): Catchment land use, Eutrophication, General degradation, Pollution by organic matter

*Has the pressure-impact-relationship been tested?*

1.10 Internet reference:

1.11 Pertinent literature of mandatory character:

n.a.

1.12 Scientific literature:

n.a.

1.13 Method developed by

joanna.faltnowicz@imgw.wroc.pl

1.14 Method reported by

Joanna Picinska-Faltnowicz

joanna.faltnowicz@imgw.wroc.pl

Institute of Meteorology and Water Management, Wroclaw Branch,  
Department of Ecology

1.15 Comments

## 2. Data acquisition

### Field sampling/surveying

2.01 Sampling/Survey guidelines

Picinska-Faltnowicz, J. & J. Blachuta, 2008. Zasady poboru i opracowania prób fitobentosu okrzemkowego z rzek i jezior. Przewodnik metodyczny. Wersja 2008. Wroclaw.

2.02 Short description

5-10 replicates scraped from different stones along a transect crossing river's bed constitute a sample

2.03 Method to select the sampling/survey site or area: Expert knowledge

2.04 Sampling/survey device: Scraper

2.05 Specification: none

2.06 Sampled/surveyed habitat: Single habitat(s)  
hard bottom

2.07 Sampled/surveyed zones in areas with tidal influence: not relevant

2.08 Sampling/survey month(s): Mountain and mid-altitude streams: February to April, Lowland streams and rivers:  
September to November

2.09 Number of sampling/survey occasions (in time) to classify site or area

One occasion per sampling season

2.10 Number of spatial replicates per sampling/survey occasion to classify site or area

5-10 replicates per sampling occasion

2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area

n.a.

### Sample processing

2.12 Minimum size of organisms sampled and processed:

2.13 Sample treatment: Organisms of the complete sample are identified.

2.14 Level of taxonomical identification: Species/species groups

2.15 Record of abundance: Relative abundance

in relation to n.a.

a total of 300-500 diatom valves counted per sample

Unit

2.16 Quantification of biomass: n.a.

2.17 Other biological data: none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Diatom Index IO = (ZTI + ZSI + GR)/3; ZTI = 1-(TI\*0.25); ZSI = 1-[(SI-1)\*0.33]; GR = Sum of relative abundance of reference taxa; TI = Trophic Index (Rott et al. 1999); SI = Saprobic Index (Rott et al. 1997); TI and SI are calculated using a weighted formula of Zelinka & Marvan (1961)

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** A total of 30 sites for different abiotic river types

**Geographical coverage:** Reference zones in natural and landscape parks in Central Highlands, Carpathians, Central Plains, Baltic Province and Eastern Plains

**Location of sites:** Karkonosze, Tatry, Bieszczady, Magurski, Drawiński and Biebrzański Natural Parks, Chojnicki, Wdzydzki, Suwalski Landscape Parks

**Data time period:** February - April and September - November 2004-2009

**Criteria:**

Absence of point pollution sources, sub-basin dominated by natural forests, meadows and wetlands; slight hydromorphological changes have not been taken into account as they do not affect diatom phytobenthos communities

**3.08 Reference community description**

Epilithic diatom communities dominated by reference species, i.e. oligo-mesotrophilous and oligosaprobic depending on a stream/river type

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** n.a.

**3.11 Boundary setting procedure**

In preparation

**3.12 "Good status" community:** In preparation

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 219

IPR, IBGN, IBMR, IPS/IBD

## 1. General information

- 1.01 GIG:** Central-Baltic
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms, Benthic Invertebrates, Fish Fauna, Macrophytes  
Helophytes and hydrophytes
- 1.04 Country:** Luxembourg
- 1.05 Specification:** only for the rivers making the borders of Luxembourg sometimes the assessment method of the neighbouring country is applied
- 1.06 Method name:** **Assessment system for rivers using fish - macrozoobenthos - macrophytes - diatoms**
- 1.07 Original name:** *Indice poissons rivière, Indice biologique global normalisé, Indice biologique des macrophytes en rivière, Indice de la spécificité River (2009), Indice biologique des diatomées*
- 1.08 Status: Method is still being used in France (2009)**
- 1.09 Detected pressure(s):** Acidification, Catchment land use, Eutrophication, Flow modification, General degradation, Habitat destruction, Heavy metals, Hydromorphological degradation, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.  
the assessment methods have not been tested especially for Luxembourg, but the indices we are using are french or international standardized methods
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
NF T90-344 Mai 2004, Qualité de l'eau - Détermination de l'indice poissons rivière (IPR), Indice de classement : T90-344  
NF T90-395 Octobre 2003, Qualité de l'eau - Détermination de l'indice biologique macrophytique en rivière (IBMR), Indice de classement : T90-395  
NF T90-354 Décembre 2007, Qualité de l'eau - Détermination de l'Indice Biologique Diatomées (IBD), Indice de classement.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
n.a.
- 1.14 Method reported by**  
Lauff Max (fish), Reichard Monique (macrozoobenthos), Thiel Marc (macrozoobenthos & macrophytes), Welschbillig Nora (macrophytes & diatoms)  
max.lauff@eau.etat.lu, monique.reichard@eau.etat.lu, marc.thiel@eau.etat.lu, nora.welschbillig@eau.etat.lu  
Administration de la Gestion de l'Eau - Ministère de l'Intérieur et à la Grande Région
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
NF T90-344 Mai 2004, Qualité de l'eau - Détermination de l'indice poissons rivière (IPR), Indice de classement : T90-344  
NF T90-350, Mars 2004, Qualité de l'eau - Détermination de l'indice biologique global normalisé (IBGN)  
GA T90-374, Décembre 2006, Qualité de l'eau - Guide d'application de la norme NF T90-350:2004, IBGN (Détermination de l'indice biologique global normalisé)  
NF T90-395 Octobre 2003, Qualité de l'eau - Détermination de l'indice biologique macrophytique en rivière (IBMR), Indice de classement : T90-395  
NF T90-354 Décembre 2007, Qualité de l'eau - Détermination de l'Indice Biologique Diatomées (IBD), Indice de classement : T90-354
- 2.02 Short description**  
Benthic invertebrates: 12 samplings depending on the representative habitats; macrophytes: the hole sampling area; diatoms: 5 to 10 stones in the river flow
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Brush, Electrofishing gear, Surber or Hess sampler  
hands and sometimes knife
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** may to october
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**

one occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

one per stream, microhabitat >5% coverage

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

minimum 100,7 square-meters (macrozoobenthos: 12x1/20 m<sup>2</sup>; macrophytes (100m<sup>2</sup>); phytobenthos minimum 100cm<sup>2</sup>). If fish are monitored, the minimum monitoring area is 100 to 150 m<sup>2</sup>

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:**

fish - all size, macrozoobenthos - 500 micrometers, macrophytes (angiosperms, bryophytes and macroalgae) - visible with the naked eye, diatoms - microscopic

**2.13 Sample treatment:** Organisms of the complete sample are identified.

For macrozoobenthos, sub-sampling is performed as described in the french guidance document "Circulaire DCE 2007/22 du 11 avril 2007 relative au protocole de prélèvement et de traitement des échantillons des invertébrés pour la mise en oeuvre du programme de surveillance sur cours d'eau. Réf. DE/MAGE/BEMA07/n<sup>o</sup>4".

**2.14 Level of taxonomical identification:** Family, Genus, Species/species groups

Fish: species; macrozoobenthos: family, order; angiosperms: species; macroalgae: genus; bryophytes: species; diatoms: species

**2.15 Record of abundance:** Abundance classes, Individual counts, Percent coverage

**in relation to** Area

**Unit** Fish : Number of individuals per sampled area; macrozoobenthos: Number of individuals per sampled area; macrophytes: abundance / sampled area; diatoms: Number of individuals per sampled area

**2.16 Quantification of biomass:** Chlorophyll-a concentration

**2.17 Other biological data:** fish: length

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

**3. Data evaluation**

**Evaluation**

**3.01 List of biological metrics**

as described in standardized methods

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Worst quality class

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

**Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:**

**Geographical coverage:**

**Location of sites:** n.a.

**Data time period:**

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?**

No

Indices and class boundaries

**Boundary setting**

**3.10 Setting of ecological status boundaries:** n.a.

**3.11 Boundary setting procedure**

**3.12 "Good status" community:** n.a.

**Uncertainty**

**3.13 Consideration of uncertainty:**

**3.14 Comments:**

We are applying the indices and methods developed mostly in France and are not involved in the development and adjustments of these methods. Therefore, we are not able to give further informations on the method-related questions.

ID: 123

MMIF

## 1. General information

- 1.01 GIG:** Central-Baltic  
R-C1, R-C4
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish region
- 1.06 Method name:** **Multimetric Macroinvertebrate Index Flanders**
- 1.07 Original name:** *Multimetrische Macro-invertebratenindex Vlaanderen*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Heavy metals, Hydromorphological degradation, Impact of alien species, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter, Riparian habitat alteration

### **Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Gabriels et al (2009) found a positive correlation of MMIF with oxygen concentration (Spearman  $R=0.45$ ,  $n=304$ ) and with oxygen saturation (Spearman  $R=0.46$ ,  $n=304$ ) and a negative correlation with Kjeldahl nitrogen (Spearman  $R=-0.66$ ,  $n=282$ ), total nitrogen (Spearman  $R=-0.43$ ,  $n=301$ ), ammonium (Spearman  $R=-0.69$ ,  $n=297$ ), nitrite (Spearman  $R=-0.41$ ,  $n=301$ ), total phosphorous (Spearman  $R=-0.61$ ,  $n=296$ ), orthophosphate (Spearman  $R=-0.53$ ,  $n=170$ ), 5 day biochemical oxygen demand (Spearman  $R=-0.62$ ,  $n=261$ ) and chemical oxygen demand (Spearman  $R=-0.43$ ,  $n=237$ ) ( $p<0.001$  in all cases).

### **1.10 Internet reference:**

### **1.11 Pertinent literature of mandatory character:**

VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. Vlaamse Milieumaatschappij, Erembodegem, Belgium.

### **1.12 Scientific literature:**

Gabriels, W., K. Lock, N. De Pauw & P.L.M. Goethals, 2009. Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). *Limnologica* (in press).

### **1.13 Method developed by**

Wim Gabriels et al.  
w.gabriels@vmm.be  
Flemish Environment Agency

### **1.14 Method reported by**

Wim Gabriels  
w.gabriels@vmm.be  
Flemish Environment Agency

### **1.15 Comments**

none

## 2. Data acquisition

### **Field sampling/surveying**

#### **2.01 Sampling/Survey guidelines**

NBN T92-402. Biological quality of watercourses. Determination of the Biotic Index based on aquatic macroinvertebrates.

#### **2.02 Short description**

With the handnet, a stretch of approximately 10-20 meters is sampled during 3 minutes for watercourses less than 2 m wide or up to 5 minutes for larger rivers. Sampling effort is proportionally distributed over all accessible aquatic habitats. This includes the bed substrate (stones, sand or mud), macrophytes (floating, submerged, emerged), immersed roots of overhanging trees and all other natural or artificial substrates, floating or submerged in the water. Each aquatic habitat is explored, either with the handnet or manually, in order to collect the highest possible diversity of macroinvertebrates. For this purpose, kick-sampling is performed by vertically positioning the handnet on the bed and turning over bottom material located immediately upstream by foot or hand. In addition to the handnet sampling, animals are manually picked from stones, leaves or branches along the same stretch. If a site is too deep to be sampled with the handnet method, macroinvertebrates can alternatively be sampled using the so-called Belgian artificial substrates. These are composed of a plastic netting filled with medium-sized (4-8 cm) pieces of brick, with a total volume of approximately 5 L. Per sampling site, three substrates are placed in the water, anchored with a rope to a fixed point located on the bank. The substrates should not be placed in open water but along the banks: in protected sites among the vegetation near the surface, in unprotected sites, which are exposed to surface turbulence, in deeper water. After an exposure time of at least 3 weeks, the substrates are lifted from the water and transferred into a closed container.

#### **2.03 Method to select the sampling/survey site or area:** Expert knowledge

#### **2.04 Sampling/survey device:** Artificial substrate, Hand net Standard method is handnet; a

#### **2.05 Specification:** Handnet: standard handnet with 500 $\mu\text{m}$ mesh size / Artificial substrates: a plastic netting filled with medium-sized (4-8 cm) pieces of brick, with a total volume of approximately 5 L

#### **2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant  
**2.08 Sampling/survey month(s):** April - november  
**2.09 Number of sampling/survey occasions (in time) to classify site or area**  
1  
**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1  
**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Sampling duration of 3-5 minutes depending of the size of the watercourse

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** All animals retained after sieving with 500 µm mesh size  
**2.13 Sample treatment:** Organisms of the complete sample are identified.  
**2.14 Level of taxonomical identification:** Family, Genus, Other  
Plathelminthes, Hirudinea, Mollusca, Hemiptera, Megaloptera, Odonata, Ephemeroptera, Plecoptera: genus; Polychaeta, Oligochaeta, Coleoptera, Trichoptera, Crustacea: family; Diptera (Chironomidae): group (thummi-plumosus or non thummi-plumosus); Diptera (other): family; Acari: presence (i.e. counted as one taxon)  
**2.15 Record of abundance:** Individual counts  
in relation to n.a.  
Total sample  
Unit number of individuals per sample  
**2.16 Quantification of biomass:** n.a.  
**2.17 Other biological data:** none  
**2.18 Special cases, exceptions, additions:** Flemish river type 'Mlz' (tidal rivers) is not addressed using this method; for this type, see transitional waters method  
**2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Total number of present taxa; number of EPT taxa; number of other sensitive taxa; Shannon-Wiener diversity index; mean tolerance score (the mean of the tolerance scores of all encountered taxa; the tolerance score is predefined for each taxon)  
**3.02 Does the metric selection differ between types of water bodies?** No  
**3.03 Combination rule for multi-metrics:** Average metric scores  
**3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific  
**3.06 Key source(s) to derive reference conditions:**  
Expert knowledge  
**3.07 Reference site characterisation**  
Number of sites: n.a.  
Geographical coverage: n.a.  
Location of sites: n.a.  
Data time period: n.a.  
Criteria:  
n.a.  
**3.08 Reference community description**  
Reference conditions are assumed to correspond to an EQR value of 1, which is associated with expert-based type-specific metric values reflecting high taxa richness, sensitivity and diversity.  
**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** n.a.

Originally, equidistant division of the EQR gradient was applied (boundaries at 0.8, 0.6, 0.4, 0.2); these values were modified for most types as a result of the intercalibration exercise

**3.11 Boundary setting procedure**

EQR gradient is assumed to represent a continuous correlation with general degradation.

**3.12 "Good status" community:** The EQR values at good status reflect metric values that are only slightly lower than at (expert-based) reference state, hence the community can be characterised as only slightly different from reference in terms of taxa richness, sensitivity and diversity.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 16

IBGN

## 1. General information

- 1.01 GIG:** Central-Baltic  
RC3 (only this type has been taken in account in intercalibration exercise) but we have also data
- 1.02 Category:** from RC1, RC5, RC4, RC6
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Belgium (Wallonia)
- 1.05 Specification:** none
- 1.06 Method name:** *Global biological index normalized*
- 1.07 Original name:** *Indice Biotique Global Normalisé*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Norm AFNOR NF - T90 350 (1992-2004).
- 1.12 Scientific literature:**  
Bossche, V. & U. Polatera, 2005. Characterization, ecological status and type-specific reference conditions of surface water bodies in Wallonia using bioecotoxic metrics based on benthic macroinvertebrates communities. *Hydrobiologia* 551: 253-271.
- 1.13 Method developed by**  
0  
Service Public de Wallonie. DEMNA  
Pierre.Gerard@spw.wallonie.be
- 1.14 Method reported by**  
Christine KEULEN  
Christine.Keulen@spw.wallonie.be  
Service Public de Wallonie - DEMNA - 5030 Gembloux (Belgium)
- 1.15 Comments**  
The methodology used in Wallonia is based on the IBGN protocol norm AFNOR NF T 90-350 (1992-2004). Guide technique : Ministère de l'Environnement - Agence de l'Eau, Conseil supérieur de la Pêche - Cabinet Gay Grenoble. Vandenberghe & Usseglio Polatera

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Norm AFNOR NF - T90 350 (1992-2004).
- 2.02 Short description**  
Multi-habitats sampling designed for sampling the major substrates and current velocity (microbiotopes) is carried out. A sample consists of a maximum of microbiotopes classified according to the norm AFNOR and the collect effort depending of the substrate (B11)
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Artificial substrate, Dredge, Grab, Hand net
- 2.05 Specification:** Hand net size 500µm - Van Veen grab
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** may to october
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
at least one time by sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
ive kicks 1/20 m<sup>2</sup> for sand, gravel, mud, silt ; one kick for liter; three tufts 1:20 m<sup>2</sup> for spermaphytes; about ten stones by hand
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
surface related to the number and type of microbiotope present on station

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 500µm
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Family, Other  
other level species only for exotic potentially invasive taxa

**2.15 Record of abundance:** Abundance classes

**in relation to** n.a.

**Unit**

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** large rivers : application of IBGA

**2.19 Comments**

1. Faval indicator group (IG) : the group code corresponds to the sensitivity of the most sensible family of the sample in 38 selected families.
2. Taxonomic diversity or richness (n) total number of relevant families in the sample categorized in variety classes  $\Sigma$ IBGN or IBGA = GI + variety class (1 < or = 20)

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

n.a.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** 18 for RC3

**Geographical coverage:** South part of Wallonia (RC3 - Ardenne) but the other types exist and are not yet taken in account in intercalibration exercise

**Location of sites:** Ardenne (RC3), Condroz, Famenne (for RC5 and RC6)

**Data time period:** <2007

**Criteria:**

The references sites were selected on basis of lower anthropic pressures; the physico-chemical quality of water is also taken in account. The sites must be in high biological status for the specific indicator.

**3.08 Reference community description**

The reference communities were described following national typology for macroinvertebrates (7 types in Wallonia - see Vanden Bossche & Usseglio-Polatera for more details - A22).

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

A typology of walloon streams based on macroinvertebrates composition as been done. The presence of very sensitive families has been taken in account to define high status.

**3.12 "Good status" community:** A good community is relevant of the local typology without effects of human pressures.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 217

DVFI

## 1. General information

- 1.01 GIG:** Central-Baltic
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Denmark
- 1.05 Specification:**
- 1.06 Method name:** *Danish Streamfauna Index*
- 1.07 Original name:** *Dansk Vandløbsfaunaindeks*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Hydromorphological degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Organic matter (BOD) - 3600 datasets - however the pressure-response-analysis was conducted in the 1990ies and the datasets are not easy available anymore.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
<http://www2.mst.dk/udgiv/Publikationer/1998/87-7810-995-7/pdf/87-7810-995-7.pdf>
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Jens Skriver  
  
NERI
- 1.14 Method reported by**  
Lars Kjellerup Larsen  
lla@blst.dk  
Danish Ministry of the Environment, Agency of Spatial and Environmental Planning
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
<http://www2.mst.dk/udgiv/Publikationer/1998/87-7810-995-7/pdf/87-7810-995-7.pdf>
- 2.02 Short description**  
The sampling procedure is standardised and includes sampling of all microhabitats at the site. Sampling is undertaken using a standard hand net with a 25 x 25 cm opening and a tapering netbag with a mesh-size of 0.5 mm. Sampling is done at three transects across the stream lying about 10 m apart, four kick samples are taken at each transect 25%, 50%, 75% and 100% from one of the stream banks. If stream width is less than 1 m, the transects should be placed diagonally in an upstream direction. The 12 kick samples are pooled for further analysis. In deep rivers it is recommended to sample at least all available substrate types present along the banks. Animals adhering firmly to the substrate are sampled by 5 min of hand-picking from submerged stones and large wooden debris. This collection is kept separately from the kick sample. The macroinvertebrates are sorted and identified in the laboratory.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** February to April
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
One
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
depending on river size

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 0.5 mm
- 2.13 Sample treatment:** n.a.

**2.14 Level of taxonomical identification:** Family, Genus, Species/species groups

**2.15 Record of abundance:** Individual counts, Relative abundance

**in relation to** n.a.

**Unit**

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Danish Stream Fauna Index calculated using a matrix with 6 indicator groups along one axis and 4 diversity groups along another axis.

**3.02 Does the metric selection differ between types of water bodies?**

**3.03 Combination rule for multi-metrics:** n.a.

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:**

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** No reference sites are existing in Denmark.

**Geographical coverage:** No reference sites are existing in Denmark.

**Location of sites:** n.a.

**Data time period:**

**Criteria:**

REFCOND criteria

**3.08 Reference community description**

No missing groups and only minor changes in abundance. No or only a very minor loss in sensitive taxa. No or only a very minor loss in diversity.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise

**3.11 Boundary setting procedure**

**3.12 "Good status" community:** Typically most major taxonomic groups (orders) are found. But several families especially in important groups like Ephemeroptera, Plecoptera, Trichoptera and Coleoptera (EPTC) are missing. Abundance of some insensitive taxa could increase. The number of species and individuals of sensitive taxa of Plecoptera (genus-level), Ephemeroptera (family-level) and other sensitive groups are highly reduced at the good/moderate boundary. As a consequence the proportion of insensitive taxa becomes higher compared to the reference state. Loss in species diversity has been estimated for the EPTC families (see above). As a mean only about 45% of the EPTC species present at high quality will be found at the good/moderate boundary. At the family level (all families) as a mean about 70% of the families can be found at the good/moderate boundary.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

**3.14 Comments:**

none

ID: 59

DIUF

## 1. General information

- 1.01 GIG:** Central-Baltic  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Lithuania
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment system for rivers using macrozoobenthos indicators (Danish Stream Fauna Index)*
- 1.07 Original name:** *Upių ekologinės būklės vertinimo sistema naudojant makrozoobentoso rodiklius (Danijos indeksas upių faunai)*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation
- Has the pressure-impact-relationship been tested?**  
0
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**
- LAND 57, 2003. Makrozoobentoso tyrimo metodika paviršinio vandens telkiniuose (Valstybės žinios, 2004-12-11, Nr.: 53 - 1827, 2005-08-02, Nr.: 93 - 3469, 2007-01-09, Nr.: 3 - 138)
- LAND 70, 2005. Vandens kokybė. Biologinių mėginių ėmimo metodika. Nurodymai, kaip imti bentalės bestuburių mėginius (Valstybės žinios, 2006-01-17, Nr.: 6 - 226.).
- LST EN 27828: 2000
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
DSFI authors - Friberg, Larsen, Christensen, Rasmussen, Skriver. Daina Akinienė and Kestutis Arbaciauskas have modified this index, but its performance still is not tested. Tomas Virbickas has estimated the boundaries of quality classes in accordance arbas@ekoi.lt, tvirbickas@takas.lt, dakinienė@gmail.com  
Institute of Ecology of Vilnius University (Kestutis Arbaciauskas and Tomas Virbickas), Lithuanian Environmental Protection Agency (Daina Akinienė).
- 1.14 Method reported by**  
Jelena Titova  
j.titova@aaa.am.lt  
Lithuanian Environmental Protection Agency
- 1.15 Comments**  
Collected data were insufficient for pressure-impact analysis

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Friberg, N., S.E. Larsen, F. Christensen, J.V. Rasmussen & J. Skriver, 1996. Dansk Fauna Indeks: Tekst og Modifikationer. – Faglig rapport fra DMU, nr. 181, Danmarks Miljøundersøgelser, 58 pp.
- 2.02 Short description**  
Multi-habitat integrated sampling designed for sampling major habitats in proportion to their presence within a sampling reach is carried out. A sample consists of 10 "sampling units" taken from all habitat types at the sampling site with a share of at least 10 % coverage. A "sampling unit" is a stationary sampling performed by positioning the net and disturbing the substrate in a quadratic area that equals 0,4 x 0,25 m (1 minute). + 1 sample "by collecting" (we must collect 1 sample about 5 min.).
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** Hand net (length of net - 40 cm., span of frame - 25 cm, height of frame – 25 cm).
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** April - May, September - October.
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
2 times per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
10 replicates (in microhabitats >10% coverage) distributed in proportion to coverage.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

40x25 cm x 10 spatial replicates = around 1 square meter, plus qualitative collection sample per 5 min.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 0,5 mm (mesh - size of hand net).
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
All levels are so, which must be to calculate DSFI and circumstantialier.
- 2.15 Record of abundance:** Individual counts  
in relation to Area  
Unit Number of individuals per 1 m<sup>2</sup>
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** Species structure, number of individuals of taxons in one sample, number of species in one sample
- 2.18 Special cases, exceptions, additions:** Data of monitoring of rivers of makrozoobenthos was analyzed. Result - some data was not representative. -> Method of sampling was modified. Since this year are 10 (was 5) replicates (one per stream microhabitat > 10 % coverage). Plans -> analyze of new data.
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Species structure Number of individuals of taxons in sample "by collecting" Number of individuals of taxons in sample "by kicking"
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
n.a.  
Existing near-natural reference sites, collection of data ongoing.
- 3.07 Reference site characterisation**  
Number of sites: n.a.  
Geographical coverage: n.a.  
Location of sites: n.a.  
Data time period: n.a.  
Criteria:  
n.a.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** n.a.
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

### **Uncertainty**

**3.13 Consideration of uncertainty:** n.a.

**3.14 Comments:**  
none

ID: 149

KRW-maatlatten

## 1. General information

- 1.01 GIG:** Central-Baltic  
R-C1 and R-C4
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Netherlands
- 1.05 Specification:** none
- 1.06 Method name:** *WFD-metrics for natural watertypes*
- 1.07 Original name:** *KRW-maatlatten voor natuurlijke watertypen*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Hydromorphological degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
WFDmetric for small rivers was tested for a hydromorphology pressure (5 nominal classes). n=279 samples. R2=0.61. Relations with total-P (0-5.5 mgP/l) showed no clear correlation, but high tot-P values limit EQR to 0.4 (n=177 sites). Relations with total-N (1-16 mg/l;n=177) showed no clear correlation. Relation with oxygen saturation values showed no correlation, but gradient was limited to 45-140%. Van Riel & Knobben (2006) The Dutch assessment of macroinvertebrates in international comparison. For very large rivers no sufficient gradient is present to test pressure impact relationship.
- 1.10 Internet reference:** [http://themas.stowa.nl/thema/ecologische\\_beoordeling/krw-maatlatten.aspx?mid=7213&rid=817](http://themas.stowa.nl/thema/ecologische_beoordeling/krw-maatlatten.aspx?mid=7213&rid=817)
- 1.11 Pertinent literature of mandatory character:**  
Besluit Kwaliteitseisen en Monitoring Water, 2009. Ministry of Housing, Spatial Planning and the Environment (presently under public consultation).
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
development by national expert group commissioned by  
STOWA, Bas van der Wal & RWS Waterdienst, Diederik van der Molen  
b.van.der.wal@stowa.nl  
STOWA Foundation for Applied Water Management Research &  
Rijkswaterstaat Waterdienst
- 1.14 Method reported by**  
Roel Knobben  
r.knobben@royalhaskoning.com  
Rijkswaterstaat Waterdienst
- 1.15 Comments**  
Description of KRWmaatlatten in Dutch.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) Quality Handbook Hydrobiology (in prep). 2009. STOWA.
- 2.02 Short description**  
Multihabitat sampling in all habitats present in proportion to their presence. Active moving of handnet through vegetation and bottom substrates. Due to low current velocity no kick sampling.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** small rivers: handnet 30\*15 cm. Very large rivers:
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)  
very large rivers:
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** small rivers: march till 15 june
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
minimum one occasion per year (spring), but classification preferably averaged over three years.
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1 in small rivers, 10 in very large rivers
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
small rivers: 5 m handnet = 1,5 m2

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 500 um

- 2.13 Sample treatment:** Organisms of the complete sample are identified.  
only if some organisms occur in extreme high number, subsampling is done and total number is estimated.
- 2.14 Level of taxonomical identification:** Species/species groups  
Oligochaetes and Hydracarina may sometimes be determined at genus/family level.
- 2.15 Record of abundance:** Individual counts  
in relation to Area  
Unit numbers in standard sample. (5 m handnet)
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
Biological WFD monitoring is performed by 26 regional water boards. Small differences may occur in sampling strategies etc.

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
small streams:  $EQR = \{200 * (\%KM / KMmax) + 2 * 100 - \%DN\} + \% (DP + KM) / 500$  where  $\%KM$  = relative number of typical (for watertype) species in a sample  $\%KMmax$  - maximum achievable number of typical species under reference conditions  $\%DN$  = relative abundance of dominant negative species  $\% (DP + KM)$  = sum of relative abundances of dominant positive species and typical species  
Abundances are converted first to abundance (log) classes  
Very large rivers:  $EQR = fEPT * \{200 * (\%KM / KMmax) + 200 / (1 - \%DN / \%DNmax) + (\%KM + \%DP)\} / 500$  where  $fEPT$  = correctionfactor for contribution of Ephemeroptera, Plecoptera and Trichoptera
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions  
no actual existing natural sites in rivers; spatial references from foreign countries
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** Belarus, Ukrain  
**Location of sites:** Prypyat  
**Data time period:** n.a.  
**Criteria:**  
Dutch sites were tested against reference criteria by Wasson (2006) and all rejected.
- 3.08 Reference community description**  
Regarding the metric: High status of small rivers is characterized by a high abundance of dominant positive species and a high diversity and abundance of typical species. Dominant negative species are nearly absent. Furthermore a general description is given (in Dutch) in: STOWA (2009) Referenties en maatlaten voor natuurlijke watertypen. report 2007-32
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
The boundaries for the different EQR-classes (bad, poor, moderate, good and high) are set, based on expert judgement and follow a more or less equal division of quality. The WFDi and its class-boundaries were validated by experts judging species lists from anonymous sites, using normative definitions. In the validation of the method the response of the WFD-classes to pressures was tested. WFD-classes responded negatively to hydromorphologic pressure. According to the studied chemical pressures, EQR is most related to oxygen content. EQR and oxygen availability are positively correlated. Influences of other chemical pressures considered (phosphate and nitrogen content) were less clear. Water bodies in the Netherlands

are hydro-morphologically altered, making physical pressure an important factor in assessment of Dutch water bodies.

**3.12 "Good status" community:** Good status is characterized by a high diversity and abundance of typical species and an increasing abundance of dominant positive species. The abundance of dominant negative species is low.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Precision and uncertainty is regarded in Van Herpen, van Tongeren, Knobben, Baggelaar, van Loon (2009). Quick scan precision and confidence of KRW assessment (in Dutch). This study resulted in a statistical method to assess the level of precision and confidence monitoring results and status classifications (including identifying outliers and estimates for missing values). The confidence of a status classification is expressed as the probability of exceeding a chemical limit value or the biological status classification moderate/good. Recommendations from this study are incorporated in the Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) (see question B.0). In the metric abundance is expressed in abundance classes to reduce the impact of extreme abundance of one species on the calculated EQR.

**3.14 Comments:**

none

ID: 11

ES-BI-RI

## 1. General information

- 1.01 GIG:** Central-Baltic  
R-C2 , R-C3 , R-C5
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Spain
- 1.05 Specification:** Only to some waterbody types. North of Spain
- 1.06 Method name:** *Type specific multimetric*
- 1.07 Original name:** *Multimétrico específico del tipo*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Pardo, I., M. Álvarez & E. Roselló, 2007. Índices Multimétricos del Norte de España . Asistencia científica y técnica para la aplicación de los anejos 2 y 5 de la Directiva Marco del Agua en la Demarcación Hidrográfica del Norte. Technical report. University of Vigo, Vigo.
- 1.13 Method developed by**  
Isabel Pardo  
  
University of Vigo
- 1.14 Method reported by**  
Carmen Coleto Fiaño  
ccoletto@mma.es  
Subdirección General de Gestión Integrada del Dominio Público  
Hidráulico - Ministerio de Medio ambiente, Medio Rural y Marino
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Barbour, M.T., J. Gerritsen, B.D. Snyder & J.B. Stribling, 1999. Quantitative Sampling Protocol (20 kicks) based on USA Environmental protection Agency procedure.
- 2.02 Short description**  
Multihabitat quantitative sampling protocol. 20 sampling units taken from all habitats present at the sampling point.
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** Hand net (500 µm - 0,25 m base and equal or higher height)
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** Spring and summer-autum
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Two samples every year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
20 replicates (one per stream microhabitat >5% coverage)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Sum of 20 spatial replicates

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 500 µm sampled
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Wrona, F.J., Culp, J.M. y R.W. Davies (1982). Macroinvertebrate subsampling: a simplified apparatus and approach. Can.J.Fish.Aquat.Sci., 39:1051 – 1054
- 2.14 Level of taxonomical identification:** Family
- 2.15 Record of abundance:** Individual counts, Relative abundance  
**in relation to** Area

The effort unit is the kick

Unit Number of individuals

2.16 Quantification of biomass: n.a.

2.17 Other biological data: none

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

Number of families EPT Number of families PT Number of sensible families Similarity index Bray – Curtis % of sensible families Abundance of PT families % de 3 dominant families Oligoqueta Abundancia de clases familias EPT Margalef diversity index % of 6 dominant families

3.02 Does the metric selection differ between types of water bodies? Yes

3.03 Combination rule for multi-metrics: Weighted average metric scores

##### 3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

#### Reference conditions

3.05 Scope of reference conditions: Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Expert knowledge, Modelling (extrapolating model results)

##### 3.07 Reference site characterisation

Number of sites: n.a.

Geographical coverage: North of Spain. Only some water body types could be sampled to obtain reference conditions

Location of sites: n.a.

Data time period: Historical data from 2005

##### Criteria:

Refcond Guidance + Central-Baltic GIG criteria

##### 3.08 Reference community description

No reference community description yet.

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: Using discontinuities in the relationship of anthropogenic pressure and the biological response.

##### 3.11 Boundary setting procedure

n.a.

3.12 "Good status" community: n.a.

#### Uncertainty

3.13 Consideration of uncertainty: No (to be done)

##### 3.14 Comments:

none

ID: 52

IBI

## 1. General information

- 1.01 GIG:** Central-Baltic  
Lowland-midland
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish Region
- 1.06 Method name:** *Flemish Index of Biotic Integrity*
- 1.07 Original name:** *Vlaamse Index voor Biotische Integriteit*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, Flow modification, General degradation, Habitat destruction, Heavy metals, Hydromorphological degradation, Impact of alien species

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. Vlaamse Milieumaatschappij, Erembodegem, Belgium.

**1.12 Scientific literature:**

Belpaire, C., R. Smolders, I. Vanden Auweele, D. Ercken, J. Breine, G. Van Thuyne & F. Ollevier, 2000. An Index of Biotic Integrity characterizing fish populations and the ecological quality of Flandrian waterbodies. *Hydrobiologia* 434 (1-3): 17-33. Breine, J., I. Simoens, P. Goethals, P. Quataert, D. Ercken, C. Van Liefferinghe & C. Belpaire, 2004. A fish-based index of biotic integrity for upstream brooks in Flanders (Belgium). *Hydrobiologia* 522 (1-3): 133-148.

**1.13 Method developed by**

Jan Breine  
jan.breine@inbo.be  
Research Institute for Nature and Forest

**1.14 Method reported by**

Jan Breine  
jan.breine@inbo.be  
Research Institute for Nature and Forest

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

CEN and guidelines as presented in Breine et al., 2006: FAME consortium.

**2.02 Short description**

Electric fishing: (1) Rivers < 0.7 m depth = wadable rivers; Waveform selection: DC or PDC; Number of anodes: one anode per 5 m width; Number of hand-netters: each anode followed by 1 or 2 hand-netters (mesh size of 6 mm maximum) and 1 suitable vessel for holding fish; Number of runs: one run; Time of the day: daylight hours; Fishing length: 10-20 times the wetted width, with a minimum length of 100 m; Fished area: river width <15 m: the whole site surface / river width >15 m: several separated sampling areas are selected and prospected within a sampling site, with a minimum of 1000 m<sup>2</sup> (partial sampling method); Fishing direction: upstream; Movement: slowly, covering the habitat with a sweeping movement of the anodes and attempt to draw fish out of hiding; Stop nets: used if necessary and feasible. (2) Rivers > 0.7 m depth = non-wadable rivers (boat fishing); Waveform selection: DC or PDC; Number of anodes: Depending on boat configuration; Number of runs: one run; Time of the day: daylight hours; Fishing length: 10-20 times the wetted width, with a minimum length of 100 m; Fished area: both banks of the river or a number of sub-samples proportional to the diversity of the habitats present with a minimum of 1000 m<sup>2</sup> (partial sampling method); Fishing direction: normal flow: downstream in such a manner as to facilitate good coverage of the habitat, especially where weed beds are present or hiding places of any kind are likely to conceal fish / high flow: upstream / low flow: not necessary to match boat movement to water flow, and the boat can be controlled by ropes from the bank side if required; Movement: slowly, covering the habitat with a sweeping movement of the anodes or drifting with the boom along selected habitats and attempting to draw fish out of hiding.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Electrofishing gear, Fyke net, Seine netting

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** March - November

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

1 per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

3

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

100 - 250 m electric (depends on river width) and 24 h with fykes (Remark: only electric catches are used to assess river status)

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:** 1 mm but all fish are processed (weighed and measured)

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

in relation to Area

Unit kg/ha

**2.16 Quantification of biomass:** n.a.

balance

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** Flemish river type 'Mlz' (tidal rivers) is not addressed using this method; for this type, see transitional waters

**2.19 Comments**

method is WFD proof

**3. Data evaluation**

**Evaluation**

**3.01 List of biological metrics**

see Belpaire et al., 2000; Breine et al., 2004

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

Data from single spatial replicate

**Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Historical data, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** n.a.

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**

n.a.

**3.08 Reference community description**

Reference conditions are assumed to correspond to an EQR value of 1, which is associated with expert-based type-specific metric values reflecting high taxa richness, sensitivity and diversity.

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** The EQR values at good status reflect metric values that are only slightly lower than at (expert-based) reference state, hence the community can be characterised as only slightly different from reference in terms of taxa richness, sensitivity and diversity.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 17

IBIP

## 1. General information

**1.01 GIG:** Central-Baltic  
Central Baltic (Lowlands and Midlands)

**1.02 Category:** Rivers

**1.03 BQE:** Fish Fauna

**1.04 Country:** Belgium (Wallonia)

**1.05 Specification:** none

**1.06 Method name:** *Biological Index for Fish Integrity*

**1.07 Original name:** *Indice Biotique d'Intégrité Piscicole*

**1.08 Status: Method is/will be used in** First RBMP (2009)

**1.09 Detected pressure(s):** General degradation

*Has the pressure-impact-relationship been tested?*

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Indice Biotique d'Intégrité piscicole (IBIP) pour évaluer la qualité écologique des écosystèmes aquatiques - Convention RW N° 2095 (dans le cadre du projet Life Haute-Meuse) - Mars 1993 - Février 1997.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Didier, J. , Kestemont P. & JC Micha  
Thierry.Demol@spw.wallonie.be  
Facultés Universitaires Notre Dame de la Paix à Namur  
(Belgique) - Prof. P. Kestemont

**1.14 Method reported by**

Christine KEULEN  
C.Keulen@spw.wallonie.be  
Service Public de Wallonie - DEMNA - 5030 Gemboux - Belgium

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Guidelines for Fish monitoring in Fresh Waters - EIFAC.

**2.02 Short description**

The sampling area includes several microhabitats relevant from the water body. For the natural WB, the whole width of the stream is sampled. The electrofishing is practised by foot. For the large heavily modified water bodies, the electrofishing is practised by boat along the banks (2 meters width).

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Electrofishing gear

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** May to October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

one /3 or 6 years

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

2 (but only one is taken in account for the Index)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

The minimum size of the sampling area = 20 X width of the stream

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 10 mm

**2.13 Sample treatment:** n.a.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

in relation to Area

Unit number of individuals

- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** individual length and individual weight
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments:**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
number of native species, number of benthic species, percentage of intolerant fishes, number of Bullhead / number of Bullhead + , number of specialized layers, presence-absence of alevines of the dominant and intolerant species
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** 48 sites  
**Geographical coverage:** Wallonia  
**Location of sites:** Wallonia especially in the river basins : Meuse, Seine and Rhin  
**Data time period:** 1985 to 2007  
**Criteria:**  
The reference sites were selected on basis of lower anthropic pressures; the physico-chemical quality of water is also taken in account. The sites must be in high biological status for the specific indicator.
- 3.08 Reference community description**  
A reference community corresponds to the community of fish expected for the fish zone ( following Huet classification).
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure**  
in progress
- 3.12 "Good status" community:** A good fish community is composed from the native species of the fish zone and is not disturbed by anthropogenic pressures or exotic species.

#### Uncertainty

- 3.13 Consideration of uncertainty:** Yes  
The efficacy of the sampling method is calculated from the score obtained for the two occasions on the same area and in the same time.
- 3.14 Comments:**  
none

ID: 61

LŽI

## 1. General information

**1.01 GIG:** Central-Baltic  
n.a.

**1.02 Category:** Rivers

**1.03 BQE:** Fish Fauna

**1.04 Country:** Lithuania

**1.05 Specification:** none

**1.06 Method name:** *Assessment method of rivers using Lithuanian fish index*

**1.07 Original name:** *Upių ekologinės būklės vertinimo metodas pagal Lietuvos žuvų indeksą*

**1.08 Status: Method is/will be used in** First RBMP (2009)

**1.09 Detected pressure(s):** General degradation

***Has the pressure-impact-relationship been tested?***

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

LFI was developed analyzing response of fish metrics to pressures in 152 sites with near natural hydromorphology. Correlation between 8 fish metrics and 8 water quality metrics ranged within 0.2 – 0.63, that of LFI and water quality metrics – -0.35 - -0.52. Relationship of LFI was tested on 130 independent sites. Correlation of LFI and general degradation metric (covering water quality, channel morphology, hydrological alterations, impact of dams) is -0.73, LFI and water quality alone (52 sites) - -0.6, LFI and channel morphology (20 sites) – -0.8.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

CEN, 2003. Water Quality – Sampling of Fish with Electricity. EN 14011, European Committee for Standardization, Brussels. □ LAND 85 - 2007 Lietuvos žuvų indekso apskaičiavimo metodika. (2007, Nr. 47-1812).

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Tomas Virbickas  
tvirbickas@takas.lt  
Institute of Ecology of Vilnius University

**1.14 Method reported by**

Jelena Titova  
j.titova@aaa.am.lt  
Lithuanian Environmental Protection Agency

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

n.a.

**2.02 Short description**

streams up to 5-6 m width – the whole cross-section, more than 6 m width – partial random sampling.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Electrofishing gear

**2.05 Specification:** Producer: HANS GRASSL GmbH, device IG 200/2.

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** July - October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

1 time per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

n.a.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

length of sampled stretch is more than 10 times greater than stream width, but not less than 100 m.

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** mesh size of hand net – 6 mm.

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

in relation to Area

**Unit** Number of individuals per 1 ha (ind./ha)

- 2.16 Quantification of biomass:** n.a.  
fishes are weighted individually, or (in case of many individuals) – fish in sub sample are weighted individually, the rest are grouped to length groups and total weight of length group is recorded.
- 2.17 Other biological data:** organism length (cm), the masses of individuals (g), age structure (%), species composition
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Relative abundance of individuals of intolerant, lithophilic, rheophilic, tolerant and omnivorous fish, relative number of tolerant and lithophilic fish species, number of intolerant species
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** 41 site  
**Geographical coverage:** All area of Lithuania  
**Location of sites:** n.a.  
**Data time period:** 2000-2005  
**Criteria:**  
No pressures that could have an impact on community structure and composition. Characteristics of the river site are typical for a certain river type (channel shape and slope, bottom structure, riparian vegetation).
- 3.08 Reference community description**  
All type-specific intolerant species (*Salmo* sp., *Lampetra* sp., *Thymallus thymallus*, *Cottus gobio*, *Alburnoides bipunctatus*; list of intolerant species depends on river type), are present in high abundances, only type specific tolerant species are present in respective abundances.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Using discontinuities in the relationship of anthropogenic pressure and the biological response.
- 3.11 Boundary setting procedure**  
Class boundary for each individual metric was set calculating averages between 25% (higher status class) and 75% (lower status class) (for increasing metrics, e.g. abundance of individuals of tolerant species the opposite scheme was applied).
- 3.12 "Good status" community:** Nearly all type specific intolerant species are present, however in lower abundances. Community is dominated by lithophilic and reophilic fish individuals, diversity and abundance of less sensitive omnivorous fish is higher than at reference conditions.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 152

NLFISR

## 1. General information

- 1.01 GIG:** Central-Baltic  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Netherlands
- 1.05 Specification:** none
- 1.06 Method name:** *Netherlands References and Metrics for Fish in Small Rivers*
- 1.07 Original name:** *Nederlandse Referenties en Maatlatten voor Vis in kleine Rivieren*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Acidification, Eutrophication, General degradation, Hydromorphological degradation, Riparian habitat alteration  
River connectivity at water body scale (segment)
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).  
Yes; pressures: combination of acidification, hydromorphological degradation and connectivity segment score; impact metrics: relative abundance of eurytopic, rheophilic, habitat sensitive and migrating species; amount of data used: 2199 sites; statistical significance (R2): eurytopics = 0,45; rheophilics = 0,47; habitat sensitive species = 0,55; migrating species = 0,18; average abundance metrics = 0,54.
- 1.10 Internet reference:** <http://www.stowa.nl>
- 1.11 Pertinent literature of mandatory character:**  
Evers, C.H.M., H. de Mars, A.J.M. van den Broek, R. Buskens, M. Klinge & N. Jaarsma, 2005. Validatie en verdere operationalisering van de concept KRW-maatlatten voor de natuurlijke rivier- en meertypen. Consortium (Royal Haskoning, Taken Landschapsplanning, Witteveen+Bos) in opdracht van RWS-RIZA. Jaarsma, N., M. Klinge & R. Pot (eds), 2007. Achtergronddocument Referenties en Maatlatten Vissen ten behoeve van de Kaderrichtlijn Water. STOWA, Utrecht. Van der Molen, D.T. & R. Pot (eds), 2007. Referenties en maatlatten voor natuurlijk watertypen voor de Kaderrichtlijn Water. STOWA 2007-32; RWS-WD 2007-018
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Marco Beers, Tim Vriese & Tom Buijse  
m.beers@brabantsedelta.nl  
STOWA, RWS-RIZA (renamed to RWS-Waterdienst)
- 1.14 Method reported by**  
Tom Buijse & Marco Beers  
tom.buijse@deltares.nl ; m.beers@brabantsedelta.nl  
Deltares ; Waterschap Brabantse Delta
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Klinge, M., G. Hensens, A. Brenninkmeijer & L. Nagelkerke, 2003. STOWA Handboek Visstandbemonstering. Voorbereiding, bemonstering en beoordeling. STOWA, Utrecht.
- 2.02 Short description**  
Electrofishing by wading in streams/ rivers <= 3 m; by boat in streams/ rivers > 3m. In case the width of the stream/river > 8m then only the shoreline is sampled. Remark: not one, but many organisations responsible (regional waterboards, state-managed waters). Sampling is mostly performed by consultancy firms.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Electrofishing gear
- 2.05 Specification:** backpack, barge or boat-mounted electrofishing models depending on sampling conditions; one or two anode with size 0.5 m; current type: (P)DC
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** March - May; September - November
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Stream length / 2500 m with a minimum of 2; one replicate is 250 m
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
10% of the stream/river length

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Mesh size anode electrofishing gear: 8 mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to Area  
Unit number per ha
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** Length (either fork length or total length); According to the guidelines (answer B01) total length of fishes should be recorded.
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### **3. Data evaluation**

#### **Evaluation**

- 3.01 List of biological metrics**  
Number of species and relative abundance of Rheophilic, Eurytopic, Migratory and Habitat Sensitive species. Metric (NoS; RA) = [(rheophilic + eurytopic)/2 + migratory + habitat sensitive]/3. Metrics are calculated separately for i) number of species and ii) relative abundance. The final EQR is the average of these two metrics.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** 0; there are no reference sites in the Netherlands.  
**Geographical coverage:** Data from FAME were used to improve the method (pressure level 1 or 2). Ecoregion 13 and 14 below 300 m ASL  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
No reference sites available in the Netherlands.
- 3.08 Reference community description**  
Based on expert judgement. Rivers dominated by a rheophilic (75 - 90%), migratory (20 - 70%) and habitat sensitive (>95%) fish community. The level of dominance varies per river type.
- 3.09 Results expressed as EQR?** Yes

#### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
Expert judgement. The method is sensitive to pressures (see A-13), is significantly correlated to the EFI (Fame) as well as EFI+, but has to be intercalibrated for boundary setting.
- 3.12 "Good status" community:** Based on expert judgement. Rivers dominated by a rheophilic (65 - 85%), migratory (15 - 50%) and habitat sensitive (85 - 90%) fish community. The level of dominance varies per river type.

#### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none



ID: 153

NLFILR

## 1. General information

- 1.01 GIG:** Central-Baltic  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Netherlands
- 1.05 Specification:** none
- 1.06 Method name:** *Netherlands References and Metrics for Fish in Large Rivers*
- 1.07 Original name:** *Nederlandse Referenties en Maatlatten voor Vis in Grote Rivieren*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Hydromorphological degradation, Riparian habitat alteration  
River connectivity at water body scale (segment)

**Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

An assessment was made to relate metrics to pressures, but the number of large rivers and the limited range in pressure variation did not allow to derive statistical significant relationships. Subsequently, metrics were selected based on expert judgement that are considered to respond to pressures and impacts. For species richness there are 3 metrics: number of diadromous, rheophilic and limnophilic species; for abundance there are 2 metrics: relative abundance of rheophilic and limnophilic species; for age structure there is at present not yet a suitable metric.

- 1.10 Internet reference:** <http://www.stowa.nl>
- 1.11 Pertinent literature of mandatory character:**  
Jaarsma, N., M. Klinge & R. Pot (eds), 2007. Achtergronddocument Referenties en Maatlatten Vissen ten behoeve van de Kaderrichtlijn Water. STOWA, Utrecht. [Van der Molen, D.T. & R. Pot \(eds\), 2007. Referenties en maatlatten voor natuurlijk watertypen voor de Kaderrichtlijn Water. STOWA 2007-32; RWS-WD 2007-018.](#) [Winter, H.V., W. Dekker & J.J. de Leeuw, 2006. Optimalisatie MWTL vismonitoring. IMARES Rapport C052/06 in opdracht van RWS-RIZA.](#)
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Joep de Leeuw  
joep.deleeuw@fiskeriverket.se  
STOWA, RWS-RIZA (renamed to RWS-Waterdienst)
- 1.14 Method reported by**  
Tom Buijse  
tom.buijse@deltares.nl  
Deltares
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
Electrofishing by boat (mesh size 15 mm; one or two anode 0,5 m; (P)DC); width 1 m; length of sample: P10 = 300 m, P90 = 1790 m) [Beam trawl by boat \(mesh size 20 mm; width 3 m; length of haul: P10 = 958 m, P90 = 8560 m\)](#)
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Beam trawl, Electrofishing gear, Fyke net
- 2.05 Specification:** boat-mounted electrofishing (shoreline), 3-m beam trawl (mid-channel), by-catch recording in commercial fyke nets, salmon fyke nets to record upstream migration
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** March - May; September - November
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Such analysis has not been performed; per water body multiple electrofishing (6-26) and trawl samples (12-60) are taken.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Sampling effort not standardised per water body. For electrofishing total sampling effort per water body varies between 2400 and 9800 m; for trawling between 3,4 and 20,7 ha

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Generally fish over 50 mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.  
Sub-sampling is performed in case of large samples. Generally sum-sampling is done by volume (1/2, 1/4, 1/8 etc) or by counting (subsample is measured; the remainder is counted).
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** number per ha
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** Organism length
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Number of species of Rheophilic, Diadromous and Limnophilic species;  $\text{EQR} = \frac{[(\text{species score diadromy} + \text{rheophily} + \text{limnophily})/3 + (\text{abundance score rheophily} + \text{limnophily})/2]}{2}$
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** 0; there are no reference sites in the Netherlands.  
**Geographical coverage:** To date no reference sites available  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
No reference sites available in the Netherlands.
- 3.08 Reference community description**  
Based on expert judgement. Rivers with rheophilic species (17 - 21 species; 35 - 50% relative abundance), limnophilic species (6 species; 5 - 15% relative abundance) and diadromous species (8-12 species). The level of dominance varies per river type; there are three types of large rivers.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
Expert judgement. The method is considered to be sensitive to pressures (see A-13).
- 3.12 "Good status" community:** Based on expert judgement. Rivers with rheophilic species (15 -19 species; 25 - 40% relative abundance), limnophilic species (4 species; 3 - 10% relative abundance) and diadromous species (6-10 species). The level of dominance varies per river type; there are three types of large rivers.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**

---

none

ID: 125

FL-MA-LA

## 1. General information

- 1.01 GIG:** Central-Baltic  
RC1, RC4
- 1.02 Category:** Rivers
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish region
- 1.06 Method name:** *Flemish macrophyte assessment system*
- 1.07 Original name:** *Vlaams macrofytenbeoordelingssysteem*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Acidification, Eutrophication, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter, Riparian habitat alteration  
salinity change
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. September 2009. Vlaamse Milieumaatschappij, Erembodegem, Belgium.
- 1.12 Scientific literature:**  
n.a.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Anik Schneiders, Hans Jochems & Leo Vanhecke<br>luc.denys@inbo.be<br>Research Institute for Nature and Forest | <b>1.14 Method reported by</b><br>Wim Gabriels<br>w.gabriels@vmm.be<br>Flemish Environment Agency |
|--|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
A survey of a 100 m stretch is carried out. For the survey only the water vegetation is fully recorded; for the riparian vegetation, only the characteristic species are indicated. The water vegetation includes those plants that root in the water or the water bed. For the survey of the water vegetation the present species are listed for the entire stretch, along with their abundance according to a modified Tansley-scale. The survey is done, if possible, from the water. Wading is always done against the flow in order to avoid that the loosened sediment layer would impede the view on the vegetation. If the field conditions do not allow this, the survey is carried out from the bank. In this situation, the macrophytes are sampled by means of a rake, mounted on a telescopic pole. In this case, raking is done three times for each 10 m-strip. Additionally, submerged vegetation development is recorded if the stretch under consideration belongs to a river type for which this metric is used. This is done using a four-class cover scale.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Rake
- 2.05 Specification:** a rake is used for submerged vegetation if necessary
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** june-september
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
at least 1
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1 per site ( 3 sites per water body)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
100 m stretch

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** all macrophytes present in 100 m stretches except for mosses
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Other, Species/species groups  
species level except for filamentous algae: genus or unspecified
- 2.15 Record of abundance:** n.a.  
Modified Tansley scale for individual taxa (rare/occasional/frequent/low-abundant/abundant/co-dominant/dominant); presence/absence for growth forms; ECOFRAME-like scale for submerged plant abundance  
**in relation to** n.a.  
100 m-transect  
**Unit** Modified Tansley scale, presence/absence; ordinal
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** observed growth forms for specified taxa
- 2.18 Special cases, exceptions, additions:** Flemish river type 'Mlz' (tidal rivers) is not addressed using this method; for this type, see transitional waters method
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Type specificity (the abundance-weighted mean of all (predefined, species-specific) type specificity values of all present species); disturbance (the abundance-weighted mean of all (predefined, species-specific) disturbance values of all present species); growth forms (a type specific evaluation of number of present growth forms); submerged vegetation development (based on a four-class cover scale)
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Worst metric score
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
Reference conditions are characterised by high proportions of type-specific species, low proportions of species associated with disturbance, presence of most growth forms associated with the river type in question, and if applicable to the river type in question, also a high submerged vegetation development
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
EQR gradient is assumed to represent a continuous trend with general degradation.

**3.12 "Good status" community:** The EQR values at good status reflect metric values that are only slightly lower than at (expert-based) reference state, hence the community can be characterised as only slightly different from reference in terms of taxa richness, sensitivity and diversity.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 15

IBMR

## 1. General information

- 1.01 GIG:** Central-Baltic  
RC1, RC4, RC3, RC5 & RC6
- 1.02 Category:** Rivers
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Belgium (Wallonia)
- 1.05 Specification:** macrophytes are not relevant in heavily modified WB
- 1.06 Method name:** *Macrophyte Biological Index for Rivers*
- 1.07 Original name:** *Indice Biologique macrophytique en Rivière*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

There is a link between the IBMR score and the PO4-- concentration. See also :A new method to assess water trophy and organic pollution - The macrophyte Biological Index for rivers (IBMR) : its application to different types of river and pollution. Haury et al. Hydrobiologia (2006) 570 : 153-158

- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
AFNOR norm NF T 90-395.
- 1.12 Scientific literature:**  
Haury et al., 2006. A new method to assess water trophy and organic pollution. The macrophyte Biological Index for rivers (IBMR) : its application to different types of river and pollution. Hydrobiologia 570: 153-158.
- 1.13 Method developed by**  
Haury, J.  
  
Agrocampus Rennes - UMR INRA - F 35042 Rennes cedex  
(France)
- 1.14 Method reported by**  
Christine KEULEN  
Christine.keulen@spw.wallonie.be  
Service Public de Wallonie - DEMNA - 5030 Gembloux - Belgium
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
AFNOR norm NF T 90-395.
- 2.02 Short description**  
A survey of a 100 m stretch is carried out. This one includes shaded and brightened parts and lotic/lentic facies. For the survey only the water vegetation is fully recorded; The water vegetation includes those plants that root in the water or the water bed. The survey is done from the water. Wading is always done against the flow in order to avoid that the lost sediment layer would impede the view on the vegetation. Macrophytes are sampled and identified on the whole width of the stream. The % coverage of all the species is estimated on the survey. For more details see AFNOR norm NF T 90-395 or Haury et al. (2006) (see A22).
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.  
hand
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** may to september
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion / 3 years
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
width of the stream X 100 meters

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 2 mm

- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Percent coverage  
**in relation to** Area  
**Unit** % coverage
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** plant growth form
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
see AFNOR norm NF T 90-395 and annexes
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** whole Wallonia  
**Location of sites:** especially in the river basins : Meuse, Rhin & Seine  
**Data time period:** from 2005 to 2009 (may to september)  
**Criteria:**  
The reference sites were selected on basis of the lower anthropic pressures; the physico-chemical quality of water is also taken in account. The sites must be in high biological status for the specific indicator. The EQR = 1 for macrophytes on this site.
- 3.08 Reference community description**  
The reference community is relevant of the river type and includes sensitive species.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** The EQR values at good status reflect metric values that are only slightly lower than at (expert-based) reference state, hence the community can be characterised as only slightly different from reference in terms of taxa richness, sensitivity and diversity.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 156

KRW-maatlatten

## 1. General information

- 1.01 GIG:** Central-Baltic  
R-C1 and R-C4
- 1.02 Category:** Rivers
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Netherlands
- 1.05 Specification:** none
- 1.06 Method name:** *WFD-metrics for natural watertypes*
- 1.07 Original name:** *KRW-maatlatten voor natuurlijke watertypen*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, Flow modification, General degradation, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

- 1.10 Internet reference:** [http://themas.stowa.nl/thema/ecologische\\_beoordeling/krw-maatlatten.aspx?mid=7213&rid=817](http://themas.stowa.nl/thema/ecologische_beoordeling/krw-maatlatten.aspx?mid=7213&rid=817)

**1.11 Pertinent literature of mandatory character:**

Besluit Kwaliteitseisen en Monitoring Water, 2009. Ministry of Housing, Spatial Planning and the Environment (presently under public consultation).

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

development by national expert group commissioned by STOWA, Bas van der Wal & RWS Waterdienst, Diederik van der Molen  
b.van.der.wal@stowa.nl  
STOWA Foundation for Applied Water Management Research & Rijkswaterstaat Waterdienst

**1.14 Method reported by**

Roelf Pot  
roelfpot@wxs.nl  
Rijkswaterstaat Waterdienst

**1.15 Comments**

Description of KRWmaatlatten in Dutch.

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) Quality Handbook Hydrobiology (in prep). 2009. STOWA.

**2.02 Short description**

Cover estimation of all present species in (minimum 3) classes in whole area suitable for macrophyte growth, up to the mark of the highest yearly water level in 100 m length stretches; cover estimate of 5 growth forms in percentage in the same area (1 of the growth forms being filamentous algae, which are in fact phytobenthos); estimate of the percentage well developed riparian vegetation of the whole waterbody

- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Stratified sampling/surveying

- 2.04 Sampling/survey device:** Rake  
non-destructive survey

- 2.05 Specification:** visual recognition of species and estimate of cover; assisted by boat (large rivers); rake is additionally used

- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

- 2.08 Sampling/survey month(s):** june- august

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

one occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

per waterbody: 6 in small rivers, 20 in very large rivers

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

6 - 20 surveys of 100 meter river length each

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 1 cm

- 2.13 Sample treatment:** Organisms of the complete sample are identified.

- 2.14 Level of taxonomical identification:** Other, Species/species groups
- 2.15 Record of abundance:** Abundance classes, Percent coverage  
**in relation to** Area  
**Unit** Abundance class (related to percentage cover) for every species; Percentage cover for growth forms.
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
 Biological WFD monitoring is performed by 26 regional water boards. Small differences may occur in sampling strategies etc.

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
 1. Weighted number of characteristic species, weight 1-4 depending on species indication value, species abundance and species consistence over 6 - 20 sampled stretches. EQR = total score/expected score in reference. 2. Deviation of growth form cover from expected cover in reference in suitable area. EQR derived from class boundaries 3. final EQR = (EQR species + (mean of EQRs growth forms) + EQR fyto benthos species (diatoms) ) / 3
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
 Aggregated data from multiple spatial replicates

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
 Expert knowledge, Historical data, Least Disturbed Conditions  
 no actual existing natural sites in rivers; spatial references from foreign countries
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** Western and Central, temperate Europe  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
 All rivers in The Netherlands are (very) high hydromorphologically impacted and most of them are moderately to highly impacted by eutrophication, at least over >80% of the length. No rivers are assumed to meet the criteria of (almost) unimpacted.
- 3.08 Reference community description**  
 High status of small rivers is characterized by a high variety of species, growing at diverse habitats. Pressure tolerant species are present but only in low abundance and a few sites; total cover of vegetation is moderate or low and type-specific. Furthermore a general description is given (in Dutch) in: STOWA (2009) Referenties en maatlaten voor natuurlijke watertypen. report 2007-32
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
 The reference score for the sum of the scores of the species is derived from frequency data in the national vegetation database on well developed plant communities in The Netherlands (Schaminée et al.) , which is considered a good estimate for the probability of finding the species in a fixed amount of samples. The fraction of species at G/M and H/G are estimated with expert judgment and adjustment may be needed because of too low number of reference sites. Final adjustment of the reference scores are based on intercalibration results.
- 3.12 "Good status" community:** Good status of small rivers is characterized by a variety of species, growing at several habitats. Pressure tolerant species are present but only in low abundance; total cover of vegetation is moderate and type-specific.

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**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 220

MIR

## 1. General information

- 1.01 GIG:** Central-Baltic  
R-C4x2, R-C1x2
- 1.02 Category:** Rivers
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Poland
- 1.05 Specification:**
- 1.06 Method name:** *Macrophyte Index for Rivers*
- 1.07 Original name:** *Makrofitowa Metoda Oceny Rzek*
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
nutrient concentration, land use
- 1.10 Internet reference:** <http://www.au.poznan.pl/keios/html/MMOR.html>
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Pietruczuk, K. & K. Szoszkiewicz, 2008. Ramowa Dyrektywa Wodna w praktyce – Makrofitowa metoda oceny rzek w monitoringu wód płynących w Wielkopolsce. *Gospodarka wodna*, 10: 408-410. [Pietruczuk, K. & K. Szoszkiewicz, 2009. Ocena stanu ekologicznego rzek i jezior w Wielkopolsce w oparciu o makrofity zgodnie z wymogami Ramowej Dyrektywy Wodnej. \*Nauka Przyroda Technologie\* 3: 3-96. \[Szoszkiewicz, K., J. Zbierska, Sz. Jusik & T. Zgoła, 2008. Metoda oceny rzek oparta na makrofitach realizowana w Polsce na potrzeby Ramowej Dyrektywy Wodnej. \\*Wiad. Mel. i Łąk.\\* 4 \\(419\\): 163-165.\]\(#\)](#)
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Krzysztof Szoszkiewicz<br>kszoszk@up.poznan.pl<br>Department of Ecology and Environmental Protection, Poznan<br>University of Life Sciences | <b>1.14 Method reported by</b><br>Krzysztof Szoszkiewicz<br>kszoszk@up.poznan.pl<br>Department of Ecology and Environmental Protection, Poznan<br>University of Life Sciences |
|--|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
n.a.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Grapnel, Rake
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** July-mid September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Generally one occasion per sampling season. Second occasion required to catch different vegetation form.
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
one
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
100 meters of the river section

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:**
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Genus level is limited to algae
- 2.15 Record of abundance:** Abundance classes

in relation to Area

Unit area covered by species

2.16 Quantification of biomass: n.a.

2.17 Other biological data: none

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

3.01 List of biological metrics

Macrophyte Index for Rivers

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: n.a.

3.04 From which biological data are the metrics calculated?

Data from single spatial replicate

#### Reference conditions

3.05 Scope of reference conditions: Surface water type-specific

3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites

3.07 Reference site characterisation

Number of sites: 40

Geographical coverage: Whole country

Location of sites: National parks, nature reserves, landscape parks, near natural sites out of protected areas

Data time period: 2002-2009

Criteria:

The absence of pressures near the sites and in the whole catchment, protected areas, lack or very limited/sustainable agriculture, very high hydromorphological quality

3.08 Reference community description

n.a.

3.09 Results expressed as EQR?

No

MIR based assessment is indirect EQR classification

#### Boundary setting

3.10 Setting of ecological status boundaries: Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient

3.11 Boundary setting procedure

MIR index was calibrated against nutrient content of rivers. Indicative scores of macrophyte species were calculated analyzing against nutrient gradient.

3.12 "Good status" community: At good status stands indicators of high status play an important role in the area-cover overgrown by vegetation.

#### Uncertainty

3.13 Consideration of uncertainty: Yes

Multi sampling campaign, visiting sites by various surveyors, comparing hydromorphologically impacted and unimpacted sites, shaded and unshaded, testing different section length

3.14 Comments:

none

ID: 19

LEAFPACS

## 1. General information

- 1.01 GIG:** Central-Baltic  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Macrophytes
- 1.04 Country:** United Kingdom
- 1.05 Specification:** none
- 1.06 Method name:** *Ecological Classification of Rivers using Macrophytes*
- 1.07 Original name:** *Ecological Classification of Rivers using Macrophytes*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, Hydromorphological degradation

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

The relationship of all metrics with pressure (SRP, total oxidised nitrogen [TON], NH<sub>4</sub>, %intensive land cover) were investigated using 6600 macrophyte surveys of almost 1000 UK rivers. The nutrient metric (RMNI) was the most significantly related to nutrient pressure (Correlation of RMNI to SRP r-squared = 47.5; correlation to TON r-squared = 58.2); diversity metrics were only significantly related to nutrients in high alkalinity rivers, but were related to other BQEs (Invertebrates p<0.001). Filamentous algal cover was significantly related to nutrient pressure (p<0.05)

- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/river%20macrophytes%20nw](http://www.wfduk.org/bio_assessment/bio_assessment/river%20macrophytes%20nw)
- 1.11 Pertinent literature of mandatory character:**  
Water Framework Directive- United Kingdom Technical, 2009. UKTAG river assessment methods macrophyte and phytobenthos macrophytes river leafpacs. Version 2. [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/river%20macrophytes%20nw](http://www.wfduk.org/bio_assessment/bio_assessment/river%20macrophytes%20nw) Willby, N.J., J. Pitt & G.L. Phillips, 2010. The ecological classification of UK rivers using aquatic macrophytes. Environment Agency Science Report.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Nigel Willby  
n.j.willby@stir.ac.uk  
  
University of Stirling
- 1.14 Method reported by**  
Damien Hicks, Imelda O'Neill, Geoff Phillips  
Damien.Hicks@sepa.org.uk, imelda.oneill@doeni.gov.uk,  
Geoff.phillips@environment-agency.gov.uk  
Scottish Environment Protection Agency, Northern Ireland  
Environment Agency, Environment Agency(England & Wales)
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Willby, N.J., Pitt, J & G.L. Phillips, 2010. The ecological classification of UK rivers using aquatic macrophyte. Environment Agency Science Report.
- 2.02 Short description**  
At the 100 m stretch surveyors record presence and abundance of macrophytes in permanently submerged parts of the channel or within the saturated zone at the margins or the lower part of the inundation zone.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Grapnel  
bathyscope
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** June – September inclusive
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Dependent on confidence required: recommended 1 year of the 6-year RBMP reporting period
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Dependent on confidence required: recommended 3 sites per waterbody. 2 sites per water body can achieve 95% confidence of being worse than Good if the class is at the middle point of moderate. For the 1st RBP 1-3 sites per water body have been used in dif
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Surveyed area is 100m. Between one and three 100m stretches are surveyed and mean EQR for each 100m stretch is determined for river waterbody

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Macroalgal filaments
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Most macroalgae to genus level only
- 2.15 Record of abundance:** Abundance classes  
**in relation to** n.a.  
cover  
**Unit** Rank 1-9 Species Cover Value: C1 <0.1% C2 0.1 to 1% C3 1 to 2.5% C4 2.5 to 5% C5 5 to 10% C6 10 to 25% C7 25 to 50% C8 50 to 75% C9 >75%
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** Non-wadable rivers are surveyed by 5m wide sections on each side of the river
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
River Macrophyte Nutrient Index; River Macrophyte Hydraulic Index, Functional Group Diversity, Number of Taxa, Filamentous Algal Cover
- 3.02 Does the metric selection differ between types of water bodies?** n.a.
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores, Worst metric score  
Worst diversity metric, worst nutrient and hydraulic index are combined with each other and algal metric using weighted average depending on location of water body on natural fertility gradient
- 3.04 From which biological data are the metrics calculated?**  
Data from single spatial replicate

### **Reference conditions**

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Modelling (extrapolating model results)  
Cover of highly sensitive and sensitive taxa. Taxa defined using modelled (CCA) relationships with pressure, subsequently verified and adjusted by comparing regression of metric scores of these indicative taxa groups against a morpho edaphic index to ensure that UK data used to select groups were drawn from a full gradient of pressure.
- 3.07 Reference site characterisation**  
**Number of sites:** C400 surveys (mixture of historic and contemporary surveys)  
**Geographical coverage:** Surveys from whole of UK (England, Wales, Scotland & Northern Ireland)  
**Location of sites:** Available on request  
**Data time period:** Surveys selected from data set covering 1976-2003  
**Criteria:**  
Sites selected by iterative application of biological and physicochemical criteria. <15% total cover of pressure tolerant taxa, highly pressure sensitive species present, cover of highly tolerant species <10% total cover, number of aquatic taxa and functional groups > 25th percentile of type specific richness, total hydrophyte cover & mean cover score per species within type and method specific 10-90th percentile range. No individual taxa with cover score > 6 (10-25%), no established invasive alien or translocated species, dominant acid tolerant taxa <50% cover, filamentous green algae < 2.5% absolute cover and <20% relative cover. Mean annual concentration of N-NH<sub>4</sub> < 0.05-0.1mg/l, SRP < 20-40 µ, N-NO<sub>3</sub>/l <2-4 mg/l depending on type, River Habitat Survey Class 1 or 2, No resectioning of reaches, flows within 10% of naturalised flow, impacted land cover <20% of catchment area.
- 3.08 Reference community description**  
Macrophyte community dominated by highly sensitive taxa, tolerant taxa are strongly subordinate and highly tolerant taxa occur only as transients and are never established. Typical macrophyte mediated functions (habitat support, bed and bank stabilisation, biogeochemical cycling, aesthetics) are intact.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites Using paired metrics that respond in different ways to the influence of the pressure Approach to boundary setting set out in Phillips et al 2003. The assessment of ecological quality of lakes in the Great Britain Ecoregion: an update on thinking and a possible approach for phytoplankton. TeemaNord 2003, 547, 35-39.

#### **3.11 Boundary setting procedure**

The relative positions of High-Good and Poor-Bad boundaries are effectively symmetrical with sensitive species overwhelmingly dominant at one and tolerant species overwhelmingly dominant at the other. Using the same standard error from logistic regressions, a ratio of sensitive:tolerant species of 85:15 is used as the High-Good boundary, since this represents the upper error when tolerant species are predicted to be absent. These ratios are reversed at the Poor-Bad boundary with 15% sensitive species representing the lower error when sensitive species are predicted to be absent.

**3.12 "Good status" community:** Sensitive taxa dominate, highly sensitive taxa are scarcer and account for about half the contribution of sensitive taxa. Tolerant taxa are present, but remain subordinate. Highly tolerant taxa, if present are rare. Macrophyte functions at high status all remain intact, undesirable disturbances are rare and macrophyte cover is stable over time.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

If the modelled relationship between observed mean EQR and EQR SD, taking into account sampling, temporal and spatial sources of variation is accepted as the best available estimate of the error associated with a given EQR. We can combine this with information on class boundaries and therefore predict the confidence with which a site can be assigned to a given class. This approach effectively assumes that the errors associated with a given EQR are normally distributed about that mean with a distribution equivalent to the modelled EQR SD. Given this information one can assess the impact of different survey frequencies on confidence of class. The procedure for calculating confidence of class is outlined by Ellis (2006). The risk of face-value misclassification (i.e. of assigning a site to the wrong class) is then computed as the sum of confidences of membership of all classes except for the observed class. The risk of misclassification will always be at least 50% for an EQR that lies exactly on a class boundary but will fall to a minimum moving towards the middle of that class. It should be noted that this approach differs slightly from that trialled previously using the STARBUGS software (Clarke, 2005). In STARBUGS the EQR SD is considered constant and confidence of class is based on the result of multiple simulations in which a random error derived from the distribution defined by the SD is added to each observed EQR. The probability that a site belongs to a specific class is based on the statistical distribution of these simulated values.

#### **3.14 Comments:**

Further information on variability can be found in Environment Agency report SC070051/SR4 Davey & Garrow (2010) Variability components for macrophyte communities in rivers.

ID: 64

PhytoFluss

## 1. General information

- 1.01 GIG:** Central-Baltic  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish region
- 1.06 Method name:** *German phytoplankton assessment method for rivers*
- 1.07 Original name:** *Gesamtindex Phytoplankton PhytoFluss*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. Vlaamse Milieumaatschappij, Erembodegem, Belgium.
- 1.12 Scientific literature:**  
n.a.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Jeroen Van Wichelen<br>jeroen.vanwichelen@UGent.be<br>Ghent University | <b>1.14 Method reported by</b><br>Jeroen Van Wichelen<br>jeroen.vanwichelen@UGent.be<br>Ghent University |
|---|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
Water, ideally from the middle of the stream, is collected in a large container using a large plastic bucket and a rope. After the sample is taken, subsamples are taken from the large container for microscopic and pigment analysis. The water should be thoroughly stirred in advance in order to homogenize floating organisms.
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** Surface water sample taken with a bucket
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Surface water
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** April-september
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
at least one occasion per month during the growing season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3 to 5
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Total volume sampled (prior to subsampling) is (bucket volume) x (3-5 samples per occasion) x (6 months) x (number of monthly samples; at least one)

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** All cells in the sample, including picocyanobacteria
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Subsamples are taken from a thoroughly homogenised sample
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
To the species level where possible, otherwise genus
- 2.15 Record of abundance:** n.a.

- counts of individuals or, where applicable, colonies
- in relation to** Volume
- Unit** biomass per volume
- 2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** Flemish river type 'Mlz' (tidal rivers) is not addressed using this method; for this type, see transitional waters method
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Biomass (chlorophyll a); relative proportion of pennate diatoms; relative proportion of green algae; relative proportion of cyanobacteria; Typspezifischen Indexwertes Potamoplankton
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
Reference conditions are characterised by a relatively low biomass per volume, a relatively even distribution of proportions of different phytoplankton groups such as diatoms and green algae, and the absence of cyanobacterial blooms
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
Expert judgement
- 3.11 Boundary setting procedure**  
EQR gradient is assumed to represent a continuous trend with general degradation.
- 3.12 "Good status" community:** The EQR values at good status are characterised by metric values that are only slightly lower than at (expert-based) reference state, hence a slightly increased biomass per volume, a slightly disturbed distribution of proportions of different phytoplankton groups such as diatoms and green algae, and a slight increase of cyanobacteria are possible.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 229

PhytoFluss

## 1. General information

**1.01 GIG:** Central-Baltic

**1.02 Category:** Rivers

**1.03 BQE:** Phytoplankton

**1.04 Country:** Germany

**1.05 Specification:**

**1.06 Method name:** *Index Phytoplankton PhytoFluss*

**1.07 Original name:** *Gesamtindex Phytoplankton PhytoFluss*

**1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)

**1.09 Detected pressure(s):** Eutrophication, Hydromorphological degradation

*Has the pressure-impact-relationship been tested?*

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

mischke@igb-berlin.de

**1.14 Method reported by**

Ute Mischke

mischke@igb-berlin.de

Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB Berlin)

**1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Mischke, U. & H. Behrendt, 2007. Handbuch zum Praxistest eines Bewertungsverfahrens von Fließgewässern mittels Phytoplankton zur Umsetzung der EU-Wasserrahmenrichtlinie in Deutschland. Press: Weißensee Verlag, Berlin: 1-88.

**2.02 Short description**

Sampling of at least 2 litre of river water by water samplers (not specified) preferable from bridges in the mid of the river flow. The sampling is carried out monthly between April and October. The samples must be preserved with Lugol solution at once when sampled and stored in glass bottles.

**2.03 Method to select the sampling/survey site or area:** n.a.

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** Single habitat(s)

0.5 m water depths in the middle of the river bed

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** April to October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

6 survey occasions

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

n.a.

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 2 liters

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

Phytoplankton counts are made according to the Utermöhl-technique with inverse microscopes (see. DIN EN 15204 (2006) and at least 20 taxa and 400 objects and two magnifications, and with cell biovolume estimations (see handbook).

**2.14 Level of taxonomical identification:** n.a.

The level of required taxa determination for 1600 phytoplankton taxa is described in a special column of the German taxa list for phytoplankton available as a download file in <http://www.igb-berlin.de/abt2/mitarbeiter/mischke> and in printed version as a table annex of the handbook of the method (Mischke & Behrendt 2007)

**2.15 Record of abundance:** Individual counts

in relation to Volume

Unit taxa biovolume in liter

**2.16 Quantification of biomass:** Utermöhl technique**2.17 Other biological data:** none**2.18 Special cases, exceptions, additions:** none**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Biomass metric: chlorophyll\_a (uncorrected) concentration in seasonal mean  
Composition metrics: Relative abundance of Pennales, Chlorophyceae and / or Cyanobacteria and of indicator taxa.  
Pennales Index, Chloro-Index, Cyano-Index, TIP (Typspecific Index Potamoplankton)

**3.02 Does the metric selection differ between types of water bodies?** Yes**3.03 Combination rule for multi-metrics:** Average metric scores**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific**3.06 Key source(s) to derive reference conditions:**

Least Disturbed Conditions, Modelling (extrapolating model results)

**3.07 Reference site characterisation****Number of sites:** 5**Geographical coverage:** lowland streams with low run-off are not covered, modelling necessary**Location of sites:** Donau near Böfingen (10.1); Rhine near Kleve-Bimmen (20.1), Schwarzer Schöps, Sprey (15.1); Spree, Niedergurig (17.1); Maurine below Schönberg (type 23)**Data time period:****Criteria:**

For reference sites only limiting factor total phosphor (TP) was checked. Additionally the trophic (pelagic) status was proven by the new defined rule-out criteria for TP and chlorophyll a both in seasonal mean from April - October:  
Criteria: TP concentration below 50 - 54 µg/l in all relevant river types (9.2, 15, 17, 10, 20, 23)  
A) Chl\_a concentration below 10.1 µg/l in streams with high run-off (national type 10.1, 20.1 Rhine, Danube)  
B) Chl\_a concentration below 20.0 µg/l in middle sized rivers (national type 9.2, 15, 17)  
C) Chl\_a concentration below 30.0 µg/l in streams with low run-off (national type 10.2, 20.2, 23 Elbe, Odra)

**3.08 Reference community description**

In reference status with mean TP concentrations below 50 – 54 µg/l, the phytoplankton composition and biomass is assumed to respond river type specific:  
Type 9.2 (large low mountain rivers): In phytoplankton community Pennales contributes more than 30% of total biovolume (Diatoma vulgaris, Navicula, Surirella), because of high slope and flow velocity. Cyanobacteria are less than 10%. Monoraphidium contortum and species of Scenedesmus are common within species rich Chlorophytes. Phytoplankton biomass reflects the mesotrophic status (Donau bei Böfingen) in potamal, so seasonal mean of chlorophyll a-concentration remains below 20 µg/l.  
Types 10.1 and 20.1 (large rivers and streams with high run-off > 10l/s/km<sup>2</sup>): In phytoplankton community Pennales contributes more than 25% of total biovolume (Suriella; Fragilaria ulna var. acus; Diatoma vulgaris; Cocconeis placentula, Fragilaria crotonensis). Cyanobacteria are not relevant, but small Chrysophytes and Haptophytes can contribute significant to the total biomass. Oligotrophic status in potamal, so seasonal mean of chlorophyll a-concentration remains below 10 µg/l.  
Type 10.2 and 20.2 (large rivers and streams with low run-off (< 10l/s/km<sup>2</sup>)): Chlorophytes contributes less than 5% of total biovolume, but increase with eutrophication. Cyanobacteria are rare. Species richness is very high, but plankton is dominated strongly by the group of centric large diatoms. Indicator species are Amphora, Fragilaria, small Chrysophytes as Kephyrion und Pseudokephyrion and dinoflagellates. Eutrophic status in potamal, so seasonal mean of chlorophyll a-concentration remains below 30 µg/l.  
Type 15.2 and 17.2 (lowland rivers 5,000 - 10,000 km<sup>2</sup> catchment): Pennales contributes more than 25% of total biovolume. Portion of Cyanobacteria increase with eutrophication and is below 20% in reference. Indicator species (28 taxa) are Pennales as Cocconeis, Diatoma, Fragilaria crotonensis, Navicula, Nitzschia, Gomphonema, Rhoicosphenia, Surirella and some flagellates as Chlamydomonas, Ceratium, Chrysophytes, Chrysochromulina and Gymnodinium. Mesotrophic status in potamal, so seasonal mean of chlorophyll a-concentration remains below 20 µg/l.  
Type 15.1 and 17.1 (lowland rivers 1000 - 5000 km<sup>2</sup> catchment): Pennales contributes more than 20% of total biovolume. Portion of Cyanobacteria increase with eutrophication and is below 10% in reference. Indicator species (28 taxa) are Amphora, Asterionella, Diatoma, Fragilaria

ulna – Sippen, *F. crotonensis*, *Navicula lanceolata*, *Nitzschia acicularis*- Formenkreis, *Stenopterobia*, *Surirella*, *Scenedesmus*, *Euglena*, *Chlamydomonas*, *Cryptomonas* and limnoplankton as *Ceratium* und *Gymnodinium*. Mesotrophic status in potamal, so seasonal mean of chlorophyll a-concentration remains below 20µg/l. Type 23 (rivers to Baltic Sea, all with extreme low slope and sporadically tidal influence): Chlorophytes contributes less than 5% of total biovolume, but increase with eutrophication. Cyanobacteria are rare (<0.001 mm<sup>3</sup>/l). Plankton is dominated strongly by the group of diatoms with more than 20% Pennales. Indicator species are *Nitzschia sigmoidea*, *Amphora*, *Fragilaria* and small Chrysophytes. Eutrophic status in potamal, so seasonal mean of chlorophyll a-concentration remains below 30µg/l.

**3.09 Results expressed as EQR?** No Equidistant score from 0.5-1.5 (high) to 4.5 - 5.5 (bad)

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.

#### **3.11 Boundary setting procedure**

No or few reference sites. Background limiting factor TP was reconstructed by modelling (MONERIS) for all types with a value of smaller than 50µg/l. HG boundary: On the base of background TP, the maximal potential of the phytoplankton biomass was reconstructed for each relevant river types by exponential regression line from existing data. PB boundary: Point with no further effect of pressure increase (about 250µg/l TP). GM and MP boundaries by equidistant division of range between HG and PB boundary. GM boundary was checked and corrected by three methods: 1) Comparison with values in the few existing near natural sites (the 90percentile) 2) Identification of discontinuity in chl<sub>a</sub> / TP ratio (GM boundary was set before a sudden increase of chl<sub>a</sub> can be observed in a high portion (50%) of the cases in some river types. 3) Habitat reconstruction including grazing pressure (macrozoobenthos) and habitat structure (shading, charge range, flow velocity, mean water retention time etc.) and checking by historical data (see Rhine (historical museum filters from 1870 with diatom valves, Havel by paleolimnological studies etc).

**3.12 "Good status" community:** see ranges of index values in handbook. Above 0.090 mg/l total phosphor (pressure) a sudden increase of total biomass of phytoplankton (chlorophyll a & phaeophytin) can be observed in a relevant portion of cases (maximal potential) for the trophic sensitive national river types 9.2, 15.2, 17.2, 10.2, 20.2 und 23. The river types with high run-off (10.1 / 20.1) or with small catchment areas (15.1 / 17.1 with 1000 - 5000km<sup>2</sup>) are less sensitive for pressure, so TP concentrations less than 0.135mg/l allow good status for phytoplankton.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

#### **3.14 Comments:**

Uncertainty is very high because further factors can limit growth and biomass of phytoplankton. In such cases with unsuitable conditions for phytoplankton (high run-off; frequent change in flow conditions; inorganic turbidity) the trophic status can be presented better by phytobenthos or macrophytes in river sections.

ID: 245

CZ1

## 1. General information

- 1.01 GIG:** Central-Baltic, Eastern Continental
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Czech Republic
- 1.05 Specification:**
- 1.06 Method name:** *Czech national method of the river ecological status classification according to the fish biocoenosis*
- 1.07 Original name:** *Ceský index hodnocení ekologické kvality toku pomocí rybích společenstev*
- 1.08 Status: Method is/will be used in** neither first nor second RBMP
- 1.09 Detected pressure(s):** General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic compounds (e.g. DDT, PCB)

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

CZI value decreases with increasing number of obstacles on the particular river and with decreasing relative distance between two consecutive obstacles in relation to river length ( $F1, 108 = 4.72; P < 0.0321$ ). In other words, the more obstacles and the shorter distance between them the more degraded fish assemblage is present

- 1.10 Internet reference:** [http://circa.europa.eu/Members/irc/jrc/jrc\\_eewai/library?l=/intercalibration\\_1/newupdated\\_national/national\\_methodpdf/\\_EN\\_1.0\\_](http://circa.europa.eu/Members/irc/jrc/jrc_eewai/library?l=/intercalibration_1/newupdated_national/national_methodpdf/_EN_1.0_)

**1.11 Pertinent literature of mandatory character:**

Jurajda, P., O. Slavík & Z. Adámek, 2006. Metodika odlovu a zpracování vzorku pludkových společenstev tekoucích vod. CSN EN 14011-757706 Jakost vod. Odber vzorku pomocí elektrického proudu. [In Czech] Horký, P. et al., 2009. Czech national method of the river ecological status classification according to the fish biocoenosis. Report for the Ministry of Environment of the Czech Republic, Prague.

**1.12 Scientific literature:**

**1.13 Method developed by**

Pavel Horký  
pavel\_horky@vuv.cz  
T.G.Masaryk Water Research Institute, Prague

**1.14 Method reported by**

Pavel Horký  
pavel\_horky@vuv.cz  
T.G.Masaryk Water Research Institute, Prague

**1.15 Comments**

Comparative study "YOUNG-OF-THE-YEAR (YOY) ASSEMBLAGE SAMPLING AS A TOOL FOR ASSESSING THE ECOLOGICAL QUALITY OF RUNNING WATERS" submitted to Journal of Applied Ichthyology

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Jurajda, P., O. Slavík & Z. Adámek, 2006. Metodika odlovu a zpracování vzorku pludkových společenstev tekoucích vod. CSN EN 14011-757706 Jakost vod. Odber vzorku pomocí elektrického proudu. [In Czech]

**2.02 Short description**

Partial sampling procedure is applied, covering all types of habitats to obtain a representative sample of the site. Sampling area borders are determined with help of the portable GPS receiver. All sampling occasions are undertaken during late summer, to assure efficiency of YOY sampling (Copp, 1989). Electrofishing of YOY is conducted by wading the bank in an upstream direction, regardless of the river size (electroshocker maximum output 225 - 300 V, 6 A, pulsed D.C.). Although point abundance and continuous sampling of YOY are comparable in terms of qualitative analyses, continuous sampling is preferred in order to allow quantitative interpretation of results (Janáč & Jurajda, 2007). Most fish are identified to species and immediately released at the site of capture. Specimens that could not be reliably identified are fixed in 4% formaldehyde solution for laboratory identification.

- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Stratified sampling/surveying

- 2.04 Sampling/survey device:** Electrofishing gear

- 2.05 Specification:** Efko or Bednár electrofishing units tuned for sampling of YOY

- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:**

- 2.08 Sampling/survey month(s):** Late summer (preferably August)

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

At least one sampling occasion per microhabitat present is required (depending on the structure of the site - uniform vs. heterogenous sites).

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** cca. 0,5 cm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** n.a.  
overall abundance and also relative abundance of particular fish ecological guild  
**in relation to** n.a.  
meters of shoreline sampled  
**Unit** number of individuals per one meter of shoreline
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:**
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**

The Czech method is focused on the young-of-the-year (YOY) sampling. Separate study was performed in order to verify its applicability for the ecological quality assessment. YOY sampling was validated as a tool that is comparable with sampling of adults. Furthermore, it was suggested as a useful method for assessing river ecological quality with the ability to provide a sensitive response to several pressures regardless of the effect of stocking or river size. Functional river typology and multimetric index were also developed, suggesting the Czech national method as a relevant tool according to the Water Framework Directive requirements. Method description including full results of statistical analyses is downloadable from the above mentioned link (Circa database) or could be sent via e-mail if needed.

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
presence of typical species; overall abundance; relative abundance of rheophilic species; relative abundance of eurytopic species
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** 82  
**Geographical coverage:** 28 river types representing 84.26 % of the overall area of the Czech Republic.  
**Location of sites:** All available sites from the national monitoring programme, covering almost the whole area of the Czech Republic.  
**Data time period:** 2006-2008 (late summer period)  
**Criteria:**  
Several hydromorphological and chemical variables were measured in order to define no or low level of disturbance (sediment, geomorphology, impoundment, lateral obstacles, channelization, riparian vegetation, toxicity, nutrients, standard chemical water quality).
- 3.08 Reference community description**  
Reference community depends on the river type (type-specific ref. comm. criteria). Generally is the reference community represented by the high abundance of rheophilic species (e.g. *Salmo trutta m. fario*, *Barbus barbus*...) and presence of type-specific species sensitive to disturbances (e.g. *Cottus gobio*).
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure**  
HG boundary - assemblage is clearly dominated by intolerant species (rheophilic group), however some type-specific species

sensitive to disturbances could be missing or less abundant  
GM boundary - abundance of rheophyllic species is decreasing and abundance of eurytopic species is increasing, however sensitive species are still present (cca. 50 - 60% of the whole assemblage)  
MP boundary - the situation change and tolerant eurytopic species become dominant; however cca. 30% of sensitive species is still present  
PB boundary - almost all type-specific species sensitive to disturbances are extinct and the assemblage is clearly dominated by tolerant eurytopic species

**3.12 "Good status" community:** High abundance of rheophyllic species (e.g. *Salmo trutta m. fario*, *Barbus barbus*...) and presence of type-specific species sensitive to disturbances (e.g. *Cottus gobio*). Abundance of rheophyllic species is decreasing and abundance of eurytopic species is increasing at moderate and lower classes.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

ID: 69

AIM Rivers

## 1. General information

- 1.01 GIG:** Central-Baltic, Eastern Continental  
R-C3
- 1.02 Category:** Rivers
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Austria
- 1.05 Specification:** none
- 1.06 Method name:** *Austrian Index Macrophytes for Rivers*
- 1.07 Original name:** *Austrian Index Macrophytes for Rivers*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Riparian habitat alteration  
Main focus is eutrophication and flow modification (and degree of bank fixation)
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Ecological data from 466 river sites were examined to establish pressure-impact relationship between macrophyte metric and eutrophication gradient, flow velocity, degree of bank fixation. The relationship between macrophyte metrics and the mentioned pressures showed type-specific significant correlations.
- 1.10 Internet reference:** <http://wasser.lebensministerium.at/article/archive/5659/0> "Leitfaden für die Erhebung der biologischen Qualitätselemente"
- 1.11 Pertinent literature of mandatory character:**  
Pall, K. & V. Mayerhofer, 2008. Leitfaden zur Erhebung biologischer Qualitätselemente, Teil A4- Makrophyten. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft (Hrsg.), 60pp.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Karin Pall  
karin.pall@systema.at  
systema GmbH, Vienna, Austria
- 1.14 Method reported by**  
Karin Pall  
karin.pall@systema.at  
systema GmbH, Vienna, Austria
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
The whole vegetated area of the regarded river stretch has to be investigated by wading or with the help of a boat. The abundance of all occurring species is to be estimated according to a five level scale.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Rake  
Wading trousers, rubber boat,
- 2.05 Specification:** telescopic rake (4,3m), size of boat and kind of drive mechanism depend on river size, aquascope cressi sub
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** May to September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One survey per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
n.a.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
river stretch of 100 to 500 m, depending on species richness

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** all visible plants of the regarded plant groups (charophytes, mosses, ferns, spermatophytes)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.

- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Abundance classes  
**in relation to Area**  
**Unit** 1=very rare, 2=rare, 3=common, 4=abundant, 5=very abundant, in masses
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Weighted average of species indicator taxa
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** 20  
**Geographical coverage:** Alpine region, peri-alpine region  
**Location of sites:** Alpine region, perialpine region  
**Data time period:** 1999-2007  
**Criteria:**  
CB GIG Criteria
- 3.08 Reference community description**  
type-specific
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
The class boundaries for each metric were defined according to the normative definitions and interpretations of the WFD as given in the REFCOND Guidance.
- 3.12 "Good status" community:** Type specific!

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 248

ICMi

## 1. General information

**1.01 GIG:** Central-Baltic, Mediterranean  
Mediterranean GIG II phase

**1.02 Category:** Rivers

**1.03 BQE:** Benthic Diatoms

**1.04 Country:** Italy

**1.05 Specification:**

**1.06 Method name:** *Intercalibration Common Metrics Index*

**1.07 Original name:** *Intercalibration Common Metrics Index-Italy*

**1.08 Status: Method is/will be used in** Second RBMP (2015)

**1.09 Detected pressure(s):** Eutrophication

General degradation, Eutrophication, Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

160 ecological data from diatom communities of Italian rivers were studied in relationship between metric and eutrophication gradient, with significance correlation.

**1.10 Internet reference:** [www.iss.it/binary/publ/cont](http://www.iss.it/binary/publ/cont)

**1.11 Pertinent literature of mandatory character:**

Ministerial Decree in final stage approval

**1.12 Scientific literature:**

The assessment method of the ecological status of running waters: diatom communities. Edited by Laura Mancini and Caterina Sollazzo 2009, 32 p. Rapporti ISTISAN 09/19

**1.13 Method developed by**

**1.14 Method reported by**

Laura Mancini

[laura.mancini@iss.it](mailto:laura.mancini@iss.it)

Italian National Institute of Health

**1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Standard methods: UNI EN 13946:2005; UNI EN 14407:2004:

**2.02 Short description**

Avoid heavy shade, collect diatoms from cobbles and boulders with a brush for a total area of 100 cm<sup>2</sup>.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Brush

**2.05 Specification:**

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)  
cobbles and boulders

**2.07 Sampled/surveyed zones in areas with tidal influence:**

**2.08 Sampling/survey month(s):** March to June

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Cobbles and boulders, total sampled area must be at least 100cm<sup>2</sup>

### Sample processing

**2.12 Minimum size of organisms sampled and processed:**

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

individual counts up to 400

in relation to Area

Unit

**2.16 Quantification of biomass:** n.a.

2.17 Other biological data:

2.18 Special cases, exceptions, additions: In non wadable river, sampling should be performed in the euphotic zone

2.19 Comments

### 3. Data evaluation

#### Evaluation

3.01 List of biological metrics

indice de polluosensibilite specifique IPS (Coste,1982) , Trophic Index TI (Rott,1999)

3.02 Does the metric selection differ between types of water bodies?

3.03 Combination rule for multi-metrics: Average metric scores

3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

#### Reference conditions

3.05 Scope of reference conditions: Surface water type-specific

3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Expert knowledge

3.07 Reference site characterisation

Number of sites: 3 sites for each river type

Geographical coverage: ?

Location of sites: National Protected areas

Data time period: on average 2 years

Criteria:

Very low impacts

3.08 Reference community description

?

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: Boundaries taken over from the intercalibration exercise

3.11 Boundary setting procedure

n.a.

3.12 "Good status" community: At the good status, sensitive taxa, such as Achnanidium minutissimum, Cymbella prostrata, Achnanidium biasolteanum, discrease and tolerant species grow up

#### Uncertainty

3.13 Consideration of uncertainty: No (to be done)

3.14 Comments:

ID: 154

IBMR

## 1. General information

- 1.01 GIG:** Central-Baltic, Mediterranean  
IC in progress for CB (R-C3, R-C4). IC in starting stage for Med (R-M1,R-M4)
- 1.02 Category:** Rivers
- 1.03 BQE:** Macrophytes
- 1.04 Country:** France
- 1.05 Specification:** none
- 1.06 Method name:** *Biological Macrophytes Index for Rivers*
- 1.07 Original name:** *Indice Biologique Macrophytes en Rivière*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Hydromorphological degradation

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

- 1.10 Internet reference:** <https://hydrobio-dce.cemagref.fr/> - for all national used methods

**1.11 Pertinent literature of mandatory character:**

AFNOR, 2003. Qualité de l'eau. Détermination de l'indice biologique macrophytes en rivière (IBMR). NF T90-395, octobre 2003, 10 p. Chauvir C., M.-C. Peltre & J. Haury, NN. L'Indice biologique macrophytique en rivière. Guide méthodologique. AFNOR éd. 110 p. in press. République Française. Ministère de l'écologie et du développement durable - Direction de l'eau. 13 juillet 2006. Circulaire DCE 2006/16 relative à la constitution et la mise en oeuvre du programme de surveillance (contrôle de surveillance, contrôles opérationnels, contrôles d'enquête et contrôles additionnels) pour les eaux douces de surface (cours d'eau, canaux et plans d'eau) en application de la directive 2000/60/CE du 23 octobre 2000 du Parlement et du Conseil établissant un cadre pour une politique communautaire dans le domaine de l'eau.

**1.12 Scientific literature:**

Haury, J., M.-C. Peltre, M. Termolieres, J. Barbe, G. Thiebaut, I. Bernez, H. Daniel, P. Chatenet, G. Haan-Archipof, S. Muller, A. Dutartre, C. Laplace-Treytore, A. Cazaubon & E. Lambert-Servien, 2006. A new method to assess water trophy and organic pollution: the Macrophyte Biological Index for Rivers (IBMR) : its application to different types of river and pollution. Hydrobiologia 570: 153-158.

**1.13 Method developed by**

original IBMR method : Jacques HAURY. National WFD  
assessment method development : Christian Chauvin  
christian.chauvin@cemagref.fr  
CEMAGREF groupement Bordeaux

**1.14 Method reported by**

Christian CHAUVIN  
christian.chauvin@cemagref.fr  
CEMAGREF groupement de Bordeaux

**1.15 Comments**

IBMR is the official assessment macrophytes method applicable for the whole French territory. Its using in the quality classification and reporting will be effective as soon as the EQR will be available.

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Norme AFNOR NF T90-395, octobre 2003. Prescriptions from the application guidance (Guide méthodologique AFNOR in press).

**2.02 Short description**

Exhaustive record on field of a 100m stretch. All vegetal taxa totally or partially in the water surface are recorded. Direct observation through an aquascope (by wading). Sampling protocol by contact-points investigation is available as an adaptation for large and/or deep rivers (as well for high turbidity rivers), by boat and telescopic rake.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Rake  
Aquascope (glass-bottom box) f

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** June to September (adaptation according to the river type)

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

one per year (in vegetation season)

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

total record of the 100 m stretch.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

100 m long stretch (area = 100m x stream width)

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** n.a.

- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Phanerogams, pteridophytes, lichens, bryophytes and Characea algae : species level  
Macroscopic algae (filamentous, gelatinous, thallose) : genus level  
Bacterial tufts : genus level
- 2.15 Record of abundance:** Percent coverage  
abundance classes are required by the standard, but the percent of cover is required for WFD networks survey.  
**in relation to** Area  
**Unit** percent cover
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** covers of multiple vegetal layers (submerged, floating, leave\_floating, emerged)
- 2.18 Special cases, exceptions, additions:** Some river types are not surveyed because of the absence of macrophytes : e.g. intern Alps streams, very mobil bed rivers.
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
K = abundance (translated in 5 classes) CS = trophic score (0-20) E = stenoecy coefficient (1-3) IBMR =  $\Sigma(K.CS.E)/\Sigma(K.E)$
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** 234  
**Geographical coverage:** Whole French hydrosystem (but some river types don't provide reference sites)  
**Location of sites:** Whole French hydrosystem (but some river types don't provide reference sites)  
**Data time period:** 2005 to 2007 (3 years)  
**Criteria:**  
1/ base : national reference network (first refcond guidance criteria) 2/ qualitative criteria's list at the basin, reach, site scale evaluated by local experts. 3/ GIS criteria based on Corine Land Cover data (artificial, intensive agriculture, agriculture in the watershed).
- 3.08 Reference community description**  
Not available yet
- 3.09 Results expressed as EQR?** No in the IBMR scale (1 to 20). EQR will be available in 2010

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.
- 3.11 Boundary setting procedure**  
The boundaries will be derive from EQR scales as soon as EQR will be available (2010).
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 244

IBMR

## 1. General information

- 1.01 GIG:** Central-Baltic, Mediterranean  
R-C1; R-C2; R-C3; R-C4; R-C6; R-M1; R-M2; R-M4; R-M5
- 1.02 Category:** Rivers
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Italy
- 1.05 Specification:**
- 1.06 Method name:** *Macrophyte Biological Index for Rivers*
- 1.07 Original name:** *Indice Biologique Macrofitique en Rivière*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

The assessment method has been developed in France (see cited literature) in 2003, mainly to determine rivers trophic level. The sensitive score of each taxon indicator was defined after researches in whole France. Sensitive scores for indicators taxa are related to concentrations of nutrients but also general alteration. Italian applications had been pointed out significant likeness with chemical data deriving from French studies.

- 1.10 Internet reference:** [www.sintai.sinanet.apat.it/view/index.faces](http://www.sintai.sinanet.apat.it/view/index.faces)

**1.11 Pertinent literature of mandatory character:**

Italian Law AFNOR, 2003 Qualité de l'eau: Détermination de l'Indice Biologique Macrofitique en Rivière (IBMR) - NF T 90-395 MINCIARDI M.R., SPADA C. D., ROSSI G.L., ANGIUS R., ORRU' G., MANCINI L., PACE G., MARCHEGGIANI S., PUCCINELLI C.; 2009. Metodo per la valutazione e la classificazione dei corsi d'acqua utilizzando la comunità delle Macrofite acquatiche. Rapporto Tecnico ENEA RT/2009/23/ENEA: 35pp.

**1.12 Scientific literature:**

Haury J., Peltre M.C., Trémolières M., Barbe J., Thiebaut G., Bernez I., Daniel H., Chatenet P., Haan-Archipof G., Muller S., Dutartre A., Laplace-Treytore C., Cazaubon A., Lambert-Servien E., 2006. A new method to assess water trophy and organic pollution. The Macrophyte Biological Index for Rivers (IBMR): its application to different types of river and pollution. *Hydrobiologia*: 153-158

**1.13 Method developed by**

IBMR has been developed in France by Groupement d'Interet Scientifique and has been formalized as AFNOR norm  
(For the develop of italian applications and the definition of the EQR) [mariarita.minciardi@enea.it](mailto:mariarita.minciardi@enea.it)

**1.14 Method reported by**

Maria Rita Minciardi  
[mariarita.minciardi@enea.it](mailto:mariarita.minciardi@enea.it)

ENEA Agency for New Technologies, Energy and sustainable Development

**1.15 Comments**

IBMR has been applied in Italy since 2004 first of all in North Western regions, afterwards the method has been tested in whole country in the most of river typologies; these studies have allowed to determine type specific EQR and to take IBMR as national method

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

UNI EN 14184, 2004. Linee guida per la valutazione delle macrofite acquatiche nelle acque correnti. APAT, 2007. Protocollo di campionamento ed analisi per le macrofite delle acque correnti. In: "Metodi Biologici per le Acque. Parte I" Manuali e Linee Guida APAT

**2.02 Short description**

Survey of total coverage of macrophyte community in identified river reach in aquatic zone; coverage of each macrophyte taxon (algae, briophytes, pteridophytes and phanerogames). Sampling of one or more specimen for each taxon.

- 2.03 Method to select the sampling/survey site or area:** Expert knowledge

- 2.04 Sampling/survey device:** Grapnel

**2.05 Specification:**

- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:**

- 2.08 Sampling/survey month(s):** April to June; July to September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Two occasions per vegetative season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

One replicate

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

reach 50-100 m length

### **Sample processing**

**2.12 Minimum size of organisms sampled and processed:**

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Genus, Species/species groups

Algae: Genus level; Others: Species level

**2.15 Record of abundance:** Percent coverage

in relation to Area

Unit % coverage

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:**

**2.18 Special cases, exceptions, additions:** Deep and not wadables rivers could be sampled with a grapnel or a rake using boat or from the edges.

**2.19 Comments**

## **3. Data evaluation**

### **Evaluation**

**3.01 List of biological metrics**

For each indicator taxa coverage is translated in abundance score  $\text{Sum of (Abundance score * Sensitivity score * Reliability score) / sum of (Abundance score * Reliability score) = index value}$

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

### **Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** 78 sites in whole country

**Geographical coverage:** West alpine region, east alpine region, lowlands in Po plain, Northern Appennine region, Adriatic hill, Southern Appennine region, Sicily

**Location of sites:** Mountain alpine regions in Valle d'Aosta and Piemonte in national and regional parks, Parco del Po in Piemonte region, Parco del Ticino, Natura 2000 sites in Veneto, Tagliamento basin (Friuli Venezia Giulia), Entella and Trebbia basins (Liguria), Natura 2000 sites in Appennine region in Emilia Romagna, Parco del Circeo (Lazio), Biferno basin (Molise), Parco del Pollino (Calabria), Alcantara basin (Sicilia)

**Data time period:** sampling data from 2002 till 2009

**Criteria:**

The reference sites have been selected on the basis of pressures analysis (land use, hydrodynamism, morphological alteration, physical and chemical features) at site, water body and catchment scales; in sampling sites also macrophyte communities have been detected to evaluate structural likeness with reference type-specific communities and to evaluate presence and abundance of alien species, intensity and presence of natural disturbances.

**3.08 Reference community description**

Different typologies are characterized by different reference communities. In mountain typologies bryophytes and red algae should be dominant while in lowland regions the communities should be dominated by phanerogams with different abundances of submerged, amphiphytic and helophytic vegetation mainly related with hydrological features and channel substrate.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

Boundary setting has been identified using data from sites belonging to different quality level assessed on expert judgement, others BQE (macrobenthos), historical series of pressure data (land use, hydrological, morphological and chemical features).

□

The HG boundary has been identified also assessing IBMR variability range in references sites for each considered river typology.

- 3.12 "Good status" community:** Good status communities are different in different river typologies and are closely related with type-specific references communities; good status community is mainly compounds by taxa characterized by ecological optimum trophic level consistent with type specific water body trophic level; good status communities are characterized by significant diversity level, low coverage of filamentous algae (except red algae and charophytes) and low coverage of tolerant taxa (also in lowland areas).

### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)

- 3.14 Comments:**

ID: 78

kiselalgsanalys

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
R-C1, R-C2, R-C3, R-C4, R-C6
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Benthic algae in running water - diatom analysis*
- 1.07 Original name:** *Påväxt i rinnande vatten – kiselalgsanalys*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Acidification, Eutrophication, Pollution by organic matter  
Acidification index under development, right now actually only ACIDITY, not acidification.
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Ecological data from 200 streams with a pressure gradient of P, N, pH, and pollution by organic matter, among other parameters, also land use. Strong relationship between diatom indices and nutrients/land use (amount of agricultural land), pH, and indicators of organic pollution.
- 1.10 Internet reference:** [http://www.naturvardsverket.se/upload/02\\_tillstandet\\_i\\_miljon/Miljoovervakning/undersokn\\_typ/sotvatten/pavaxtdf](http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/sotvatten/pavaxtdf)
- 1.11 Pertinent literature of mandatory character:**  
<http://www.naturvardsverket.se/sv/Arbete-med-naturvard/Vattenforvaltning/Lagstiftning-och-vagledning/Vagledning/NFS-20081-och-Handbok-20074/>[http://www.naturvardsverket.se/Documents/foreskrifter/nfs2008/nfs\\_2008\\_01.pdf](http://www.naturvardsverket.se/Documents/foreskrifter/nfs2008/nfs_2008_01.pdf)
- 1.12 Scientific literature:**  
n.a.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Amelie Jarlman & Maria Kahlert<br>maria.kahlert@vatten.slu.se<br>Dept. of Environmental Assessment, Swedish University of<br>Agricultural Sciences | <b>1.14 Method reported by</b><br>Maria Kahlert<br>maria.kahlert@vatten.slu.se<br>Dept. of Environmental Assessment, Swedish University of<br>Agricultural Sciences |
|---|---|
- 1.15 Comments**  
The method has been translated into English. Ask Mikaela.Gonczy@naturvardsverket.se for the file.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
[http://www.naturvardsverket.se/upload/02\\_tillstandet\\_i\\_miljon/Miljoovervakning/undersokn\\_typ/sotvatten/pavaxtdf](http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/sotvatten/pavaxtdf) SS-EN 1394, 2003. Water quality. Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers (= Vattenundersökningar. Vägledning för provtagning och förbehandling av bentiska kiselalger i vattendrag). SS-EN 1440, 2005. Water quality. Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters (= Vattenundersökningar. Vägledning för identifiering och utvärdering av prover av bentiska kiselalger från vattendrag).
- 2.02 Short description**  
site most representative for reach, preferably with stones, preferably riffle, NOT directly at shoreline, 5-10 stones are sampled throughout the whole river width, if possible, otherwise as long in as possible. Stones are scraped with toothbrush, all scraped material pooled in one sample. Sample is settled and preserved with alcohol.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Brush
- 2.05 Specification:** toothbrush
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
stones (cobbles) if possible, otherwise preferably macrophytes
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** autumn (august to late september/early october)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
once per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
5-10 stones
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
5-10 stones

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 5-10 stones
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Bottle is shaken and part of it taken out for diatom slide preparation.
- 2.14 Level of taxonomical identification:** Species/species groups  
As specified as possible.
- 2.15 Record of abundance:** Relative abundance  
**in relation to** n.a.  
relative  
**Unit** %
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** % cover of other algae than diatoms is noted in field protocol if > 75%, and a fresh sample of these is analysed. Bacterial tufts is noted in the field, if present.
- 2.18 Special cases, exceptions, additions:** Non-wadable rivers are sampled only as long in as possible.
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
IPS, TDI, %PT, ACID
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates

### **Reference conditions**

- 3.05 Scope of reference conditions:** n.a.
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge
- 3.07 Reference site characterisation**  
**Number of sites:** 80  
**Geographical coverage:** Mostly northern Sweden, but some also in the South.  
**Location of sites:** contact author  
**Data time period:** autumn, since 2000  
**Criteria:**  
High/good boundary: IPS=17,5. High status: River/stream fulfils the national reference criteria, e.g. Tot-P < 10 µg/l or no eutrophication (areal specific loss of Tot-P = class 1; in case of missing data for calculation of areal specific loss: Tot-P < 20 µg/l AND colour > 100 mg Pt/l), no acidification, land use: < 20 % farming, < 0,1 % urban area.
- 3.08 Reference community description**  
species not evaluated yet, IPS > 17.5
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites  
Using paired metrics that respond in different ways to the influence of the pressure  
High-good boundary derived from preclassified reference state see C-13
- 3.11 Boundary setting procedure**  
High-good boundary see C-13. Good/moderate boundary: IPS=14.5. The G/M boundary was set to the IPS value where the nutrient tolerant and pollution tolerant species exceed a relative abundance of ca. 30 % (and the amount of sensitive species falls below ca. 30 %).
- 3.12 "Good status" community:** Relative abundance of sensitive species at least around 30 %, of nutrient tolerant and pollution tolerant species not more than ca. 30 %.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Derived from literature (studies in France) and from national intercalibrations.

**3.14 Comments:**

none

ID: 54

TDI 3

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** United Kingdom
- 1.05 Specification:** none
- 1.06 Method name:** **WFD River Diatom method or Trophic Diatom Index version 3 Method**
- 1.07 Original name:** *WFD River Diatom method or Trophic Diatom Index version 3 Method*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Data has been presented in EA Science Report SCO301030/SR1 Environment Agency 2007 (Kelly et al, 2007, Use of diatoms for evaluating ecological status in UK freshwaters), and by Kelly et al, 2008, Assessment of ecological status in U.K. rivers using diatoms, Freshwater Biology (2008) 53, 403–422

- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/river%20phytobenthos%20method%20statement](http://www.wfduk.org/bio_assessment/bio_assessment/river%20phytobenthos%20method%20statement)

**1.11 Pertinent literature of mandatory character:**

Water Framework Directive - United Kingdom Advisory Group (WFD-UKTAG), 2008. UKTAG river assessment methods macrophytes and phytobenthos. Phytobenthos- diatom assessment for River ecological status (DARES1).

[http://www.wfduk.org/bio\\_assessment/bio\\_assessment/river%20phytobenthos%20method%20statement](http://www.wfduk.org/bio_assessment/bio_assessment/river%20phytobenthos%20method%20statement)

Kelly, M.G., S. Juggins, H. Bennion, A. Burgess, M. Yallop, H. Hirst, L. King, J. Jamieson, R. Guthrie & B. Rippey, 2007. Use of diatoms for evaluating ecological status in UK freshwaters. Environment Agency Science report SCO301030/SR.

Environment Agency England & Wales use these operational instructions (regularly reviewed):

EA Ref. No. 027\_07 Sampling diatoms from rivers and lakes

EA Ref. No. 087\_07 Fixing phytoplankton and diatom samples with Lugol's iodine

EA Ref. No. 028\_07 Diatom sample digestion and slide preparation

EA Ref. No. 029\_07 Diatom slide analysis, recording and archiving

EA Ref. No. 198\_07 Quality Assurance Scheme for diatom samples

EA Ref. No. 387\_09 Interpreting and reporting freshwater ecology data

**1.12 Scientific literature:**

Kelly et al., 2008. Assessment of ecological status in UK rivers using diatoms. Freshwater Biology 53: 403-422. Kelly, M.G., H. Bennion, A. Burgess, J. Ellis, S. Juggins, R. Guthrie, B.J. Jamieson, V. Adriaenssens & M. Yallop, 2009. Uncertainty in ecological status assessments of lakes and rivers using diatoms. Hydrobiologia 63: 5-15. Kelly, M.G., L. King, G. Clarke, H. Bennion & M. Yallop, 2006. Recommendations for sampling littoral diatoms in lakes for ecological status assessments. Journal of Applied Phycology 18: 15-25. Kelly, M.G., L. King, R. Jones, P. Barker & B.J. Jamieson, 2008. Validation of diatoms as proxies for phytobenthos when assessing ecological status in lakes. Hydrobiologia 610: 125-129. Yallop, M., H. Hirst, M. Kelly, S. Juggins, B.J. Jamieson & R. Guthrie, 2009. Validation of ecological status concepts in UK rivers using historic diatom samples. Aquatic Botany 90: 289-295.

**1.13 Method developed by**

first point of contact, Dr Martyn Kelly  
MGKelly@bowburn-consultancy.co.uk

Bowburn Consultancy

**1.14 Method reported by**

Jan Krokowski, Imelda O'Neill, Jane Jamieson

Jan.krokowski@sepa.org.uk, Imelda.oneill@doeni.gov.uk,

jane.jamieson@environment-agency.gov.uk

Scottish Environment Protection Agency (SEPA), Northern Ireland Environment Agency (NIEA), Environment Agency (EA; England and Wales)

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Kelly, M.G., A. Cazaubon & E. Coring et al., 1998. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. Journal of Applied Phycology 10: 215–224.

EN 13946, 2003. Water Quality – Guidance Standard for the Routine Sampling and Pretreatment of Benthic Diatoms from Rivers.

EN 14407, 2004. Water Quality – Guidance Standard for the Identification, Enumeration and Interpretation of Benthic Diatom Samples from Running Waters.

Environment Agency England & Wales also uses these operational instructions (regularly reviewed):

EA Ref. No. 027\_07 Sampling diatoms from rivers and lakes

EA Ref. No. 087\_07 Fixing phytoplankton and diatom samples with Lugol's iodine  
EA Ref. No. 028\_07 Diatom sample digestion and slide preparation  
EA Ref. No. 029\_07 Diatom slide analysis, recording and archiving  
EA Ref. No. 198\_07 Quality Assurance Scheme for diatom samples  
EA Ref. No. 387\_09 Interpreting and reporting freshwater ecology data

## 2.02 Short description

Cobbles are the recommended substratum because they are stable (allowing diatom communities to develop) and manoeuvrable. Cobbles are available in most river types. Five cobbles/small boulders, free from algae, are collected from mid-stream and placed into a tray with a little stream water and the top surface of each brushed with a clean toothbrush to remove the biofilm. The resulting suspension was collected in a plastic bottle, fixed with Lugol's iodine and stored prior to analysis. Step Action 1 From the sampling area, collect at least five cobbles (64 to 256 mm) or small boulders (> 256 mm) that have an obvious diatom film (brown colour and slimy texture). In standing waters, collect samples from depths where cobbles are permanently submerged and that you can reach wearing thigh waders. Note: If suitable substrata are very abundant, select each cobble from a separate location within reach or within the sampling area. 2 Gently agitate the cobbles in river or lake water to remove loosely attached surface contamination (this will not dislodge the biofilm). Surface contamination might include small particles of organic matter or sediment. 3 Place the stones in a tray with about 50 ml of river or lake water. 4 Wash a stiff toothbrush in clean river or lake water and rub it on waders or a similar surface to remove any diatoms from previous samples. 5 Brush the upper surface of the stone vigorously to remove the diatom film, rinsing the toothbrush periodically in the tray water to transfer the diatoms. If there are filamentous algae or silt deposits on the stone, try to remove diatoms from the stone where it is free of contaminants. they don't, try brushing them for a sample. 6 7 8 Replace the stone in the river or lake and repeat the steps above for other stones. Transfer the tray water (which should be brown and turbid from the diatoms) from the tray to the sample bottle. If samples will be stored for some time, you can concentrate the suspension by: 1. allowing it to settle overnight; 2. decanting the supernatant; transferring the sediment to a smaller (60 - 100 ml) bottle.

**2.03 Method to select the sampling/survey site or area:** n.a.

**2.04 Sampling/survey device:** n.a.

Toothbrush

**2.05 Specification:** toothbrush; strong scissors; white plastic tray; wide-mouthed plastic sample bottles with watertight lids; waterproof permanent marker pen or another means of labelling samples; (house bricks with holes in, and polypropylene rope – only if using introduced substrata in absence of cobbles)

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

Generally cobbles but other habitats when cobbles are not present. Sample habitat is

**2.07 Sampled/surveyed zones in areas with tidal influence:** which list appropriate for optimising the presence of diatoms at a site.

**2.08 Sampling/survey month(s):** SEPA: Spring: mid-April to end May and autumn: September to end November EA/NIEA: summer: June to end August

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

6 samples/survey occasions in a classification period

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

one

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

5 randomly selected cobbles/small boulders free of algae Environment Agency (England & Wales) and SEPA use different sampling methods for different substrata, in order of preference: 5 randomly selected cobbles/small boulders, free of algae  
Algae-covered

## Sample processing

**2.12 Minimum size of organisms sampled and processed:** n.a.

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts, Relative abundance

**in relation to** n.a.

sampled 5 cobbles/small boulders

**Unit** Number of valves

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** Other photosynthetic organisms e.g. filamentous algae (% in 10M reach) Cover of sewage fungus above and below stones, presence and density

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

The TDI3 is based on the weighted average equation of Zelinka and Marvan (1961), where  $a_j$  is the abundance or proportion of valves of species  $j$  in sample;  $s_j$ , the revised nutrient sensitivity class (1–5) of species  $j$ ;  $WMS_j$ , the weighted mean score. The second step was performed to present the TDI on a score ranging from 0 (very low nutrients) to 100 (very high nutrients).

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

##### 3.04 From which biological data are the metrics calculated?

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Expert knowledge, Historical data, Modelling (extrapolating model results)

##### 3.07 Reference site characterisation

**Number of sites:** 169 sites across Scotland, England and Wales and Northern Ireland were used to derive reference conditions for the method.

**Geographical coverage:** Scotland, England and Wales and Northern Ireland

**Location of sites:** Large numbers of reference sites were found in Scotland, Wales and south-west England – almost none in densely populated areas of the midlands and southern England

**Data time period:** Reference sites were identified from the DARES database – comprising subsets of data from 1970's through to 2005

##### Criteria:

The process of identifying reference sites from the DARES was iterative, as data were screened and hypotheses tested. Guidelines from UK studies associated with the Habitats Directive (European Community, 1992) set limits no higher than 30 µg l<sup>-1</sup> SRP in rivers without significant anthropogenic influences (Pitt et al., 2002) and this value was used to filter out an initial pool of potential reference sites. A further criterion used in the first iteration was that the invertebrate biology, as evaluated by RIVPACS, had to fall into the top two classes. The precise limits varied between the Environment Agency, SEPA and EHS but all correspond, approximately, to 'good status' or better. Following this, a further iteration (based on discussions with other experts in the UK) set a threshold of 20 µg l<sup>-1</sup> SRP for sites with total alkalinity < 50 mg l<sup>-1</sup> CaCO<sub>3</sub> and 30 µg l<sup>-1</sup> SRP for sites with alkalinity ≥ 50 mg l<sup>-1</sup> CaCO<sub>3</sub>. As more sites with high resolution SRP data become available, these limits will need to be revisited. The data were also screened to remove sites with high nitrate-N concentrations. A value of 2 mg l<sup>-1</sup> nitrate-N was applied to Low Alkalinity sites while a higher value (4 mg l<sup>-1</sup> nitrate-N) was applied to sites with total alkalinity ≥ 50 mg l<sup>-1</sup> CaCO<sub>3</sub> for the same reasons as described above, though this will almost certainly include some slightly impacted sites. Initial analysis of the resulting reference sites showed some to have high TDI values, suggesting that even after screening using chemical criteria the reference groups still contained sites suffering from the impacts of elevated nutrient concentrations. We therefore applied a further screening and removed sites with TDI scores > 50. The above screening identified a subset of 278 reference samples from 169 sites, from the total database of 1051 samples. Figure 4.1 shows the spatial distribution of reference and non-reference samples, and the distribution of reference samples in relation to alkalinity and altitude. Figure 4.2 summarises additional environmental characteristics of the reference samples. Reference sites are distributed primarily around the periphery of Great Britain, with large numbers in Scotland, Wales and north and south-west England. There are almost no reference sites in the densely populated areas of the midlands and southern England. This geographic bias is also reflected in the hydrochemistry: while 661 (63%) samples in the total database are from sites with mean annual alkalinity

##### 3.08 Reference community description

High relative abundance of *Achnanthes* spp. (many sites also contained *A. biasolettiana* and/or *A. microcephalum*), attached taxa *Gomphonema* spp. and loosely-attached *Fragilariophyceae* (*Fragilaria capucina* was the most abundant, but *Meridion circulare*, *Hannaea arcus* and *Tabellaria flocculosa* were all common at lower alkalinities), but few motile taxa. A few lower alkalinity sites were dominated by *Achnanthes oblongella*, and *Cocconeis placentula* was also abundant on some occasions.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites Using paired metrics that respond in different ways to the influence of the pressure

##### 3.11 Boundary setting procedure

The high/good boundary is set at the 75th percentile of EQR values for reference sites within a particular type. 'Crossover' between nutrient-sensitive and nutrient-tolerant species (Pollard and van de Bund, 2005). Biological metrics tend to show gradual change as the level of nutrient/organic pressure increases, with no distinct discontinuities that could act as criteria for setting class boundaries. An alternative approach – based on the proportions of nutrient-tolerant, nutrient-sensitive and indifferent taxa within samples – was used to define status class boundaries in the UK, with the good/moderate boundary set at the point where the proportion of sensitive taxa falls below that of tolerant taxa. In ecological terms, the diatom flora at high and good status is characterised by a number of taxa, often with relatively broad niches (e.g. *Achnanthes minutissimum*, *Fragilaria capucina*) which occur at different phases of a microsuccession from colonisation of bare rock up to a mature biofilm (see Biggs et al., 1989). At high status, these are accompanied by other nutrient-sensitive taxa but as nutrient concentrations increase, the most sensitive of these taxa disappear whilst the numbers of nutrient tolerant taxa increases. Therefore 'crossover' is the point at which the taxa which form the 'association' characteristic of a site in the absence of pressure become subordinate to taxa which are favoured by a pressure (nutrients, in this case). The EQR gradient below the good/moderate boundary is then divided into three equally-spaced portions from which the moderate/poor and poor/bad boundaries are derived.

- 3.12 "Good status" community:** *A. minutissimum*, *F. capucina*, *F. vaucheriae* and *N. dissipata* were present in a majority of sites at 99.5%, 69.2%, 70.6% and 70.6% respectively, but the maximum relative abundance recorded was lower than in samples at high status (62.5%, 25.7%, 26.0% and 41.6% relative abundance respectively). Other species including *G. parvulum*, *A. pediculus*, *Planorbulina lanceolata*, *Reimeria sinuata* and motile species including *N. gregaria*, *N. lanceolata*, *Navicula minima* and *N. dissipata* were present in over 70% of all samples in this status class. Concerning these species the highest maximum of relative abundance was recorded for *G. parvulum* (61.4%).

### **Uncertainty**

- 3.13 Consideration of uncertainty:** Yes

Detailed in chapter 6 of the report: Use of diatoms for evaluating ecological status in UK freshwaters. Environment Agency Science report SC0301030/SR1. In this chapter we ask two questions: What is the uncertainty associated with a single sample as an estimate of ecological status on the day that the sample was collected? How well does this sample reflect the long-term average condition of the biology? These questions are addressed separately. The former uses a nested analysis of variance that examines variation in metrics associated with variability on a slide nested within variability at a site. No attempt has been made to separate (natural) spatial variability from variability introduced by the operator but the latter sources of error were minimised by use of standard methods. Errors associated with making slides are relatively small and differences between lakes and rivers are minor. If analysts adhere to protocols, one slide per sample is sufficient to estimate the taxonomic composition and derived indices from a sample. The variance between replicate samples taken at one time from one location in lakes was much smaller than in rivers. There is a large amount of temporal variation at single sampling locations in rivers and reliable indications of status class will need to be based on repeated sampling from the same location. Results suggest that at least six replicates (i.e. two per year for three years or three per year for two years) will be required in order to provide a firm basis for regulation. A sampling intensity greater than this might be at risk of 'pseudo-replication'. The risk of misclassification depends on the proximity of the mean EQR for a site to the status class boundary. When the EQR value is very close to the boundary, the risk of misclassification will be approximately 50%, regardless of the number of samples available.

- 3.14 Comments:**

none

ID: 96

ASPT

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Average Score per Taxon*
- 1.07 Original name:** *Average Score per Taxon*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Johnson, R.K. & W. Goedkoop, 2007. Bedömningsgrunder för bottenfauna i sjöar och vattendrag- Användarmanual och bakgrundsdokument, Swedish University of Agricultural Sciences, Report 2007: 4, 84 p. [Background report for benthic fauna in lakes and watercourses - User manual and background document]. Report 2007: 4. Department of Environmental Analysis Swedish University of Agricultural Sciences (SLU).
- 1.12 Scientific literature:**  
Armitage, P.D., D. Moss, J.F. Wright & M.T. Furse, 1983. The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-waters. *Water Research* 17: 333-347.
- 1.13 Method developed by** Armitage et al.
- 1.14 Method reported by** Richard K. Johnson  
richard.johnson@vatten.slu.se  
Dept. Aquatic Sciences and Assessment, SLU, Uppsala, Sweden
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Standardized Kick-sampling, SSEN-27828 (60 s x 1 m; 0,5 mesh; 5 replicates taken in autumn).
- 2.02 Short description**  
Substratum is disturbed by kicking for 60 s and moving a distance upstream of 1 m
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** Kick net
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
hard bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** September to November
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
5 replicates per site
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
0.25 (width of kick net) x 1 m

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 0.5 mm mesh
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** Time  
**Unit** Catch per unit effort
- 2.16 Quantification of biomass:** n.a.

- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

ASPT exploits the differences in tolerance among different families of benthic macroinvertebrates and the order Oligochaeta (earthworms). Very sensitive families give high indicator values, while those with high tolerance give low indicator values. The index value for ASPT is a mean value for included taxa and is calculated by adding indicator values and dividing them by the number of included taxa (families). Table 7.3. Indicator values for ASPT for different families. Indicator value Family

10 Aphelocheiridae, Beraeidae, Brachycentridae, Capniidae, Chloroperlidae, Ephemeridae, Ephemerellidae, Goeridae, Heptageniidae, Lepidostomatidae, Leptoceridae, Leptophlebiidae, Leuctridae, Molannidae, Odontoceridae, Perlidae, Perlodidae, Phryganeidae, Potamanthidae, Sericostomatidae, Siphonuridae, Taeniopterygidae

8 Aeshnidae, Astacidae, Agriidae, Cordulegasteridae, Corduliidae, Gomphidae, Lestidae, Libellulidae, Philopotamidae, Psychomyiidae

7 Caenidae, Limnephilidae, Nemouridae, Polycentropodidae, Rhyacophilidae (incl. Glossosomatidae)

6 Ancylidae, Coenagriidae, Corophiidae, Gammaridae, Hydroptilidae, Neritidae, Platycnemididae, Unionidae, Viviparidae

5 Chrysomelidae, Clambidae, Corixidae, Curculionidae, Dendrocoelidae, Dryopidae, Dytiscidae, Elminthidae, Gerridae, Gyrinidae, Haliplidae, Heledidae, Hydrophilidae (incl. Hydraenidae), Hydropsychidae, Hygrobiidae, Hydrometridae, Mesoveliidae, Naucoridae, Nepidae, Notonectidae, Planariidae, Pleidae, Simuliidae, Tipulidae (inkl. Pediciidae)

4 Baetidae, Piscicolidae, Sialidae

3 Asellidae, Erpobdellidae, Glossiphoniidae, Hirudidae, Hydrobiidae, Lymnaeidae, Planorbidae, Physidae, Sphaeriidae, Valvatidae

2 Chironomidae

1 Oligochaeta

The ecological quality ratio (EQR) is calculated as follows:  $EQR = \text{calculated ASPT} / \text{reference value}$

Reference values and class boundaries are given in Table 7.4.

- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Worst quality class
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**
- Number of sites:** ca 300
- Geographical coverage:** whole of Sweden
- Location of sites:** whole of Sweden
- Data time period:** 2000 national survey and Trend Streams (national monitoring programme)
- Criteria:**  
Use of pressure filter to identify reference conditions.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** Yes  
Determination of type 2 error frequency using independent data.
- 3.14 Comments:**  
none

ID: 98

DJ-index

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *DJ-index*
- 1.07 Original name:** *DJ-index*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Johnson, R.K. & W. Goedkoop, 2007. Bedömningsgrunder för bottenfauna i sjöar och vattendrag- Användarmanual och bakgrundsdokument, Swedish University of Agricultural Sciences, Report 2007: 4, 84 p. [Background report for benthic fauna in lakes and watercourses - User manual and background document]. Report 2007: 4. Department of Environmental Analysis Swedish University of Agricultural Sciences (SLU).
- 1.12 Scientific literature:**  
Dahl, J. & R.K. Johnson, 2004. A multimetric macroinvertebrate index for detecting organic pollution of streams in southern Sweden. Archiv für Hydrobiologie 160: 487-513.
- |  |  |
|--|--|
| <b>1.13 Method developed by</b><br>Joakim Dahl and Richard K. Johnson<br>Joakim.Lucke@naturvardsverket.se<br>Dept. of Aquatic Sciences and Assessment, SLU | <b>1.14 Method reported by</b><br>Richard K. Johnson<br>richard.johnson@vatten.slu.se<br>Dept. Aquatic Sciences and Assessment, SLU, Uppsala, Sweden |
|--|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Standardized Kick-sampling, SSEN-27828 (60 s x 1 m; 0,5 mesh; 5 replicates taken in autumn).
- 2.02 Short description**  
Substratum is disturbed by kicking for 60 s and moving a distance upstream of 1 m
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** Kick net
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
hard bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** September to November
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
5 replicates per site
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
0.25 (width of kick net) x 1 m

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 0.5 mm mesh
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** Time  
**Unit** Catch per unit effort
- 2.16 Quantification of biomass:** n.a.

- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

The multimetric DJ index (Dahl & Johnson 2005) for determining the effects of eutrophication on macroinvertebrate assemblages is constructed from five different simple indices. These are (1) the number of taxa of mayflies, stoneflies and caddis flies (Ephemeroptera, Plecoptera and Trichoptera), (2) the relative abundance (%) of Crustaceans (Crustacea), (3) the relative abundance (%) of mayflies, stoneflies and caddis flies, (4) ASPT, and (5) the Saprobic index according to Zelinka and Marvan (1961). Values for these simple indices must be normalised so that each has a value 1, 2 or 3 according to the criteria in Table 7.5. Table 7.5. Criteria for normalising simple index values to values of 1, 2 or 3 for calculation of the DJ index. Index Criteria  
Mayflies, stoneflies and caddis flies (Number of taxa)  $\leq 5$  5 – 12  $> 12$  % crustaceans (of total abundance)  $\geq 22.2$   $0.5 \leq 22.2 < 0.5$  % mayflies, stoneflies and caddis flies (of total abundance)  $\leq 10.4$   $10.4 - 52.1 \geq 52.1$  ASPT  $\leq 5$   $5 - 6.3 \geq 6.3$  Saprobic index  $\geq 2.5$   $1.9 - 2.5 \leq 1.9$  Indexnorm = 1 = 2 = 3 The DJ index is calculated by adding the normalised values a can assume a minimum value of 5 and a maximum value of 15.

- 3.02 Does the metric selection differ between types of water bodies?** No

- 3.03 Combination rule for multi-metrics:** Worst quality class

##### 3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites

##### 3.07 Reference site characterisation

**Number of sites:** ca 300

**Geographical coverage:** whole of Sweden

**Location of sites:** whole of Sweden

**Data time period:** 2000 national survey and Trend Streams (national monitoring programme)

**Criteria:**

Use of pressure filter to identify reference conditions.

##### 3.08 Reference community description

n.a.

- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

##### 3.11 Boundary setting procedure

n.a.

- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** Yes

Determination of type 2 error frequency using independent data.

##### 3.14 Comments:

none

ID: 100

MISA

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Multimetric Index for Stream Acidity*
- 1.07 Original name:** *Multimetric Index for Stream Acidity*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Acidification

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Johnson, R.K. & W. Goedkoop, 2007. Bedömningsgrunder för bottenfauna i sjöar och vattendrag- Användarmanual och bakgrundsdokument, Swedish University of Agricultural Sciences, Report 2007: 4, 84 p. [Background report for benthic fauna in lakes and watercourses - User manual and background document]. Report 2007: 4. Department of Environmental Analysis Swedish University of Agricultural Sciences (SLU).

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

R.K. Johnson and W. Goedkoop  
richard.johnson@vatten.slu.se  
Dept. of Aquatic Sciences and Assessment, SLU

**1.14 Method reported by**

Richard K. Johnson  
richard.johnson@vatten.slu.se  
Dept. Aquatic Sciences and Assessment, SLU, Uppsala, Sweden

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Standardized Kick-sampling, SSEN-27828 (60 s x 1 m; 0,5 mesh; 5 replicates taken in autumn).

**2.02 Short description**

Substratum is disturbed by kicking for 60 s and moving a distance upstream of 1 m

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying

**2.04 Sampling/survey device:** Hand net

**2.05 Specification:** Kick net

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
hard bottom

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** September to November

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

one occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

5 replicates per site

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

0.25 (width of kick net) x 1 m

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 0.5 mm mesh

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

in relation to Time

Unit Catch per unit effort

**2.16 Quantification of biomass:** n.a.

- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

MISA is constructed from six different simple indices and responds to acidity. The input indices are (1) the number of families, (2) the number of mollusc taxa (Gastropoda), (3) the number of mayfly taxa (Ephemeroptera) (4) the ratio between the relative abundance (%) of mayflies and the relative abundance (%) of stoneflies (Plecoptera), (5) the AWIC index (Acid Waters Indicator Community index; Davy-Bowker et al (2005) and (6) the relative abundance (%) of shredders. Values for these simple indices must be normalised so that each has a value (indexnorm) between 0 and 10 according to Table 7.7. The normalised values are then added together and re-scaled by dividing the sum of the normalised index values by the number of simple indices included (a mean value) and multiplying this mean value by 10 according to the following:  $MISA = 10 * \frac{\sum \text{indexnorm}}{6}$  MISA thus acquires a value that can vary between 0 and 100. Table 7.7. Normalisation of index values (Indexnorm) for the six simple indices to values between 0 and 10. In the next step MISA is calculated as a mean value for these normalised indices. "ASTERICS nomenclature" relates to the software program at <http://www.aqem.de>. Index ASTERICS- nomenclature Indexnorm=10 if the index Indexnorm=0 if the index Otherwise Indexnorm=Number of families/Number of Families >43 <21 Molluscs/(number of taxa) - Gastropoda >3 <0 mayflies/(number of taxa) - Ephemeroptera >16 <3 Mayflies/stoneflies (% abundance)\* - Ephemeroptera [%] and Plecoptera [%] >7 <0

AWICfamily index AWIC Index >4.6 <3.8 Shredders - [%] Shredders <1.4 >14 \*Please note that the Mayflies/stoneflies (%abundance) index is not included in MISA in those cases where there are no stoneflies in the sample! The absence of stoneflies makes it impossible to calculate this simple index. When there are no stoneflies MISA is instead calculated as a mean value of 5 normalised index values.

- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Worst quality class

##### 3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**
- Number of sites:** ca 300
- Geographical coverage:** whole of Sweden
- Location of sites:** whole of Sweden
- Data time period:** 2000 national survey and Trend Streams (national monitoring programme)
- Criteria:**  
Use of pressure filter to identify reference conditions.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** Yes  
Determination of type 2 error frequency using independent data.
- 3.14 Comments:**

none

ID: 20

RICT

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
Intercalibrated against all CB GIG and NGIG river types.
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** United Kingdom
- 1.05 Specification:** none
- 1.06 Method name:** *River Invertebrate Classification Tool*
- 1.07 Original name:** *River Invertebrate Classification Tool*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Acidification, General degradation, Hydromorphological degradation, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter  
The answers above relate to SEPA. NIEA indicated the following pressures: acidification, aquatic habitat destruction, general degradation, impact of alien species, pollution by organic matter.
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Tested against: BOD, DO Ammonia. See  
[http://www.wfduk.org/stakeholder\\_reviews/Standards\\_Jan\\_2006/TRReports/LibraryPublicDocs/DevtofoxyConandAmmonRegValinUKRivers](http://www.wfduk.org/stakeholder_reviews/Standards_Jan_2006/TRReports/LibraryPublicDocs/DevtofoxyConandAmmonRegValinUKRivers)
- 1.10 Internet reference:** <http://rict.org.uk/>, [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/rivers\\_invertebrates](http://www.wfduk.org/bio_assessment/bio_assessment/rivers_invertebrates),  
<http://www.sniffer.org.uk/Default.aspx>
- 1.11 Pertinent literature of mandatory character:**  
United Kingdom Advisory Group, 2008. UKTAG river assessment methodsbenthic invertebrate faunariver invertebrate classification tool (RICT).[http://www.wfduk.org/bio\\_assessment/bio\\_assessment/rivers\\_invertebrates](http://www.wfduk.org/bio_assessment/bio_assessment/rivers_invertebrates)
- 1.12 Scientific literature:**  
Clarke, R.T., J.F. Wright & M.T. Furse, 2003. RIVPACS Models for predicting the expected Macroinvertebrate fauna and assessing the ecological quality of Rivers. Ecological Modelling 160: 219-233.[Davy-Bowker, J. & R.T. Clarke, 2006. Development of the Scientific Rationale and Formulae for altering RIVPACS predicted indices for WFD Reference Condition. Scotland & Northern Ireland Forum for Environmental Research report. Project WFD72b.](#)[Davy-Bowker, J., R.T. Clarke, M.T. Furse, C.E. Davies, T.A. Corbin, J.F. Murphy & N.T. Kneebone, 2005. RIVPACS Database Documentation. Scotland & Northern Ireland Forum for Environmental Research report. Project WFD46.](#)[Davy-Bowker, J., R.T. Clarke, T.A. Corbin, M.T. Furse, H. Vincent, J. Pretty, A. Hawczak, J.F. Murphy & I. Jones, 2008. River Invertebrate Classification Tool. Scotland & Northern Ireland Forum for Environmental Research report. Project WFD72C.](#)
- 1.13 Method developed by**  
John Davy-Bowker  
jdb@fba.org.uk  
Bulk of work carried out while lead scientist at Centre for Ecology & Hydrology, lead scientist now with Freshwater Biological Association, Dorset
- 1.14 Method reported by**  
David Colvill, Imelda O'Neill  
David.colvill@sepa.org.uk, imelda.oneill@doeni.gov.uk  
Scottish Environment Protection Agency; Northern Ireland Environment Agency
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
United Kingdom Advisory Group, 2008. UKTAG river assessment methodsbenthic invertebrate faunariver invertebrate classification tool (RICT).[http://www.wfduk.org/bio\\_assessment/bio\\_assessment/rivers\\_invertebrates](http://www.wfduk.org/bio_assessment/bio_assessment/rivers_invertebrates)
- 2.02 Short description**  
To apply the method, benthic macro-invertebrates should be collected from shallow flowing waters by disturbing the substratum with the feet ("kick" sampling) upstream of a hand net (nominal mesh size: 1 mm) held vertically on the riverbed.[All habitats in the chosen sampling site in the river should be sampled within a 3-minute period. In addition, a manual search, lasting one minute, should be performed and any invertebrates found attached to submerged plant stems, stones, logs or other solid surfaces should be removed and placed in the net. Rivers that are too deep to be sampled by the kick sampling method described above should be sampled by:](#)[\(i\) sweeping a long-handled pond net \(nominal mesh size: 1 mm\) through any aquatic vegetation within reach of the banks of the river;](#)[and](#)[\(ii\) kick sampling in any shallow areas;](#)[or](#)[\(iii\) sampling using a naturalist's dredge or an air-lift sampler.](#)
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Stratified sampling/surveying
- 2.04 Sampling/survey device:** Airlift sampler, Dredge, Hand net
- 2.05 Specification:** pond net, Naturalists dredge/ Airlift sampler in deep rivers
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** Spring (01-March to 31-May), Autumn (01-September to 31-November)

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

2 samples per year (1 sample in spring/Autumn for each year classified)

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

2 x 3 minute kick samples per year classified (NIEA: indicated that this is the minimum area sampled and this is accompanied by 1-minute active searches)

### **Sample processing**

**2.12 Minimum size of organisms sampled and processed:** 1mm

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Family

**2.15 Record of abundance:** Abundance classes

Log scale abundances are recorded but ARE NOT YET USED IN CLASSIFICATION. It is planned to use abundances in classification in the future.

**in relation to** Time

N/A

**Unit** Log 10 categories of numbers per sample

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** Non wadeable rivers may be sampled either by sampling in shallow areas, sweeping with a long handled net, or by using a naturalists dredge or airlift sampler.

**2.19 Comments**

none

## **3. Data evaluation**

### **Evaluation**

#### **3.01 List of biological metrics**

SEPA: NTAXA & ASPT  
NTAXA = The observed value of the parameter, NTAXA, should be the sum of the number of different taxa listed in Column 1 of Table 2 and present in one or more of the samples obtained from the sampling site in the same calendar year. Due to sample sorting and identification errors, the calculated observed value for NTAXA may be underestimated. In order to account for this, the observed values should be converted to bias-corrected observed values. This should be done using the following procedure. An NTAXA bias value should be determined representing an estimate of the average under-estimation of the observed number of taxa listed in Column 1 of Table 2 in a sample. Separate bias values should be determined for each season (i.e. Spring, Summer and Autumn). The values should be based on proper analysis (e.g. an external audit of samples taken and analysed) and determined by the quality systems and procedures in place where the samples were analysed. The observed value of the parameter should then be calculated using the applicable equation in Column 2 of Table 1. ASPT = To calculate the observed value of the parameter, ASPT, each taxon listed in Column 1 of Table 2 and identified as present in a sample should be assigned the corresponding pressure sensitivity score in Column 2 of that Table. The observed value of the parameter should then be calculated using the following equation: Observed value of ASPT =  $\sum \text{PSS} \div \text{NTAXA}$  where "PSS" is the sum of the pressure sensitivity scores assigned to each taxon present in one or more of the samples obtained from the sampling site in the same calendar year and listed in Column 1 of Table 2. The observed value should then be converted to bias-corrected values as follows: The value of ASPT for taxa missed because of sample sorting and identification errors should be estimated using the equation: Estimated ASPT of missed taxa =  $4.29 + 0.077 \times \text{observed value of NTAXA}$  where the observed value of NTAXA is the value prior to bias correction. The bias-corrected value of ASPT is then given by the following equation: Bias-corrected observed value of ASPT =  $\frac{(\text{Observed value of NTAXA} \times \text{observed value of ASPT}) + (\text{NTAXA bias value} \times \text{estimated ASPT of missed taxa})}{\text{bias corrected observed value of NTAXA}}$  where the NTAXA bias value depends on the sampling data used to calculate the observed value of the parameter as follows: Observed value calculated using sampling data collected during single season NTAXA bias value = NTAXA bias value for the season. Observed value calculated using combined sampling data from two seasons NTAXA bias value =  $0.51 \times (\text{sum of NTAXA bias values for the two seasons})$ . Observed value calculated using combined sampling data from three seasons NTAXA bias value =  $0.37 \times (\text{sum of NTAXA bias values for the three seasons})$ . See United Kingdom Advisory Group (2008) "UKTAG RIVER ASSESSMENT METHODS: BENTHIC INVERTEBRATE FAUNA: RIVER INVERTEBRATE CLASSIFICATION TOOL (RICT)" Table 2 pp 7-10 available at [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/rivers\\_invertebrates](http://www.wfduk.org/bio_assessment/bio_assessment/rivers_invertebrates). NIEA response: ASPT = Average biological monitoring working party Score Per Taxon. N-TAXA = Number of Scoring Taxa (i) Number of Taxa (NTAXA). The observed value of the parameter, NTAXA, should be the sum of the number of different taxa listed in Column 1 of Table 2 present in two samples collected from the sampling site in the Spring and Autumn of the same calendar year. (ii) Average Score Per Taxon (ASPT). To calculate the observed value of the parameter, ASPT, each taxon listed in Column 1 of Table 2 and identified as present in the two samples collected as per (i) above should be assigned the corresponding Biological Monitoring Working

Party Pressure Sensitivity score (BMWPPSs) in Column 2 of that Table. The observed value of the parameter should then be calculated using the following equation: Observed value of ASPT = BMWPPSs ÷ NTAXA where "BMWPPSs" is the sum of the pressure sensitivity scores assigned to each taxon present in the two samples collected in the Spring and Autumn of the same calendar year and listed in Column 1 of Table 2. See United Kingdom Advisory Group (2008) "UKTAG RIVER ASSESSMENT METHODS: BENTHIC INVERTEBRATE FAUNA: RIVER INVERTEBRATE CLASSIFICATION TOOL (RICT)" Table 2 pp 7-10

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Worst quality class

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

**3.05 Scope of reference conditions:** Site-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Modelling (extrapolating model results)

NIEA: Indicated expert knowledge, least disturbed conditions

**3.07 Reference site characterisation**

**Number of sites:** 793 total (110 in Northern Ireland and 685 in Great Britain)

**Geographical coverage:** Representative rivers throughout UK

**Location of sites:** Representative rivers throughout UK

**Data time period:** Samples collected over 24 years (1978-2002) for the method. Some more recent data also used for classification.

**Criteria:**

Reference sites used for the RIVPACS model were screened according to REFCOND guidance as detailed in: Davy-Bowker, J., Clarke, R., Furse, M., Davis, C., Corbin, T., Murphy, J., Kneebone, N., (2007) "RIVPACS Pressure Data Analysis" SNIFFER Publication. Available at:

<http://www.sniffer.org.uk/Webcontrol/Secure/ClientSpecific/ResourceManagement/UploadedFiles/WFD46%20RIVPACS%20Pressure%20Data%20Analysis.pdf>

**3.08 Reference community description**

Precise reference community descriptions yet to be undertaken, however short descriptions of the reference typologies & biotic scores available in: Murphy, J., Davy-Bowker, J., Clarke, R., Corbin, T., Vincent, H., Pretty, J., Hawzac, A., Blackburn, J., Jones, I., (2008) "River Invertebrate Classification Tool" SNIFFER. Available at:

<http://www.sniffer.org.uk/Webcontrol/Secure/ClientSpecific/ResourceManagement/UploadedFiles/WFD72C%20FINAL%20REPORT%20with%20security.pdf>

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites Using paired metrics that respond in different ways to the influence of the pressure Multiples of the standard deviations from the mean EQRs of un-stressed sites. NIEA response: None of the above highlighted. Comment under other: For ASPT the boundaries are the median observed/expected ratio (EQI), 5th percentile and multiples thereof. F

**3.11 Boundary setting procedure**

Boundaries were checked against pressures as described in UK-TAG web site:-

[??](#)

See: [http://www.wfduk.org/stakeholder\\_reviews/Standards\\_Jan\\_2006/TRReports/LibraryPublicDocs/DevtofoxyConandAmmonRegValinUKRivers](http://www.wfduk.org/stakeholder_reviews/Standards_Jan_2006/TRReports/LibraryPublicDocs/DevtofoxyConandAmmonRegValinUKRivers)

**3.12 "Good status" community:** This is highly dependent on reference typology as one might expect and in this limited space there is not room to describe "Good" conditions at the 43 Mainland UK groups & 11 Northern Ireland groups. However, <http://www.sniffer.org.uk/Webcontrol/Secure/ClientSpecific/ResourceManagement/UploadedFiles/WFD72b.pdf> & <http://www.wfduk.org/LibraryPublicDocs/rivers-macroinvertebrate-riect> give the following short descriptions: High/ Good boundary: Pressures may just be picked up in the biology Middle of Good: Most Expected taxa present, with noticeable impact from pressures Good / Moderate boundary: Major Taxonomic groups (dependent on typology) begin to be lost. NIEA response: There are slight changes in the composition and abundance of invertebrate taxa from the type-specific communities. The ratio of disturbance-sensitive taxa to insensitive taxa shows slight alteration from type-specific levels. The level of diversity of invertebrate taxa shows slight signs of alteration from type-specific levels.

## **Uncertainty**

### **3.13 Consideration of uncertainty:** Yes

Described in: Davy-Bowker, J., Clarke, R., Corbin, T., Vincent, H., Pretty, J., Hawke, A., Blackburn, J., Jones, I., (2008) "River Invertebrate Classification Tool" SNIFFER. Available at:  
<http://www.sniffer.org.uk/Webcontrol/Secure/ClientSpecific/ResourceManagement/UploadedFiles/WFD72C%20FINAL%20REPORT%20with%20security.pdf>

### **3.14 Comments:**

SEPA: Although this is a description of the current Macro-invertebrate method used for classification in relation to general pressure (indicated in A-12) in the UK, further refinements are imminent notably: Further validation against pressures & testing of abundance-weighted metric (WHPT) based on ASPT/NTAXA.

ID: 158

CroTroph-D

## 1. General information

- 1.01 GIG:** Eastern Continental  
R-E2, R-E3, R-E4, R-E6, R-EX1, R-EX2, R-EX3, R-EX7, R-EX8
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Croatia
- 1.05 Specification:** none
- 1.06 Method name:** *Croatian Diatom Trophic Index*
- 1.07 Original name:** *Hrvatski trofički indeks dijatomeja*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, Flow modification, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Ács, E., G. Borics, G. Feher, K.T. Kiss, N.M. Rescone, C. Stegner-Kovacs & G. Vabrio, 2009. Implementation of European Water Framework Directive to assessment the water quality of Hungarian running waters with diatoms. Diatomedeligen 33. Ecosurv BQE report phytobenthos ministry of Environment and water, Hungary, October 2005, EuropeAid/114951/D/SV/2002-000-180-04-01-02-02. [Nijboer, R.C., R.K. Johnson, P.F.M. Verdonshot, M. Sommerhäuser & A. Buffagni, 2004. Establishing reference conditions for European streams Hydrobiologia 516: 91-105.](#) [Nijboer, R.C. & A. Schmidt-Kloiber, 2004. The effect of excluding taxa with low abundances or taxa with small distribution ranges on ecological assessment Hydrobiologia 516: 347-363.](#) [Verdonshot, P.F.M. & R.C. Nijboer, 2004. Testing the European stream typology of the Water Framework Directive for macroinvertebrates Hydrobiologia 516: 35-54.](#)
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Anđelka Plenković-Moraj<br>aplenk@zg.biol.pmf.hr<br>University of Zagreb, Faculty of Science, Division of Biology | <b>1.14 Method reported by</b><br>Dagmar Šurmanović<br>dagmar.surmanovic@voda.hr<br>Hrvatske vode |
|--|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Habđijija et al., 2008. Ecological Research of freshwater in Croatia regarding criteria of the Water Framework Directive of EU.  
[Mihaljević et al.: Testing of biological methods of ecological status assessment \(Water Framework Directive 2000/60/EC\) in representative River basins of the Pannonian and Dinaric ecoregions \(in preparation\)](#) [Instruction Protocol for the ecological Assessment of Running Waters SFOR Implementation of the CE Water Framework Directive: Macrophytes and Phytobenthos \(2006\).](#)
- 2.02 Short description**  
3 large stones from different river parts, strong tooth brush and scraper, all to one sample bottle and preserving with formaldehyde
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Brush, Scraper  
direct sampling from soft sedi
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Hard bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** May to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
once per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
minimum 3
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
3 hard surfaces

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** All diatoms, no matter the size
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Diatoms - 400 individuals
- 2.14 Level of taxonomical identification:** Genus, Species/species groups
- 2.15 Record of abundance:** Relative abundance  
**in relation to** n.a.  
**Unit** percentage
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
In preparation (TDICRO and IPS)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** n.a.
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** In preparation  
**Geographical coverage:** In preparation  
**Location of sites:** Several spring sources, National Park Plitvice Lakes and some others, but in preparation  
**Data time period:** Research project started in 2006  
**Criteria:**  
According to WFD Ref. Cond. final version
- 3.08 Reference community description**  
In preparation
- 3.09 Results expressed as EQR?** n.a.

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** n.a.
- 3.11 Boundary setting procedure**  
under development
- 3.12 "Good status" community:** Under development.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 221

HUNGPHYTOBENTRIVER

## 1. General information

- 1.01 GIG:** Eastern Continental
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Hungary
- 1.05 Specification:**
- 1.06 Method name:** *Improvement of the Hungarian ecological water qualification system - Phytobenthos in Rivers*
- 1.07 Original name:** *A magyarországi ökológiai minősítési rendszer továbbfejlesztése, fitobentosz*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

On basis of the pressure data (TP, BOD, CODCr, Electrical Conductivity) the LDS were selected. The relationship between the phytobenthos metrics and BOD, EC, COD, TN, ox. sat and SRP showed significant correlations in several types (Spearman Correlation Coefficient ranging from 0,17 to 0,61 if the relationship was significant).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Ács, É., G. Borics, G. Fehér, K.T. Kiss, N.M. Reskóné, M. Nagy, C. Stenger-Kovács, A. Tóth & G. Várbíró, 2009. A fitobentosz élőlénycsoport zárójelentése az ökológiai minősítési rendszer továbbfejlesztéséről.

**1.12 Scientific literature:**

Ács, É., G. Borics, G. Fehér, K.T. Kiss, N.M. Reskóné, C. Stenger-Kovács & G. Várbíró, 2009. Implementation of the European Water Framework Directive to assess the water quality in Hungarian running waters with diatoms. *Diatomededelingen* 33: 29-32. ISSN1872-9673. Szilágyi, F., É. Ács, G. Borics, B. Halasi-Kovács, P. Juhász, B. Kiss, T. Kovács, Z. Müller, G. Lakatos, J. Padišák, P. Pomogyi, C. Stenger-Kovács, K. É. Szabó, E. Szalma & B. Tóthmérész, 2008. Application of Water Framework Directive in Hungary: Development of Biological Classification Systems. *Water Science and Technology* 58: 2117-2125.

**1.13 Method developed by**

Eva Acs and Gábor Borics  
evaacs@freemail.hu, boricsg@gmail.com  
Hungarian Danube Research Station; Environmental Protection,  
Nature Conservation and Water Authority of Transiszanian  
Region

**1.14 Method reported by**

statt 161 Eva Acs  
evaacs@botanika.hu; evaacs@freemail.hu  
Hungarian Danube Research Station, Göd

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

CEN, 2003. Water quality. Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers. European Standard EN 13946. Brussels, European Committee for Standardization, 14 pp.

**2.02 Short description**

Rivers: 5 stones or 5 macrophytes stems are randomly selected from 10 to 100 m river stretch

**2.03 Method to select the sampling/survey site or area:** Random sampling/surveying

**2.04 Sampling/survey device:** Brush

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
stones, macrophytes

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** rivers: May to October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

5

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

5x10 cm<sup>2</sup>=50 cm<sup>2</sup>

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** every diatoms

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Relative abundance  
in relation to Area

**Unit** number of valves per 400 valves

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

relative abundance of taxa with indicator and sensitivity values for organic material and nutrients (diatom indices calculated by OMNIDIA)

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** -

**Geographical coverage:** -

**Location of sites:** n.a.

**Data time period:** -

**Criteria:**

It was practically impossible to find reference conditions, especially in case of lowland rivers and large rivers that are the most of Hungarian rivers, so we used the so called "Least Disturbed Sites" for boundary setting.

**3.08 Reference community description**

-

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

Reference conditions which could be applied across rivers in Hungary have not been established yet. Nevertheless unimpacted stretches or sites with low pollution and with smaller hydromorphological alterations can be found in almost every river type. On basis of the pressure data (TP, BOD, CODCr, Electrical Conductivity) the LDS were selected. 10th percentiles of the index values of the selected LDS sites were considered as high/good (H/G) class boundaries and 75th percentiles as good/moderate G/M boundaries in every type. The rest of data was divided into 3 equal parts between the minimum value of the index in a given river groups and the G/M value in order to set the further boundaries. Theoretical EQR values (H/G= 0.8; G/M= 0.6; M/P= 0.4; P/B= 0.2) were plotted against the index boundaries for all types. By equation of the actual line of best fit the EQR values can be calculated.

**3.12 "Good status" community:** At good status stands of the sensitive taxa are well developed. They are dominant, but significantly decreasing at good-moderate boundary and replaced by tolerant taxa. The 10th percentiles of the index values of the selected LDS sites were considered as high/good (H/G) class boundaries.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 169

SK-PB-RI

## 1. General information

- 1.01 GIG:** Eastern Continental  
R-E1, R-E2, R-E3, R-E4, R-E6
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Slovakia
- 1.05 Specification:** none
- 1.06 Method name:** *Slovak assessment of benthic diatoms in rivers*
- 1.07 Original name:** *Metodika pre odvodenie referenčných podmienok a klasifikačných schém pre hodnotenie ekologického stavu vôd*
- 1.08 Status: Method is/will be used in** neither first nor second RBMP
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).  
Altogether 312 benthic diatom taxa from reference sites (115 reference sites) and 410 taxa from monitoring potentially impacted sites (313 sites): CEE, EPI-D, IPS - Strongest relation to Organic pollution (Spearman Correlation Coefficient): BOD5 - CEE and EPI-D (-0,513; -0,520), total P and total N - EPI-D (-0,611; -0,616). All indices were correlated significantly with land use. CEE, EPI-D and IPS with Agricultural Land Use (-,0461; -0,444; -0,441), Urban Areas (-0,405; -0,441; -0,444) and correlation with Hydromorphological Quality Score (-0,204; -0,178; -0,263).
- 1.10 Internet reference:** <http://www.vuvh.sk/rsv/?page=download>
- 1.11 Pertinent literature of mandatory character:**  
STN 757715. Biological analysis of surface water, 2008. Directive 2000/60/EC of the European Parliament and of the Council of 23 October establishing a framework of Community action in the field of water policy. REFCOND, 2003. Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance document No. 10. Rivers and Lakes - Typology, Reference Conditions and Classification Systems. European Communities, Luxembourg. Šporka, F., J. Makovinská, D. Hlúbiková, L. Tóthová, V. Mužík, R. Magulová, K. Kučárová, P. Pekárová & Mrafková, 2007. Method of the derivation of reference conditions and classification schemes for ecological status assessment. WIR Bratislava, SHMÚ Bratislava, ÚZ SAV Bratislava, SAŽP Banská Bystrica. www.vuvh.sk., 288 pp. National method for evaluation ES of streams based on (www.aqbios.com).
- 1.12 Scientific literature:**  
Ács, E., K. Szabó, B. Tóth & K.T. Kiss, 2004. Investigation of benthic algal communities, especially diatoms of some Hungarian streams in connection with reference conditions of the Water Framework Directives. Acta Botanica Hungarica 46 (3-4): 255-277. Descy, J.P. & M. Coste, 1991. A test of methods for assessing water quality based on diatoms. Verh. Internat. Verein. Limnol. 24: 2112-2116. Kelly, M.G., A. Cazaubon, E. Coring, A. Dell'uomo, L. Ector, B. Goldschmidt, H. Guasch, J. Hurliman, A. Jarlam, B. Kawecka, J. Kwadrans, R. Laugaste, E.A. Lindstrom, M. Leirao, P. Marvan, J. Pasisak, J. Prygiel, E. Rott, S. Sabater, H. Van Dam & J. Vizinet, 1998. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. Journal of Applied Phycology 10: 215-224. Lecointe, C., M. Coste & J. Prygiel, 1993. Omnidia software for taxonomy, calculation of diatom indices and inventories management. Hydrobiologia 269 (270): 509-513. Pantle, R. & H. Buck, 1955. Die biologische Überwachung der Gewässer und die Darstellung der Ergebnisse. Gas und Wasserfach 96: 604. Prygiel, J. & M. Coste, 1993. The assessment of water quality in the Artois Picardie water basin (France) by the use of diatom indices. Hydrobiologia 269 (270): 343-349. Rott, E., 1991. Methodological aspects and perspectives in the use of periphyton for monitoring and protecting rivers. In Whitton, B.A., E. Rott & G. Friedrich (eds), Use of Algae for Monitoring Rivers, Institut für Botanik, Universität Innsbruck, Innsbruck, pp. 9-16. Rott, E., E. Pipp & P. Pfister, 2003. Diatom methods developed for river quality assessment in Austria and a cross-check against numerical trophic indication methods used in Europe. Algological Studies 110: 91-115. Stevenson, R.J. & Y. Pan, 1999. Assessing environmental conditions in rivers and streams with diatoms. In Stroemer, E.F. & J.P. Smol (eds), The diatoms: Application for the environmental and earth sciences, Cambridge University Press, Cambridge, pp. 11-40. Van Dam, H., A. Mertens & J. Sinkeldam, 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Netherlands Journal of Aquatic Ecology 28 (1): 117-133. Zelinka, M. & P. Marvan, 1961. Zur Präzisierung der biologischen Klassifikation der Reinheit fließender Gewässer. Archiv für Hydrobiologie 57: 389-407.
- 1.13 Method developed by**  
Dr. Dáša Hlúbiková  
hlubikova@vuvh.sk  
Water Research Institute, Slovak Academy of Science
- 1.14 Method reported by**  
Matus Haviar; Emilia Misikova Elexova  
haviar@vuvh.sk; elexova@vuvh.sk  
Water Research Institute, Slovak National Water Reference Laboratory
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
EN 13946, 2003. Water quality. Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers.
- 2.02 Short description**  
According to EN 13946: 2003. Water quality. Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers, STN 757715 Biological analysis of surface water.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge

- 2.04 Sampling/survey device:** Brush  
Toothbrush
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** Single habitat(s)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** April to May; September to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
2 occasions
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
min. 100 cm<sup>2</sup> of rock surface
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
benthic diatoms min. 100 cm<sup>2</sup>

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** without size limitation - benthic diatoms
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups
- 2.15 Record of abundance:** Individual counts, Relative abundance  
in relation to Area  
Unit Number of valvae (300-500).
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** Non-wadable rivers are sampled only at the banks (riparian zones), i.e. multi-habitat-sampling is confined to the river margin habitats (benthic invertebrates and diatoms).
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
IPS, CEE, EPI-D indices
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Worst quality class  
between benthic diatoms and bacteria moduls - worst result classifies
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
(each season separately)

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Least Disturbed Conditions, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**
- Number of sites:** 60 Sites in Carpathian region
- Geographical coverage:** Carpathians, Pannonian lowland
- Location of sites:** Western (majority of territory of Slovakia) and Eastern Carpathians (Northeastern Slovakia) from more than 200 metres a.s.l. to 1000 metres a.s.l.
- Data time period:** April - May 2004, 2005, September - October 2003, 2004, 2005
- Criteria:**  
n.a.
- 3.08 Reference community description**  
Background taxa lists especially created for good status conditions as well as for any other ecological status classes are not prescribed in Slovakia.
- 3.09 Results expressed as EQR?** Yes

## **Boundary setting**

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites

### **3.11 Boundary setting procedure**

Benthic diatoms: 2 modules - benthic diatoms and filamentous bacteria. a) benthic diatoms modul-4 altitude categories, based on reference sites within 2004 . For 200-500, 500-800 and above 800 - boundary between H/G = 25. Percentile of average based on reference sites in 2004. For altitude below 200 linear model used - derived from type of altitude 200-500 by means of modelling (this procedure - applied for all 3 metrics). The other boundaries calculated using the range of metrics values within high status (best value) and minimal calculated value of metric from the data set. The whole range was equally subdivided and boundaries were stated accordingly. b) filamentous bacteria module-percentage of bacteria in phytobenthos in vivo (5-class classification). Each class is classified by Score (below 1 %-5, 1-10 %-4, 11-25%-3, 25-40%-2, above 40%-1.) Result of both modules= the worse value classifies.

**3.12 "Good status" community:** In Slovakia background taxa lists are not prescribed and especially created for good status conditions as well as for any other ES classes.

## **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Benthic diatoms: Cases of uncertainty which were calculated: Step 1: Sampling - reproducibility and repeatability, Step 2: preparing the preparete - reproducibility, repeatability and homogeneity (measured including in repeatability, Step 3: repeatability of index in analysis of the sample. Method: Four samplers took both samples from the same site (together 8 samples), analyses of each sample carried out in laboratory by one person making permanent preparete and analyses. In the last step, total expanded uncertainty was calculated from calculated relative combined uncertainty of sampling, prepares and analysis. The result was 23,21%.

**3.14 Comments:**

none

ID: 76

CZ-PB-RI

## 1. General information

- 1.01 GIG:** Eastern Continental  
R-E1, R-E3
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms, Other Phytobenthos
- 1.04 Country:** Czech Republic
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment system for rivers using phytobenthos*
- 1.07 Original name:** *Hodnocení tekoucích vod podle fyto-bentosu*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Ecological data from 120 sites (spring season) - the correlation of the Czech saprobic-trophic index was the best for BOD5, NO2, N-tot, P-tot, Cl (correlation coefficient between 0.5 and 0.6). Additional analyses with a larger dataset in preparation.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Indicators proposed for the water quality monitoring in the Czech Republic are based on indicator lists compiled by Sládeček et Sládečková, but they underwent revisions (ČSN 75 7716. Jakost vod - Biologický rozbor - Stanovení saprobiálního indexu, 1998).

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

n.a.

n.a.

**1.14 Method reported by**

Libuse Opatrilova

libuse\_opatrilova@vuv.cz

T.G. Masaryk Water Research Institute, Prague

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

EN 15708: 2009 Water quality - Guidance standard for the surveying, sampling and laboratory analysis of phytobenthos in shallow running water.

**2.02 Short description**

Single habitat sampling of epilithion (and epibryon, if present) in the sampling reach, from several microhabitats in the streamline, using brush and scraper for removing algal mats

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Brush, Scraper

**2.05 Specification:** no special equipment

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
preferably epilithic phytobenthos - mesolithal

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** April-May, June-July, September-October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

According to the methodology three occasions per year, within the operational monitoring two occasions per year (spring,

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

5 stones from different points of streamline

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

it is not possible to quantify, depends on bottom character

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** all size categories (including filamentous algae like Cladophora or Spirogyra, but excluding mosses and stoneworts)

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

For diatom identification only a part of sample is processed but not exactly specified.

- 2.14 Level of taxonomical identification:** Species/species groups  
Identification level depends on e.g. presence of respective morphological features. Czech taxalist involves separate items for species, infraspecific taxa (incl. their typical/nominate varieties or forms), groups (aggregates) of hardly distinguishable species with similar autecological demands, names of algae used in different identification compendia with different taxonomical content, in selected cases also generic names.
- 2.15 Record of abundance:** Abundance classes  
(for all components of phytobenthos, i.e. also for diatoms)  
**in relation to** Area  
(covered by individual phytobenthos taxa)  
**Unit** Abundance classes 1-7
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
One Czech saprobic-trophic index - weighted average (average of sensitivity values weighted with product of indicator weight  $g \times$  abundance  $h$  - calculated in the same way as in Omnidia for most index types involved). As an alternative median of sensitivity values is considered.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** will be used about 40 sites  
**Geographical coverage:** rather smaller streams in higher altitudes of the Czech Republic  
**Location of sites:** n.a.  
**Data time period:** will be used data from spring 2007 and 2008  
**Criteria:**  
no or minor hydromorphological impairment, land-use criteria, chemical parameters (P-PO<sub>4</sub>, N-NO<sub>3</sub>, BOD<sub>5</sub>), no pressures
- 3.08 Reference community description**  
Reference communities have not been set yet.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.
- 3.11 Boundary setting procedure**  
For the first version of methodology the reference values were derived from Austrian and German values for similar water types (because of lack of data). Confirmation or adjustment according to data from the national monitoring and procedures mentioned above will be done during January and February 2010.
- 3.12 "Good status" community:** Not available at the moment.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

It is planned to calculate metric dispersion and its approximate confidence limits. Metrics with too broad confidence limits should be excluded from evaluation. Limit values for reliable metric values have not been established yet.

**3.14 Comments:**

none

ID: 148

AQEM system

## 1. General information

- 1.01 GIG:** Eastern Continental  
R-E2, R-E3, R-E4, R-E6, R-EX1, R-EX2, R-EX3, R-EX7, R-EX8
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Croatia
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment system for rivers using macrozoobenthos*
- 1.07 Original name:** *Procjena ekološkog stanja tekućica temeljem makrozoobentosa*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** General degradation, Hydromorphological degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
AQEM Consortium, 2002. A Comprehensive Method to assess European Streams using Benthic Macroinvertebrates, developed for the Purpose of the Water Framework Directive. Version 1.0. Barbour, M.T., J. Gerritsen, B.D. Snyder & J.B. Stribling, 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C. Barbour, M.T., J.L. Plafkin, B.P. Bradley, C.G. Graves & R.W. Wiseman, 1992. Evaluation of EPA's rapid bioassessment benthic metrics: metric redundancy and variability among reference stream sites. Environ. Toxicol. Chem. 11: 437-449. Hering, D., C.K. Feld, O. Moog & T. Ofenböck, 2006. Cook book for the development of a Multimetric Index for Biological condition of aquatic ecosystems: experiences from the European AQEM and STAR projects and related initiatives. Hydrobiologia 566: 311-324. Šporka, F., Pastuchová, L. Hamerlík, M. Dobiašová & P. Beracko, 2009. Assessment of running waters (Slovakia) using benthic macroinvertebrates -derivation of ecological quality classes with respect to altitudinal gradients. Biologia 64 (6): 1196-1205.
- 1.13 Method developed by**  
Mladen Kerovec, Zlatko Mihaljević  
mkerovec@biol.pmf.hr, zmihalj@biol.pmf.hr  
University of Zagreb, Faculty of Science, Division of Biology
- 1.14 Method reported by**  
Dagmar Šurmanović  
dagmar.surmanovic@voda.hr  
Hrvatske vode
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Habdiija et al., 2008. Ecological Research of freshwater in Croatia regarding criteria of the Water Framework Directive of EU. Mihaljevic et al., 2010. Testing of biological methods of ecological status assessment (Water Framework Directive 2000/60/EC) in representative River basins of the Pannonian and Dinaric ecoregions (in preparation).
- 2.02 Short description**  
Multi-habitat sampling designed for sampling major habitats in proportion to their presence within a sampling reach is carried out. A sample consists of 20 "sampling units" taken from all habitat types at the sampling site with a share of at least 5 % coverage. A "sampling unit" is a stationary sampling performed by positioning the net and disturbing the substrate in a quadratic area that equals the frame-size upstream of the net (0.25 x 0.25 m).
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Grab, Hand net, Surber or Hess sampler
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** streams: March to May, large rivers: June to August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
once per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
20 replicates (one per stream microhabitat >5% coverage)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
sum of 20 spatial replicates; 20 x 0.0625 square-metres = 1.25 square-metres of stream bottom in total

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 500 µm (mesh-size of hand net)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Sub-sampling is performed if the sample contained more than 500 organisms. One/half to one/sixth of sampling material is separated from which 500 organisms are analyzed.
- 2.14 Level of taxonomical identification:** Family, Genus, Species/species groups  
Bivalvia, Crustacea, Coleoptera, Ephemeroptera, Gastropoda, Megaloptera, Odonata, Plecoptera, Trichoptera, Tricladica = species or genus level; Diptera = family level; Chironomidae = subfamilies, some species and species groups; Oligochaeta = families and some species
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per one square-metre
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** Non-wadable rivers are sampled only at the banks, i.e. multi-habitat-sampling is confined to the river margin habitats.
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Macroinvertebrates - taxa list with relative abundance and saprobic values of species.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores, Worst quality class
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** In preparation  
**Geographical coverage:** In preparation  
**Location of sites:** National Park Plitvice Lakes and some others, but in preparation  
**Data time period:** Research project started in 2006  
**Criteria:**  
According to WFD Ref. Cond. final version.
- 3.08 Reference community description**  
In preparation
- 3.09 Results expressed as EQR?** n.a.

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** n.a.
- 3.11 Boundary setting procedure**  
under development
- 3.12 "Good status" community:** Under development.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)

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**3.14 Comments:**

Our intention is to select appropriate metrics for each type or group of stream types. Suitable metrics will be selected according to their ability to distinguish reference and monitoring sites. Reference sites are lacking for some stream types. In such cases our intention is to apply some metrics, the value of which change along a longitudinal gradient, also react to anthropogenic stress.

ID: 214

CZ-BI-RI

## 1. General information

**1.01 GIG:** Eastern Continental  
probably will be intercalibrated types R-E1 (Carpathians, small to medium , mid altitude), R-E3 (large,  
**1.02 Category:** lowland rivers)

**1.03 BQE:** Benthic Invertebrates

**1.04 Country:** Czech Republic

**1.05 Specification:** all regions excluding non-wadable parts of large rivers

**1.06 Method name:** *Czech system for ecological status assessment of rivers using benthic macroinvertebrates*

**1.07 Original name:** *Systém pro hodnocení ekologického stavu toku podle makrozoobentosu*

**1.08 Status: Method is/will be used in** Second RBMP (2015)

**1.09 Detected pressure(s):** General degradation, Hydromorphological degradation, Pollution by organic matter

*Has the pressure-impact-relationship been tested?*

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

The relationship between biological metrics and pressure parameters (chemical parameters, hydromorphology alteration, land-use) was tested on data from 470 sites by using the statistical method Structural Equation Modeling.

**1.10 Internet reference:** [http://www.mzp.cz/prehled\\_akeptovanych\\_metodik\\_tekoucich\\_vod](http://www.mzp.cz/prehled_akeptovanych_metodik_tekoucich_vod) (only a sampling method, in Czech)

**1.11 Pertinent literature of mandatory character:**

CSN 75 7701, 2007. Jakost vod – Metodika odberu a zpracovani vzorku makrozoobentosu tekoucich vod metodou PERLA (Water quality – Methodology for sampling and treatment of macroinvertebrates from running waters using method PERLA).

**1.12 Scientific literature:**

Kokes, J., S. Zahradkova, D. Nemejcova, J. Hodovsky, J. Jarkovsky & T. Soldan, 2006. The PERLA system in the Czech Republic: a multivariate approach for assessing the ecological status of running waters. *Hydrobiologia* 566: 343– 354. (only a sampling method; assessment method has not been published yet).

**1.13 Method developed by**

Libuse Opatrilova, Denisa Nemejcova

libuse\_opatrilova@vuv.cz, denisa\_nemejcova@vuv.cz

T. G. Masaryk Water Research Institute, Public Research Institution

**1.14 Method reported by**

Denisa Nemejcova, Libuse Opatrilova

denisa\_nemejcova@vuv.cz, libuse\_opatrilova@vuv.cz

T.G.Masaryk Water Research Institute, public research institution

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Kokes, J. & D. Nemejcova, 2006. Metodika odberu a zpracovani vzorku makrozoobentosu tekoucich vod metodou PERLA. Závazná metodika programu monitoringu MZP. (Methodology for sampling and treatment of benthic macroinvertebrates from running waters using method PERLA. Mandatory method of the Ministry of Environment's monitoring programme.)

**2.02 Short description**

The sampling section is sampled using a multi habitat sampling method. Semi-quantitative 3-minute kick sample gathered with a hand net (25x40 cm aperture and 500 µm mesh size) is taken. All habitats (riffle, pool, macrophytes, woody debris, etc.) are sampled at respective time corresponding to their total area proportion in the sampling section. (Kokes, J. et al., 2006)

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Hand net

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** spring (March - May), autumn (September – November)

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Now one occasion is used for evaluation - spring; in a near future it is planned to evaluate two sampling occasions - spring

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

one replicate per sampling occasion (all habitats are sampled adequate time according to their percent coverage)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

3-minute kick sample

## **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Hand net (25x40 cm aperture and 500 µm mesh size)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Samples are pre-selected in the field (to preserve fragile organisms) and transferred to the laboratory where final sorting is done. With some quantitatively rich samples (approx. more than 1000 organisms), their half or quarter is processed and the final number of individuals is estimated by simple multiplication.
- 2.14 Level of taxonomical identification:** Genus, Other, Species/species groups  
Obligatory taxonomical level (the first proposal at the moment) has been specified in the software where data are collected and an assessment should be done. Some taxa are not identified to the species level; for example Nematoda, Ceratopogonidae. Chironomids should be identified mainly to level of genus.
- 2.15 Record of abundance:** Individual counts  
**in relation to** Time  
**Unit** Number of individuals per sample
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** For large non-wadable rivers exists a different sampling method, but within a routine monitoring non-wadable rivers are sampled according to above described method and multi-habitat sampling is done only on restricted bottom area. The assessment of non-wadable rivers is under development.
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
For 11 river types will be used multimetrics completed by choosing from following metrics: Composition/abundance metrics – % abundance of EPT taxa, Trichoptera, Plecoptera and Chironomidae taxa. Richness/diversity metrics: total number of taxa, Margalef and Shannon diversity, number of EPT taxa, number of Ephemeroptera, Trichoptera and Plecoptera taxa, number of rare taxa. Sensitivity/tolerance metrics: Czech Saprobic index, % of alfa-mesosaprobita preferences. Functional metrics: % of litorral, lithal, psammal and epirhithral preferences, Rhithron Typie Index, stone-dwelling taxa (Braukmann, with abundance classes). The combination with B index of prediction model which follows RIVPACS principles has been tested.
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**  
**Number of sites:** approx. 150 sites  
**Geographical coverage:** all of the Czech Republic  
**Location of sites:** all of the Czech Republic  
**Data time period:** 1996-2000, 2007  
**Criteria:**  
The following criteria were taken into consideration in order to meet the requirements of Czech National Standards: •The degree of urbanisation, agriculture, and silviculture in a catchment must be as low as possible. (catchment land use: intensive agriculture < 50%) •A reference site floodplain should preferably not be cultivated. •Stream bottoms and stream banks must not be fixed (old river bank fixation by a belt of trees is acceptable). •Natural riparian vegetation and floodplain conditions must still exist, making lateral connectivity between the stream and its floodplain possible. •No alterations of the natural hydrographic and discharge regime. •No hydrological alterations such as water diversion, abstraction, or pulse releases. •No (or only minor) upstream impoundments, reservoirs, weirs, or reservoirs retaining sediments may be present (a dam 20 km upstream is acceptable for some stretches of mid-sized or large streams). •Physical and chemical conditions close to natural background levels describing the baseload of a specific catchment area. •No point sources of pollution or

nutrients.☐ •No signs of acidification.☐ •No liming activities.☐ •No impairment due to physical conditions, especially the thermal conditions, which must be close to natural.☐ •There must not be any significant impairment of the allochthonous biota by introduced taxa (Crustacea or Mollusca).

**3.08 Reference community description**

Reference communities for relevant river types were not set, only reference values of biological metrics.

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

At the moment the type specific reference value was set as a median of relevant metric values from strictly chosen reference sites. The class boundaries were set as equidistant division of the EQR gradient. The preclassification based on expert judgement and comparison with results of the prediction model will be used for a confirmation of the boundaries.

**3.12 "Good status" community:** n.a.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 163

QBAP

## 1. General information

- 1.01 GIG:** Eastern Continental  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Hungary
- 1.05 Specification:** none
- 1.06 Method name:** *Macroinvertebrate-based assessment method for rivers*
- 1.07 Original name:** *Makrogerincteleneken alapuló minősítési rendszer - folyók*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Flow modification, General degradation, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Zoltán Müller et al.  
mullerz@bioaquapro.hu  
BioAquaPo Plc.
- 1.14 Method reported by**  
Béla Csányi  
bela.csanyi@gmail.com  
Inst. of Env. Protect. & Water Management Plc.
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
AQEM Consortium, 2002. Manual for the application of the AQEM system. A Comprehensive Method to assess European Streams using Benthic Macroinvertebrates, developed for the Purpose of the Water Framework Directive. Version 1.0.
- 2.02 Short description**  
Multi-habitat sampling designed for sampling major habitats in proportion to their presence within a sampling reach is carried out. A sample consists of 20 "sampling units" taken from all habitat types at the sampling site with a share of at least 5 % coverage. A "sampling unit" is a stationary sampling performed by positioning the net and disturbing the substrate in a quadratic area that equals the frame-size upstream of the net (0.25 x 0.25 m). Sediments must be disturbed to a depth of 15-20 cm (where possible) depending on substrate compactness.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** FBA pond net
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** April to September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Once a year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
10 (low diversity) or 20 (high diversity) depending on the diversity of habitats
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
0.625 or 1.25 m<sup>2</sup> (10 or 20 units)

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 950 micron (mesh-size of hand net)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
At least one/sixth of sampling material is separated (5 grid squares) - if the number of separated individuals reach 500/+-20 %. If not - additional grid squares are separated until 500 ind.(+-20 %) is separated. Number of separated grid squares is note

- 2.14 Level of taxonomical identification:** Other, Species/species groups  
Oligochaeta and Chironomidae and other Diptera are not determined to sp. (max. genus level)
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** ind/m<sup>2</sup>
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** Non-wadable rivers are sampled only in the littoral zone (MHS according to AQEM protocol, 10 sub-samples for low diversity of habitats, 20 sub-samples for high diversity of habitats)
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**
- $Q_{bap} = \text{Sum of } (K \cdot S \cdot M) / P_{max}$   
where for each species: K = character factor; S = significance factor; M = quantity factor; P<sub>max</sub> is the maximum score for each type
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge
- 3.07 Reference site characterisation**
- Number of sites:** n.a.  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** n.a.
- Criteria:**  
The best available quality sites within the given type is used.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
Determination of RefCond and pressure specificity should be further developed.

ID: 108

ECO-BENT

## 1. General information

- 1.01 GIG:** Eastern Continental  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Romania
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment method for ecological status of water bodies based on macroinvertebrates*
- 1.07 Original name:** *Assessment method for ecological status of water bodies based on macroinvertebrates*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation, Habitat destruction, Pollution by organic matter, Riparian habitat alteration
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Chiriac, G. et al., 2007. Assessment of the ecological status of various lotic ecosystems from the H.B. Jiu using biotic communities according to the WFD requirements. Oltenia. Studii și comunicări. Științele naturii, Craiova. Chiriac, G. & F. Vintilă, 2005. Inventarierea comunităților biotice acvatice din b.h. Mureș în conformitate cu cerințele Directivei Cadru a apelor, vol. Oltenia. Studii și comunicări. Științele naturii, XXI/2005, Craiova. Lungu, A. et al., 2007. Evaluarea stării ecologice a unor acumulări cu folosințe importante din b.h. Argeș conform cerințelor DCA (Stuc de caz: acumularea Golești), Lucr. Conf. Intern. Aquatic Biodiversity, Acta Oecologica, Studii și Comunicări de Ecologie și Protecția Mediului, vol. XIV (1-2): 71-81. Preda, E. et al., 2007. Aspecte teoretice și practice ale abordării multimetrice în evaluarea stării ecologice a ecosistemelor acvatice lotice din România, Conferința Națională de Ecologie, 11-14 octombrie 2007, Mamaia.
- |  |  |
|--|--|
| <b>1.13 Method developed by</b><br>Dr. Gabriel CHIRIAC<br>gabriel.chiriac@rowater.ro<br>ICIM - National R & D Institute for Environment Protection | <b>1.14 Method reported by</b><br>Serban ILIESCU<br>serban.iliescu@rowater.ro<br>Romanian Water Authority - Department of The Monitoring Water Resources |
|--|--|
- 1.15 Comments**  
Method will be tested and validated until RBMP 2015

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
AQEM - MULTIHABITAT (Modified).
- 2.02 Short description**  
Multi-habitat scheme; coverage of all representative microhabitats; 5-20 "replicates"; Rinsing-Sieving-Sorting; Sub-sampling (in some cases); Identification
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying
- 2.04 Sampling/survey device:** Grab, Hand net, Surber or Hess sampler
- 2.05 Specification:** Ponar grab, Surber sampler, Hand net.
- 2.06 Sampled/surveyed habitat:** n.a.
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** May / April; July / August; September / October.
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
2 - 3 Times / year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
5 - 20 replicates
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
0.321 - 1.25 m<sup>2</sup>

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 250 μm mesh size of hand net
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Family, Genus, Species/species groups  
Oligochaeta, Chironomidae – species levels, genus levels; Coleoptera, Heteroptera - genus levels; Plathelminthes

(Turbellaria), Gastropoda, Bivalvia, Hirudinea, Arthropoda, Ephemeroptera, Odonata, Plecoptera, Diptera, species levels.

**2.15 Record of abundance:** Individual counts

**in relation to** Area

**Unit** Number of individuals per one square-meter

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Saprobic index; No of individuals; EPT; Shannon-Wiener-Diversity

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Weighted average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

Aggregated data from multiple spatial replicates

#### Reference conditions

**3.05 Scope of reference conditions:** n.a.

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Historical data

**3.07 Reference site characterisation**

**Number of sites:** 295

**Geographical coverage:** Carpathians, Subcarpathians Hills; 4 Ecoregions

**Location of sites:** Retezat Park; Calimani Natinal Park; Maramures Zone.

**Data time period:** 1960s; 2004 – 2007.

**Criteria:**

n.a.

**3.08 Reference community description**

Presence of sensitive taxa, such as: stoneflies, mayflies, caddisflies etc.; high diversity; absence or very little presence of oligochaets.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

Organic pollution and saprobic index; ecological status boundaries H/G = 1.55; G/M = 1.80.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 164

SK-BI-RI

## 1. General information

- 1.01 GIG:** Eastern Continental  
R-E1, R-E2, R-E3, R-E4, R-E6
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Slovakia
- 1.05 Specification:** none
- 1.06 Method name:** *Slovak assessment of benthic invertebrates in rivers*
- 1.07 Original name:** *Metodika pre odvodenie referenčných podmienok a klasifikačných schém pre hodnotenie ekologického stavu vôd*
- 1.08 Status: Method is/will be used in** neither first nor second RBMP
- 1.09 Detected pressure(s):** Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration

**Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

n.a..

- 1.10 Internet reference:** <http://www.vuvh.sk/rsv/?page=download>
- 1.11 Pertinent literature of mandatory character:**  
STN 757715. Biological analysis of surface water, 2008. Šporka, F., J. Makovinská, D. Hlubíková, L. Tóthová, V. Mužík, R. Magulová, K. Kučárov P. Pekárová & L. Mrafková, 2007. Method of the derivation of reference conditions and classification schemes for ecological status assessment. WIR Bratislava, SHMÚ Bratislava, ÚZ SAV Bratislava, SAŽP Banská Bystrica, 288 pp. www.vuvh.sk.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Dr. Ferdinand Šporka  
hlubikova@vuvh.sk, makovinska@vuvh.sk, tothova@vuvh.sk,  
ferdinand.sporka@savba, kovac@fns.uniba.sk  
Water Research Institute, Slovak Academy of Science, Faculty of  
Natural Sciences
- 1.14 Method reported by**  
Matus Haviar, Emilia Misikova Elexova  
haviar@vuvh.sk; elexova@vuvh.sk  
Water Research Institute, Slovak National Water Reference  
Laboratory
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
AQEM Consortium, 2002. Manual for the application of the AQEM system. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive. Version 1.0.
- 2.02 Short description**  
Identically with example + considering ratio of riffles/pools ratio (AQEM Cons. 2002)
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** hand net: stainless steel frame 25 x25 cm, length of net 1 m, long arm, stainless rake with telescopic long arm (up to 3 m), grapnel on the rope -up to 20m), aquascope in shallow running waters and lakes
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)  
Only non-wadable rivers (deep mid-size and large rivers) - riparian zones
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** April to May, September to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
2 occasions
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
20 (1 per microhabitat with coverage more than 5 %)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
benthic invertebrates:  $20 * 0.0625 = 1.25 \text{ m}^2$

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 500 µm (mesh-size of hand net)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

At least one/sixth of sampling material is separated (5 grid squares) - if the number of separated individuals reach 500/±20 %). If not - additional grid squares are separated until 500 ind.(±20 %) is separated. Number of separated grid squares is note

**2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
species, species groups: Turbellaria, Oligochaeta, Hirudinea, Mollusca (Gastropoda), Crustacea, Plecoptera, Ephemeroptera, Odonata, Megaloptera, Trichoptera, Chironomidae, Other Diptera  
genus: Oligochaeta, Bivalvia (Pisidium), Odonata, Trichoptera (juv.), Coleoptera, Chironomidae, other Diptera  
family: Oligochaeta, Trichoptera, Coleoptera, other Diptera, Simuliidae; other level: Porifera, Bryozoa

**2.15 Record of abundance:** Individual counts  
in relation to Area  
Unit Number of individuals per one square-metre

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** Non-wadable rivers are sampled only at the banks (riparian zones), i.e. multi-habitat-sampling is confined to the river margin habitats.

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**  
Saprobic index, oligo%, Rhithron Type Index, Index of Biocoenotic Region, Aka+Lit+Psa (%), EPT-taxa, BMWP Score, Rheoindex (banning, with abundance classes), % metarhithral, Diversity (Margalef Index), % Gatherers/Collectors, Number of families

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple spatial replicates  
(each season separately, 20 replicates mixed together)

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Least Disturbed Conditions, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** 60 Sites in Carpathian region

**Geographical coverage:** Carpathians, Pannonian lowland

**Location of sites:** Western (majority of territory of Slovakia) and Eastern Carpathians (Northeastern Slovakia) from more than 200 metres a.s.l. to 1000 metres a.s.l.

**Data time period:** April - May 2004, 2005, September - October 2003, 2004, 2005

**Criteria:**  
n.a.

**3.08 Reference community description**

In Slovakia are not prescribed background taxa lists especially created for good status conditions as well as for any other classes of ecological status.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

STEP 1: selection of adequate metrics which appropriately describe community conditions, metrics calculation (ASTERICS) STEP 2: testing of metrics for each stream type (selection/ exclusion), creation of type-specific classification schemes STEP 3: calculation of reference values STEP 4: setting of boundary values for 5 ecological classes according formula  $cbv = [EQR * (trv - wv)] + wv$  (cbv – class boundary value, trv – theoretical reference value, wv – worst value, EQR gradually replaced by 0.8, 0.6, 0.4 and 0.2. Boundary values transformed into EQR because of comparability. STEP 5: calculation of multimetric index

**3.12 "Good status" community:** In Slovakia background taxa lists are not prescribed and especially created for good status conditions as well as for any other ES classes.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Cases of uncertainty which were calculated: Step 1: Sampling - reproducibility and repeatability Step 2: sub sampling - reproducibility and repeatability and homogeneity (measured including in repeatability Step 3: repeatability of index in analysis of the sample - determination and quantification. Method for step 1: Three samplers took three samples from the same site (together 9 samples), each sample analysed in laboratory by one person (6x). Following steps: quantification of uncertainty of sampling - calculation of indices Si, Oligo (Scored taxa = 100%), Index of Biocoenotic Region, Rheoindex) with calculation of average value of average analyses and deviation of analyses (repeatability of processing). Also standard deviation Sw was calculated, as the standard deviation of more analyses and Sx, as standard deviation of more samples. Before the last step total combined uncertainty of sampling (14.32%) , of sub sampling (12.2%) was calculated. In the last step total expanded uncertainty was calculated from calculated relative combined uncertainty of sampling, prepares and analysis. The result 37.63% is expanded uncertainty.

**3.14 Comments:**

none

ID: 165

SK-BI-RI large

## 1. General information

- 1.01 GIG:** Eastern Continental  
R-E2, R-E3, R-E6
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Slovakia
- 1.05 Specification:** none
- 1.06 Method name:** *Slovak assessment of benthic invertebrates in large rivers*
- 1.07 Original name:** *Metodika pre odvodenie referenčných podmienok a klasifikačných schém pre hodnotenie ekologického stavu vôd*
- 1.08 Status: Method is/will be used in** neither first nor second RBMP
- 1.09 Detected pressure(s):** Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).  
n.a.
- 1.10 Internet reference:** <http://www.vuvh.sk/rsv/?page=download>
- 1.11 Pertinent literature of mandatory character:**  
STN 757715. Biological analysis of surface water, 2008. Šporka, F., J. Makovinská, D. Hlúbiková, L. Tóthová, V. Mužík, R. Magulová, K. Kučárov P. Pekárová & L. Mrafková, 2007. Method of the derivation of reference conditions and classification schemes for ecological status assessment. WIR Bratislava, SHMÚ Bratislava, ÚZ SAV Bratislava, SAŽP Banská Bystrica, 288 pp. [www.vuvh.sk](http://www.vuvh.sk).
- 1.12 Scientific literature:**  
Graf, W., B. Csányi, P. Leitner, M. Paunovic, G. Chiriac, G., I.Stubauer, T. Ofenböck & F. Wagner, 2008. Macroinvertebrates. In Liška et al. (eds), Joint Danube Survey 2, Final Scientific Report. ICPDR, Vienna 242: 41-52. Hering, D., O. Moog, L. Sandin & P.F.M. Verdonchot, 2004. Overview and application of the AQEM assessment system. Hydrobiologia 516: 1-20. Moog, O., 1995. Fauna Aquatica Austriaca. Wassewirtschaftskataster, Bundesministerium für Land- und Fortwirtschaft, Wien.
- 1.13 Method developed by**  
Dr. Ferdinand Šporka (Slovak Academy of Sciences, benthic invertebrates)  
ferdinand.sporka@savba  
Slovak Academy of Science
- 1.14 Method reported by**  
Matus Haviar; Emilia Misikova Elexova  
haviar@vuvh.sk; elexova@vuvh.sk  
Water Research Institute, Slovak National Water Reference Laboratory
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
AQEM Consortium, 2002. Manual for the application of the AQEM system. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive. Version 1.0.
- 2.02 Short description**  
Large rivers- special homogeneous substrate (max. 2 /3 microhabitats) , but always the sampled area represents the area covered by  $20 \times 0.0625 = 1.25 \text{ m}^2$ . = related to ratio of substrate types coverage (in %). The following procedure as in wadable rivers sampling methodology (AQEM modified).
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** hand net: stainless steel frame 25 x25 cm, length of net 1 m, long arm
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)  
riparian zones
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** April to May, September to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
2 occasions
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
20 (1 per microhabitat with coverage more than 5 %)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
benthic invertebrates:  $20 * 0.0625 = 1.25 \text{ m}^2$

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 500 µm (mesh-size of hand net)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
At least one/sixth of sampling material is separated (5 grid squares) - if the number of separated individuals reach 500/+20 %. If not - additional grid squares are separated until 500 ind.(+20 %) is separated. Number of separated grid squares is note
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
species, species groups: Turbellaria, Oligochaeta, Hirudinea, Mollusca (Gastropoda), Crustacea, Plecoptera, Ephemeroptera, Odonata, Megaloptera, Trichoptera, Chironomidae, Other Diptera@genus: Oligochaeta, Bivalvia (Pisidium), Odonata, Trichoptera ( juv.), Coleoptera, Chironomidae, other Diptera@family: Oligochaeta, Trichoptera, Coleoptera, other Diptera, Simuliidae@other level: Porifera, Bryozoa
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per one square-metre
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** riparian zones
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Saprobic index, oligo%, Rhithron Type Index, Index of Biocoenotic Region, Aka+Lit+Psa (%), EPT-taxa (excl. Danube river R-E6), BMWP Score
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
(each season separately , 20 replicates mixed together)
- Reference conditions**
- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Least Disturbed Conditions, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** Pannonian lowland  
**Location of sites:** n.a.  
**Data time period:** Least disturbed since 1995 (twice per year)  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
Background taxa lists especially created for good status conditions as well as for any other ecological status classes are not prescribed in Slovakia.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure**  
STEP 1: selection of adequate metrics which appropriately describe community conditions, metrics calculation (ASTERICS)@STEP 2: testing of metrics for each stream type (selection/ exclusion), creation of type-specific classification schemes@STEP 3: calculation of reference values@STEP 4: setting of boundary values for 5 ecological classes according formula  $cbv = [EQR*(trv-wv)] + wv$  (cbv – class boundary value, trv – theoretical reference value, wv – worst value, EQR

gradually replaced by 0.8, 0.6, 0.4 and 0.2. Boundary values transformed into EQR because of comparability. STEP 5: calculation of multimetric index

**3.12 "Good status" community:** In Slovakia background taxa lists are not prescribed and especially created for good status conditions as well as for any other ES classes.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Cases of uncertainty which were calculated: Step 1: Sampling - reproducibility and repeatability; Step 2: sub sampling - reproducibility and repeatability and homogeneity (measured including in repeatability); Step 3: repeatability of index in analysis of the sample - determination and quantification. Method for step 1: Three samplers took three samples from the same site (together 9 samples), each sample analysed in laboratory by one person (6x). Following steps: quantification of uncertainty of sampling - calculation of indices Si, Oligo (Scored taxa = 100%), Index of Biocoenotic Region, Rheoindex) with calculation of average value of average analyses and deviation of analyses (repeatability of processing). Also standard deviation Sw was calculated, as the standard deviation of more analyses and Sx, as standard deviation of more samples. Before the last step total combined uncertainty of sampling (14.32%), of sub sampling (12.2%) was calculated. In the last step, total expanded uncertainty was calculated from calculated relative combined uncertainty of sampling, preparation and analysis. The result 37.63% is expanded uncertainty.

**3.14 Comments:**

none

ID: 151

EQI-HRF

## 1. General information

- 1.01 GIG:** Eastern Continental  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Hungary
- 1.05 Specification:** none
- 1.06 Method name:** *Ecological Quality Index of Hungarian Riverine Fishes*
- 1.07 Original name:** *Magyarországi vízfolyások halközösség alapú ökológiai minősítő rendszere*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Halasi- Kovacs, B., T. Erős, Á Harka, S.A. Nagy, Z. Sallai & B. Tóthmérész, 2009. A magyarországi folyóvíztestek halközösség alapú minősítése (Fish- assemblage-based ecological classification of Hungarian rivers). *Pisces Hungarici* III. 47-64.
- 1.12 Scientific literature:**  
n.a.
- |  |  |
|--|--|
| <b>1.13 Method developed by</b><br>Béla Halasi-Kovács<br>halasi1@t-online.hu<br>SCIAP Kft. | <b>1.14 Method reported by</b><br>Zoltan Szaloky<br>szaloky@vituki.hu<br>VITUKI Environmental and Water Management Research Institute<br>Non-profit Ltd. |
|--|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
EN 14011 Water quality - Sampling of fish with electricity@ECOSURV Protocol.
- 2.02 Short description**  
See: EN 14011 Water quality - Sampling of fish with electricity and ECOSURV Protocol
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Electrofishing gear
- 2.05 Specification:** Battery powered backpackers and electrofishers with engine
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** May to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
n.a.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
150-2500 m (based on river type)

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 5 mm (mesh-size of catcher bow net)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts, Relative abundance
- in relation to** Area  
**Unit** Number of individuals per one square-metre
- 2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** Length and agegroup (0+, adult) of individual specimens.

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Relative abundance of omnivorous species (%), Number of pelagic species, Rel. abundance of metaphytic species (%), Num. of benthic species, Num. of litophil species, Rel. abundance of phytophil species (%), Num. of rheophil species, Rel.abundance of stagophil species (%), Rel. abundance of specialist species (%), Rel. abundance of indigenous species (%)

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

Summed metric scores

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** n.a.

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**

The best available quality sites within the given type is used.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 230

EFI +

## 1. General information

- 1.01 GIG:** Eastern Continental
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Romania
- 1.05 Specification:**
- 1.06 Method name:** *New European Fish Index*
- 1.07 Original name:** *New European Fish Index*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration

*Has the pressure-impact-relationship been tested?*

- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**

n.a.

- 1.12 Scientific literature:**

n.a.

- 1.13 Method developed by**

efi-plus@boku.ac.at, klaus\_battes@yahoo.com

- 1.14 Method reported by**

Serban Iliescu, Claudia Pavelescu  
serban.iliescu@rowater.ro, claudia.pavelescu@dast.rowater.ro  
Romanian Water Authority - Department of The Monitoring Water Resources; Romanian Water Authority - Somes-Tisa Directorate

- 1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
SR EN 14011/2003. Water quality. Sampling of fish with electricity. SR EN ISO 14757/2005. Water quality. Sampling of fish with multi-mesh gillnets.
- 2.02 Short description**  
The fish are caught using the electrofishing gear on a known surface. At least 30 individuals are needed.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying
- 2.04 Sampling/survey device:** Electrofishing gear, Gill net
- 2.05 Specification:** Electrofishing gear; Gill net
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** late Spring- early Autumn
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
1 Time / 3 years
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1-3 replicates
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
It depends on the density of fish, habitat, river dimension (width), water deep

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** all
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per one sample
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** total length, partial length, height, thickness

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

EFI + index , metrics used to calculate: Salmonid: - density of species intolerant to oxygen depletion - density < 150mm (total length) of species intolerant to habitat degradation Cyprinid: - richness of species requiring lithophilic reproduction habitat - density of species requiring lithophilic reproduction habitat, species which spawn exclusively on gravel, rocks, stones, cobbles or pebbles

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** n.a.

The metric score is standardized distance (Miq) between the predicted value (Ti, i.e. the expected value in the absence of any significant human disturbance) and the observed value (Oi, computed from the sampled fish assemblage)

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Site-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Historical data, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** 143

**Geographical coverage:** Carpathians, Subcarpathians Hills; 4 Ecoregions

**Location of sites:** Retezat Park; Calimani National Park; Maramures Zone

**Data time period:** 1960s; 2004 – 2008

**Criteria:**

Natural (undisturbed) sites or near natural sites

**3.08 Reference community description**

Salmonid community Salmo trutta fario (dominant species), Eudontomyzon danfordi, Cottus gobio, Barbatula barbatula, Phoxinus phoxinus, Barbus petenyi, Thymallus thymallus Low number of species, well represented with one dominant species Cyprinid community No reference community or site for this category

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

EFI + criteria

**3.12 "Good status" community:**

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)  
no

**3.14 Comments:**  
none

ID: 166

FIS

## 1. General information

- 1.01 GIG:** Eastern Continental  
all available types, intercalibration is still in process
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Slovakia
- 1.05 Specification:** none
- 1.06 Method name:** *Fish Index of Slovakia*
- 1.07 Original name:** *Slovenský ichtyologický index*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Acidification, Flow modification, General degradation, Impact of alien species

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Unspecific pressures were tested using the Pressure index developed by Didier Pont. 88 sites were tested, the correlation between the FIS values and the pressure index values (mixed model) were found statically significant (Estimation Method: ML, Residual Variance Method: Profile, Fixed Effects SE Method: Prasad-Rao-Jeske-Kackar-Harville, Degrees of Freedom Method: Kenward-Roger; F Value 16.48, Pr > F <.0001). Impact of alien species on FIS is self-evident, as one of the metrics used to calculate FIS is the Relative abundance of invasive species.

- 1.10 Internet reference:** <http://www.aqbios.com>

**1.11 Pertinent literature of mandatory character:**

Dopracovanie metodiky stanovenia ekologického stavu vôd podľa rýb. Záverečná správa, 2008.

**1.12 Scientific literature:**

Balon, E.K., 1966. Príspevok k poznaniu vyváženosti rybích spoločenstiev v inundačných vodách Dunaja. *Biológia* 21 (12): 865-884. Copp, G.H., P.G. Bianco, N.G. Bogutskaya, T. Erős, I. Falka, M.T. Ferreira, M.G. Fox, J. Freyho, R.E. Gozlan, J. Grabowska, V. Kováč, R. Moreno-Amich, A.M. Naseka, M.G. Pawson, M. Penáz, M. Povž, M. Przybylski, M. Robillard, I.C. Russell, S. Stakénas, S. Šume A. Vila-Gispert & C. Wiesner, 2005. To be, or not to be, a non-native freshwater fish? *Journal of Applied Ichthyology* 21: 242-262. Karr, J.R., 1981. Assessment of biotic integrity using fish communities. *Fisheries* 6: 21-27. Kováč, V., K. Hensel, J. Černý, J. Kautman & J. Koščo, 2008. Invázne druhy rýb v povodiach Slovenska aktualizovaný zoznam 2007. *Chránené územi* 73: 30. Pont, D., B. Hugueny, N. Roset & C. Rogers, 2004. Development, Evaluation & Implementation of a Standardised Fish-based Assessment Method for the Ecological Status of European Rivers - A Contribution to the Water Framework Directive (FAME). Final Report, WP6-8, 5. Ribeiro, F., B. Elvira, M.J. Collares-Pereira & P.B. Moyle, 2007. Life-history traits of nonnative fishes in Iberian watersheds across several invasion stages: a first approach. *Biological Invasions* 1 (1): 89-102.

**1.13 Method developed by**

Vladimir Kovac  
vladimir.kovac@aqbios.com  
AQ-BIOS, s.r.o.

**1.14 Method reported by**

Vladimir Kovac  
kovac@fns.uniba.sk  
Department of Ecology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia; AQ-BIOS s.r.o.

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Hensel, K., 2001. Implementácia rámcovej smernice o vodách 2000/60/ES, časť „monitoring“ a hodnotenie povrchových vôd – ryby. *Slovenský hydrometeorologický ústav*, 22 s. Hensel, K., 2002. Pracovný postup pre odber vzoriek rýb so zreteľom na požiadavky Rámcovej smernice o vodách 2000/60/ES. *Slovenský hydrometeorologický ústav*, 16 s. Mužík, V., 2007. Ryby. In Šporka, F., J. Makovinská, D. Hlúbiková, L. Tóthová, V. Mužík, R. Magulová, K. Kučárová, P. Pekárová & L. Mrafková (eds), *Metodika pre odvolenie referenčných podmienok a klasifikačných schém pre hodnotenie ekologického stavu vôd*. VÚVH Bratislava, SHMÚ Bratislava, UZ SAV Bratislava, SAŽP Banská Bystrica. <http://www.vuvh.sk>, s. 210-247.

**2.02 Short description**

identical to EFI+, please visit <http://efi-plus.boku.ac.at> for details

- 2.03 Method to select the sampling/survey site or area:** Expert knowledge

- 2.04 Sampling/survey device:** Electrofishing gear

- 2.05 Specification:** electroshocking apparatus

- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)  
N/A

- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

- 2.08 Sampling/survey month(s):** April to November

- 2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season has been agreed among MS, though two or three occasions would provide a more realistic

#### 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area

one run has been agreed among the MS

#### 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area

depends on the size of the river; 100 m long stretch minimum, one anode per 5-7 m river width

### Sample processing

2.12 Minimum size of organisms sampled and processed: 20-30 mm

2.13 Sample treatment: Organisms of the complete sample are identified.

2.14 Level of taxonomical identification: Species/species groups

2.15 Record of abundance: Individual counts, Relative abundance

in relation to n.a.

Catch per unit effort (optional)

Unit not evaluated

2.16 Quantification of biomass: n.a.

2.17 Other biological data: Total length

2.18 Special cases, exceptions, additions: Non-wadable rivers are sampled mainly at the banks, i.e. multi-habitat-sampling is confined to the river margin habitats. However, boat sampling can be applied, if necessary.

2.19 Comments  
none

## 3. Data evaluation

### Evaluation

#### 3.01 List of biological metrics

1. Relative abundance of insectivorous species<sup>2</sup> 2. Relative abundance of phytophilous species<sup>3</sup> 3. Relative abundance of lithophilous species<sup>4</sup> 4. Relative abundance of benthic species<sup>5</sup> 5. Relative abundance of rheophilous species<sup>6</sup> 6. Relative abundance of potamodromous species<sup>7</sup> 7. Relative abundance of piscivorous species<sup>8</sup> 8. Relative abundance of salmonid species<sup>9</sup> 9. Relative abundance of invasive species<sup>10</sup> 10. Index of Equitability<sup>11</sup> FIS is finally calculated as mean value of the metrics 1-10.

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: Average metric scores

#### 3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

### Reference conditions

3.05 Scope of reference conditions: Habitat-specific

#### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Expert knowledge, Historical data

#### 3.07 Reference site characterisation

Number of sites: 37 sites in Slovakia

Geographical coverage: The whole country (Slovakia)

Location of sites: Distributed over the whole country

Data time period: Historical data, mostly before 1960s.

#### Criteria:

The absence of pressures had to be illustrated.

#### 3.08 Reference community description

Please see Table 2 (page 10) in Kovac, V., 2008: National Method for evaluation the ecological status of streams based on fishes: Fish Index of Slovakia. [http://www.aqbios.com/WFD\\_National\\_Method\\_FIS\\_Slovakia\\_V\\_Kovac\\_2009.pdf](http://www.aqbios.com/WFD_National_Method_FIS_Slovakia_V_Kovac_2009.pdf)

3.09 Results expressed as EQR? Yes

### Boundary setting

3.10 Setting of ecological status boundaries: Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites

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High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** This is expressed directly by FIS (which closely corresponds to EQR) that must be higher than 0.57.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

It was difficult to answer some questions in detail (e.g. some formulas or tables were not possible to copy into this document. Some refinements of the method are being still in process. Should you need to know more details, please do not hesitate to contact me by e-mail ([vladimir.kovac@aqbios.com](mailto:vladimir.kovac@aqbios.com)). I will be happy to answer. I also apologize for my late submission

ID: 155

CRO MAM

## 1. General information

- 1.01 GIG:** Eastern Continental  
R-E2, R-E3, R-E4, R-E6, R-EX1, R-EX2, R-EX3, R-EX7, R-EX8
- 1.02 Category:** Rivers
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Croatia
- 1.05 Specification:** none
- 1.06 Method name:** *Croatian macrophyte assessment method*
- 1.07 Original name:** *HR metoda za procjenu makrofita*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, Flow modification, Hydromorphological degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Anonymus, 2009. Metodologija vrednotenja ekološkega stanja rek s fitobentosom in makrofiti. Republika Slovenija – Ministrstvo za okolje in prostor. Ljubljana. Anonymus, 2009: Metodologija vzorčenja in laboratorijske obdelave vzorcev za vrednotenje ekološkega stanja rek s fitobentosom in makrofiti. Republika Slovenija – Ministrstvo za okolje in prostor. Ljubljana. Matoničkin, I. & Z. Pavletić, 1960. Biološke karakteristike sedrenih slapova u našim krškim rijekama. Geografski glasnik 22: 43-56. Matoničkin, I. & Z. Pavletić, 1961. Biljni i životinjski svijet n sedrenim slapovima jugoslavenskih krških voda. Biološki glasnik 14: 105-128. Matoničkin, I. & Z. Pavletić, 1972. Život naših rijeka – biologija tekućih voda. Školska knjiga, Zagreb. Schaumburg, J., C. Schranz, D. Stelzer, G. Hofmann, A. Gutowski & J. Foerster, 2006. Instruction Protocol for the ecological Assessment of Running Waters for Implementation of the EC Water Framework Directive: Macrophytes and Phytobenthos. Bavarian Environment Agency. Munich. Trémolières, M., I. Combroux, A. Herrmann & P. Nobelis, 2007. Conservation status of aquatic habitats within the Rhine floodplain using an index based on macrophytes. Ann. Limnol. – Int. J. Lim. 43: 233-244. Urbanič, G., 2008. Redelineation of European inland water ecoregions in Slovenia. Review of Hydrobiology 1: 17-25. Urbanič, G., 2008. Subekoregije in bioregije celinskih voda Slovenije. Natura Sloveniae 10: 5-19. Van de Weyer, K., 2008. Fortschreibung des Bewertungsverfahrens für Makrophyten in Fließgewässern in Nordrhein-Westfalen. LANUV-Arbeitsblätter 3. Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen. Düsseldorf.
- 1.13 Method developed by**  
Antun Alegro  
antun@botanic.hr  
University of Zagreb, Faculty of Science, Division of Biology
- 1.14 Method reported by**  
Dagmar Šurmanović  
dagmar.surmanovic@voda.hr  
Hrvatske vode
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Habdiija et al., 2008. Ecological Research of freshwater in Croatia regarding criteria of the Water Framework Directive of EU.
- 2.02 Short description**  
Field assessment of percentage cover for all submerged and floating species.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Grapnel, Rake  
Grab
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** Summer period
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Once per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1-3 depending on vegetation type
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
ca. 50 meters river section

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** one plant
- 2.13 Sample treatment:** Organisms of the complete sample are identified.

- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Abundance classes, Percent coverage  
combined scale of abundance classes and percent coverage (i.e. expanded Braun-Blanquet scale)  
**in relation to** Area  
**Unit** relative abundance
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** Plant growth form, shoot density
- 2.18 Special cases, exceptions, additions:** In the water and at the banks
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Combined indexes of abundance and coverage according to standard central European scale (extended scale according to Braun-Blanquet)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores, Worst quality class
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** in preparation  
**Location of sites:** National Park Plitvice Lakes and some others, but in preparation  
**Data time period:** Research project started in 2006  
**Criteria:**  
According to WFD Ref. Cond. final version
- 3.08 Reference community description**  
in preparation
- 3.09 Results expressed as EQR?** No under development

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
under development
- 3.11 Boundary setting procedure**  
under development
- 3.12 "Good status" community:** Under development.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 167

SK-MA-RI

## 1. General information

- 1.01 GIG:** Eastern Continental  
R-E2, R-E3, R-E6
- 1.02 Category:** Rivers
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Slovakia
- 1.05 Specification:** none
- 1.06 Method name:** *Slovak assessment of macrophytes in rivers*
- 1.07 Original name:** *Metodika pre odvodenie referenčných podmienok a klasifikačných schém pre hodnotenie ekologického stavu vôd*
- 1.08 Status: Method is/will be used in** neither first nor second RBMP
- 1.09 Detected pressure(s):** Acidification, Catchment land use, Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration  
riparian habitat alteration

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

- 1.10 Internet reference:** <http://www.vuvh.sk/rsv/?page=download>
- 1.11 Pertinent literature of mandatory character:**  
STN 757715. Biological analysis of surface water, 2008. Šporka, F., J. Makovinská, D. Hlúbiková, L. Tóthová, V. Mužík, R. Magulová, K. Kučárov P. Pekárová & L. Mrafková, 2007. Method of the derivation of reference conditions and classification schemes for ecological status assessment. WRI Bratislava, SHMÚ Bratislava, ÚZ SAV Bratislava, SAŽP Banská Bystrica. www.vuvh.sk., 288 pp.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Dr. Lívia Tóthová  
tothova@vuvh.sk  
Water Research Institute
- 1.14 Method reported by**  
Matus Haviar; Emilia Misikova Elexova  
haviar@vuvh.sk; elexova@vuvh.sk  
Water Research Institute, Slovak National Water Reference  
Laboratory
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
EN 14184, 2003. Water quality. Guidance standard for surveying of aquatic macrophytes in running waters. EN 15460, 2007. Water quality. Guidance standard for surveying of aquatic macrophytes in lakes.
- 2.02 Short description**  
Macrophytes: EN 14 184: 2003, EN 15460:2007 - Recording and quantification of macrophytes, determined on surveyed stretch.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Grapnel, Rake
- 2.05 Specification:** rake with telescopic long arm (up to 3 m),grapnel on the rope -up to 20m), aquascope in shallow running waters and lakes
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Only non-wadable rivers (deep mid-size and large rivers) - riparian zones
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** June to October (each locality 1 per year)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
1 or 2 occasions
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
survey stretch ca. 200 m -1 km
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
macrophytes-survey stretch ca. 100m - 1 km (1 km only in large rivers)

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** macrophytes easily visible with naked eyes (including macro-algae growth)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.

- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
macro-algae growth (genus level), bryophytes and vascular plants- species level
- 2.15 Record of abundance:** Abundance classes  
**in relation to** Area  
macrophytes-species abundance expressed as plant mass estimate (PME), in the scale from 1 to 5 - this is not identical with biomass (=kg/unit area), but it is equivalent to "amount of a species", to "3-D-amount"(Nieman 1980)  
**Unit** PLANT MASS ESTIMATE (PME, 3-dimensional estimation of VOLUME biomass)
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** Macrophytes: plant growth form
- 2.18 Special cases, exceptions, additions:** Non-wadable rivers are sampled only at the banks (riparian zones)
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Shannon-Weaver index, Reference index, IBMR index and Indicator taxa
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Mean quality class
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** Pannonian lowland  
**Location of sites:** least disturbed  
**Data time period:** 2008, 2009  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
In Slovakia are not prescribed background taxa lists especially created for good status conditions as well as for any other classes of ecological status.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
Extrapolation of classification scheme - based on exact data of reference sites - based on expert judgment or historical data  
1. establishing theoretical reference value of particular indices  
2. extrapolation of reference value and boundary values between 5 classes of ecological status  
3. classification of concrete locality for all indices  
4. establishing ecological status = averaging of all particular EQR values
- 3.12 "Good status" community:** In Slovakia background taxa lists are not prescribed and especially created for good status conditions as well as for any other ES classes.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 160

HRPI

## 1. General information

**1.01 GIG:** Eastern Continental  
n.a.

**1.02 Category:** Rivers

**1.03 BQE:** Phytoplankton

**1.04 Country:** Hungary

**1.05 Specification:** none

**1.06 Method name:** *Hungarian River Phytoplankton Index*

**1.07 Original name:** *Fitoplankton alapú folyóvízi minősítő rendszer*

**1.08 Status: Method is/will be used in** First RBMP (2009)

**1.09 Detected pressure(s):** Eutrophication, Flow modification, Impact of alien species, Pollution by organic matter

***Has the pressure-impact-relationship been tested?***

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Phytoplankton data (394) from 104 HU rivers (including all HU river types) were examined to establish pressure-impact relationship between the HRPI and the stressors indicating nutrient and organic load. The relationship showed significant correlation with the measures of organic pollution (BOD, COD, Oxygen saturation). R2 values ranging from 0,2-0,37 depending on river type. Significant relationship was not observed with the inorganic nutrients.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

A felszíni vizek biológiai minősítésének továbbfejlesztése, VGT Háttéranyag. Development of ecological state assessment of waters, WBMP ("grey literature").

**1.12 Scientific literature:**

Ács, É. & K.T. Kiss, 2009. Improvement of the ecological water qualification system of rivers based on first results of the Hungarian phyto-benthos and phytoplankton surveillance monitoring ISUAMR 2009: 7th Symposium "Use of Algae For monitoring Rivers". Borics, G., G. Várbíró, I. Grigorszky, E. Krasznai, S. Szabó & K.T. Kiss, 2007. A new evaluation technique of potamo-plankton for the assessment of the ecological status of rivers. Large Rivers Vol. 17, No. 3-4 Arch. Hydrobiol. Suppl. 161 (3-4): 465-486.

**1.13 Method developed by**

Gábor Borics  
boricsg@gmail.com  
Environmental Protection Nature Conservation and Water  
Inspectorate, Trans Tiszanian Region

**1.14 Method reported by**

Gábor Borics  
boricsg@gmail.com  
Environmental Protection Nature Conservation and Water  
Inspectorate, Trans Tiszanian Region

**1.15 Comments**

The first article deals with the composition metric, the second (oral presentation ) with the biomass metric and the way of combination. These (hopefully) will be published in the Hydrobiologia. (Dead line of submission is 31th December 2009).

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

MSZ EN 15204, 2006. Vízminőség. Útmutató szabvány a fitoplanktonok inverz mikroszkópiás számlálására (Ütermöhl - technika).

**2.02 Short description**

10 litres of water is taken from the thalweg. After mixing 0.33l sample is taken, and fixed on the spot by Lugol's solution.

**2.03 Method to select the sampling/survey site or area:** n.a.

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** n.a.  
from the thalweg of the river

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** April to October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

6

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

no replicates

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

10 litres

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** n.a.

- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to Volume  
Unit mg/l
- 2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
The proposed formula for calculating the Hungarian river phytoplankton index is:  
 $HRPI = 2NChla/3 + NQr/3$   
HRPI : Hungarian river phytoplankton index  
NChla: Normalised chl-a metric  
NQr: Normalised composition metric
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Least Disturbed Conditions, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
Number of sites: 93  
Geographical coverage: The whole area of Hungary  
Location of sites: The whole area of Hungary  
Data time period: 1993-2006  
Criteria:  
No off-river and in-channel reservoirs on the watershed. The species composition is close to those proposed by the model (Borics et al. 2007). Minimal organic pollution.
- 3.08 Reference community description**  
Dominance of A, B, C, D, TIB (Borics et al. 2007), functional groups in different ratio, depending on river type.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure**  
The functional groups of algae were evaluated on basis of their ecological characteristics. Nutrient status, tolerance of turbulent conditions, time sufficient for development of the given assemblage and general risk. All the groups were given a factor number (1-5). All the boundaries were set by the relative abundance of the reference (F=5) and good (F=4) taxa. These ratios were different in every river type.
- 3.12 "Good status" community:** Dominance of A, B, C, D, TIB (Borics et al. 2007) functional groups, in different ratio, depending on river type. Other groups J P X2 (that have ) can also be abundant.

#### Uncertainty

- 3.13 Consideration of uncertainty:** Yes  
The uncertainty was characterised with a number between (low 3; medium 2; high 1). The HU index is composed of two metrics. Using the proposed standards the analytical uncertainty (chl-a measurements, species identification and counting) is minimal. The main source of uncertainty is the lack of one of the metrics and the low number of samples per year. There were

three categories for sample numbers and two for the metrics. In the first step the uncertainty was estimated by the sample number depending on the sample number the site was given into one of the category. In the second step the number of metrics were considered. If all the metrics were measured in every cases, the site remained in the category proposed by the sample number. If one of the metric was missing, the uncertainty increased by 1.

**3.14 Comments:**

If the uncertainty was high the managers proposed additional monitoring for the site in the RBMP.

ID: 109

ECO-FITO

## 1. General information

- 1.01 GIG:** Eastern Continental  
No
- 1.02 Category:** Rivers
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Romania
- 1.05 Specification:** none

**1.06 Method name:** *Assessment method for the ecological status of water bodies based on phytoplankton*

**1.07 Original name:** *Metodologie de evaluare a stării ecologice a corpurilor de apă pe baza fitoplanctonului*

**1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)

**1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic matter

*Has the pressure-impact relationship been tested?*

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

Chiriac, G. et al., 2007. Assessment of the ecological status of various lotic ecosystems from the H.B. Jiu using biotic communities according to the WFD requirements. Oltenia. Studii și comunicări. Științele naturii, Craiova. Chiriac, G. & F. Vintilă, 2005. Inventarierea comunităților biotice acvatice din b.h. Mureș în conformitate cu cerințele Directivei Cadru a apelor, vol. Oltenia. Studii și comunicări. Științele naturii, XXI/2005, Craiova. Preda, E. et al., 2007. Aspecte teoretice și practice ale abordării multimetrice în evaluarea stării ecologice a ecosistemelor acvatice lotice din România, Conferința Națională de Ecologie, 11-14 octombrie 2007, Mamaia.

**1.13 Method developed by**

Dr. Mihai ADAMESCU, Dr. Gabriel CHIRIAC  
gabriel.chiriac@rowater.ro  
University of Bucharest – Department of Systemic Ecology and Sustainability ICIM - National R & D Institute for Environment Protection

**1.14 Method reported by**

Serban ILIESCU and Gabriel CHIRIAC  
serban.iliescu@rowater.ro, gabriel.chiriac@rowater.ro  
Romanian Water Authority - Department of The Monitoring Water Resources

**1.15 Comments**

Method will be tested and validated until RBMP 2015

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Guidance on quantitative and qualitative sampling of phytoplankton from inland waters. (Draft N109:2008, experimentally).

**2.02 Short description**

500 - 1000 ml from middle of the river, Alkaline Lugol's solution for preservation,

**2.03 Method to select the sampling/survey site or area:** Random sampling/surveying

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Suitable sampler

**2.06 Sampled/surveyed habitat:** n.a.

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** May / April; July / August; September / October.

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

2 - 3 Times / year based on monitoring type.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

n.a.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

500 - 1000 ml.

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 10 - 25  $\mu$ m

**2.13 Sample treatment:** n.a.

Standard SR EN ISO CEI 15204:2007 (6.6; 8.3)

**2.14 Level of taxonomical identification:** Genus, Species/species groups

Cyanobacteria – genus / species levels; Bacillariophyta - species levels; Chryptophyta, Dinophyta, Euglenopyta,

Chlorophyta - genus / species levels

**2.15 Record of abundance:** Individual counts

**in relation to** Volume

**Unit** Number of algal objects per litre

**2.16 Quantification of biomass:** Chlorophyll-a concentration

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Saprobic index, chlorophyll concentration, Simpson's diversity index, taxa number, numeric abundance (bacillariophyceae).

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Weighted average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** n.a.

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Historical data

**3.07 Reference site characterisation**

**Number of sites:** 853

**Geographical coverage:** Carpathians, Subcarpathians Hills; 4 Ecoregions

**Location of sites:** Retezat Park; Calimani Natinal Park; Maramures Zone.

**Data time period:** 1960s; 1970s; 2004 – 2007.

**Criteria:**

n.a.

**3.08 Reference community description**

Presence of sensitive taxa, high diversity, absence of algal bloom, historical data.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

Organic pollution and saprobic index; ecological status boundaries RO 01 type H/G = 1.285; G/M = 1.57.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 170

SK-PP-RI

## 1. General information

- 1.01 GIG:** Eastern Continental  
R-E2, R-E3, R-E6
- 1.02 Category:** Rivers
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Slovakia
- 1.05 Specification:** only up to 200 m a.s.l.
- 1.06 Method name:** *Slovak assessment of phytoplankton in rivers*
- 1.07 Original name:** *Metodika pre odvodenie referenčných podmienok a klasifikačných schém pre hodnotenie ekologického stavu vôd*
- 1.08 Status: Method is/will be used in** neither first nor second RBMP
- 1.09 Detected pressure(s):** Eutrophication, Flow modification, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** <http://www.vuvh.sk/rsv/?page=download>
- 1.11 Pertinent literature of mandatory character:**  
STN 757715 Biological analysis of surface water. October 2008.
- 1.12 Scientific literature:**  
n.a.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Dr. Jarmila Makovinská (WRI)<br>makovinska@vuvh.sk<br>Water Research Institute | <b>1.14 Method reported by</b><br>Matus Haviar; Emilia Misikova Elexova<br>haviar@vuvh.sk; elexova@vuvh.sk<br>Water Research Institute, Slovak National Water Reference<br>Laboratory |
|---|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Sampling (STN EN 25667-2, STN EN ISO 5667-3, STN ISO 5667- 4,6) – surface layer (0-30cm) 2. analyses: diversity, abundance (STN 757715), chlorophyll-a (STN ISO 10260).
- 2.02 Short description**  
Phytoplankton: sampling STN EN 25667-2, STN EN ISO 5667-3, STN ISO 5667- 4,6 – surface layer (0-30cm)
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Plankton net, Water sampler
- 2.05 Specification:** fytoplankton net
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
if possible - middle part (bridges, ferry), other cases-riparian zone
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** 2 times monthly from April to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
6-12 occasions
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
free water, in special cases net plankton
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
min. 250 ml

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 10µm (mesh size)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups
- 2.15 Record of abundance:** Abundance classes, Individual counts  
in relation to Volume  
Unit number of cells per ml, micrograms per l, abundance classes
- 2.16 Quantification of biomass:** Chlorophyll-a concentration

counting of cells

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** Non-wadable rivers are sampled only at the banks (riparian zones), if possible - mid-stream (bridges, ferry)

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

CYA:EUG:CHLO:CHRO abundance ratio, chlorophyll-a, total number of CYA,EUG,CHLO,CHRO cells

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Mean quality class

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time  
mean value of all samplings

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** Pannonian lowland

**Location of sites:** least disturbed in Pannonian lowland up to 200 m a.s.l.

**Data time period:** n.a.

**Criteria:**

n.a.

**3.08 Reference community description**

Background taxa lists especially created for high status conditions as well as for any other classes of ecological status are not prescribed in Slovakia.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

Phytoplankton: no reference sites for large lowland rivers, derivation of reference values based on expert judgment; confirmation by calculations/statistics. Advance setting of boundaries (data from period 2001-2005): statistical values (mean of 6 measured values within vegetation period for each metric in monitored sites) were calculated for setting of boundaries. These were verified after calculations by expert judgment and compared (correlated) to chemical quality class boundaries.

**3.12 "Good status" community:** In Slovakia background taxa lists are not prescribed and especially created for good status conditions as well as for any other ES classes.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

Reproducibility and repeatability of the sample capacity and concentrated sample; quantification of zoom interval uncertainty; uncertainty estimation counting minimally 5,000 individuals; uncertainty of spectrophotometric assessment of chlorophyll-a.

**3.14 Comments:**

none

ID: 107

SI-FI-RI

## 1. General information

**1.01 GIG:** Eastern Continental, Mediterranean  
n.a.

**1.02 Category:** Rivers

**1.03 BQE:** Fish Fauna

**1.04 Country:** Slovenia

**1.05 Specification:** none

**1.06 Method name:** *Assessment of fish fauna in rivers*

**1.07 Original name:** n.a.

**1.08 Status: Method is/will be used in** n.a.

**1.09 Detected pressure(s):** n.a.

*Has the pressure-impact-relationship been tested?*

0

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

0

**1.14 Method reported by**

Samo Podgornik

samo.podgornik@zzrs.si

Fisheries Research Institute of Slovenia

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

SIST EN 14011 - 2003: Water quality; Sampling of fish with electricity.

**2.02 Short description**

River segment is bounded with stop nets on both sides. Electrofishing with backpackers (1 backpack per 5 m width of stream) is used. Fisherman move slowly in upstream direction and fish with shifting anode from side to side. Bounded segment is sampled twice, successive with short break between.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Electrofishing gear

**2.05 Specification:** engine powered backpacker

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** beginning of june till end of september

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

n.a.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

n.a.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

10-20x stream width; at least 100 m<sup>2</sup>

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** n.a.

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

in relation to Area

Unit number of individuals per one hectare

**2.16 Quantification of biomass:** n.a.

fresh weight of individuals by weighing

**2.17 Other biological data:** length of individual specimens

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### **Evaluation**

**3.01 List of biological metrics**  
n.a.

**3.02 Does the metric selection differ between types of water bodies?** n.a.

**3.03 Combination rule for multi-metrics:** n.a.

**3.04 From which biological data are the metrics calculated?**  
n.a.

#### **Reference conditions**

**3.05 Scope of reference conditions:** n.a.

**3.06 Key source(s) to derive reference conditions:**  
n.a.

**3.07 Reference site characterisation**

Number of sites: n.a.

Geographical coverage: n.a.

Location of sites: n.a.

Data time period: n.a.

Criteria:

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** n.a.

#### **Boundary setting**

**3.10 Setting of ecological status boundaries:** n.a.

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### **Uncertainty**

**3.13 Consideration of uncertainty:** n.a.

**3.14 Comments:**

none

ID: 81

RMI

## 1. General information

- 1.01 GIG:** Eastern Continental, Mediterranean  
Mediterranean: medium, lowland mixed catchment geology (R-M2), Eastern Continental: R-E4 , R-EX6.
- 1.02 Category:** Rivers
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Slovenia
- 1.05 Specification:** 3 ecoregions: Pannonian lowland, Po lowland and Dinarids
- 1.06 Method name:** *River Macrophyte Index*
- 1.07 Original name:** *Indeks rečnih makrofitov*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Ecological data from over 100 sites were examined to establish pressure-impact relationship between biological metrics and percentage of natural land use, showing significant correlation with  $R^2 > 0.7$ .
- 1.10 Internet reference:** [http://www.mop.gov.si/si/delovna\\_podrocja/direktorat\\_z\\_okolje/sektor\\_z\\_vode/ekolosko\\_stanje\\_povrsinskih\\_voda/](http://www.mop.gov.si/si/delovna_podrocja/direktorat_z_okolje/sektor_z_vode/ekolosko_stanje_povrsinskih_voda/)
- 1.11 Pertinent literature of mandatory character:**  
Uradni list Republike Slovenije stran (pp) 832, št. (no) 10, 9.2.2009.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Gorazd Urbanič, (Mateja Germ, Alenka Gaberščik, Urška Kuhar)  
gorazd.urbanic@bf.uni-lj.si  
Biotechnical Faculty, University of Ljubljana and Institute for Water of the Republic of Slovenia
- 1.14 Method reported by**  
Mateja Germ (Gorazd Urbanič, Alenka Gaberščik, Urška Kuhar)  
mateja.germ@bf.uni-lj.si  
Biotechnical Faculty, University of Ljubljana
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Urbanc- Bercic, O., M. Germ & M. Šisko, 2005. Metodologija vzorčenja vodnih makrofitov za določanje ekološkega stanja tekočih voda v Sloveniji : predlog. Ljubljana: Nacionalni inštitut za biologijo: Oddelek za raziskovanje sladkovodnih in kopenskih ekosistemov.
- 2.02 Short description**  
Macrophytes and certain ecological parameters are sampled in 100 m long stretches marked with GPS. In shallow waters the watercourse is waded zick-zack in the upstream direction. In occasions, where we can not wade, the survey is done from the shore or in the deep waters from the boat with the help of rake with hooks and extractable pole .
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Rake
- 2.05 Specification:** rake with hooks, extractable pole
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** normally from June to September, depending from local climate characteristics, preferably July and August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
100 m

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** macroscopic
- 2.13 Sample treatment:** Organisms of the complete sample are identified.

- 2.14 Level of taxonomical identification:** Genus, Other, Species/species groups  
Most macrophytes are determined to species level. If reproductive structures are missing on the basis the determination is done, the macrophytes are determined to the genus level (Callitriche, Sparganium, Charales). Filamentous algae are marked as "Filamentous algae".
- 2.15 Record of abundance:** Abundance classes  
in relation to Volume  
Unit relative units (1-5)
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** plant growth form; S - submersed, N - natant; E- emergent
- 2.18 Special cases, exceptions, additions:** Non-wadable rivers are sampled 1. only at the banks, i.e. multi-habitat-sampling is confined to the river margin habitats; or 2. from the boat.
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
RMI was calculated according to the following equation:  $RMI = (\text{sum of Indicator Taxa Abundance group A} + 1/2 \text{ sum of Indicator Taxa Abundance group AB} - 1/2 \text{ sum of Indicator Taxa Abundance group BC} - \text{sum of Indicator Taxa Abundance group A}) / \text{Sum of all Indicator Taxa Abundance}$
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge
- 3.07 Reference site characterisation**  
**Number of sites:** 23  
**Geographical coverage:** 21 Dinarids, 2 Pannonian  
**Location of sites:** Sites: Dinarids ecoregion (14x Stržen, 2x Mali Obrh, 2x Rinža, 1x Dobljčica, 2x Krka). Pannonian ecoregion (2x) Sotla  
**Data time period:** data sets gained in the peak vegetation period of years 2002 to 2005  
**Criteria:**  
The criteria for the selection of the potential reference sites in the rivers include hydromorphological and physico-chemical condition of the site, riparian vegetation, floodplain and land use properties, saprobic index values, and some pressures presence. Potential reference sites were defined without considering the criteria of biotic pressures that includes allochthonous species and fishery management.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Using paired metrics that respond in different ways to the influence of the pressure
- 3.11 Boundary setting procedure**  
Reference value was determined as a median value of the index RMI at the reference sites. This value is 0.72. Boundary values for the five classes of the ecological status were determined on the basis of the changing of portion of the frequency of so called »good« and »bad« RMI taxa. Portions were calculated on the basis of the frequency of the taxa. Taxa from the group A and AB were taken as »good« and taxa from the group C and BC as »bad«. Boundary value between high and good ecological status was determined where so called »bad« taxa started to appear. Boundary value between good and moderate status was determined where there was a cross-point of curves of portion of »good« and »bad« taxa. Boundary value between moderate and poor ecological status was determined where the portion of frequency of »good« taxa drops below 10 %, and boundary value between poor and bad status where »good« taxa do not appear anymore.

**3.12 "Good status" community:** n.a.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

Those taxa were classified into one of the six ecological groups. Taxa present only at the reference sites (percentage of natural areas >70 %) were classified into the group A, taxa that were present only at the moderately loaded sites (percentage of natural areas 30-70 %) were classified into the group B, and into the group C we classified taxa present only at the heavily loaded sites (percentage of natural areas <30 %). Taxa present at both reference and moderately loaded sites were classified into the group AB and taxa present both at moderately and heavily loaded sites were classified into the group BC. We classified the taxa found at the heavily loaded sites as well as at the reference sites into the group ABC. Taxa in that group do not have indicator value and are not included in the RMI calculation.

ID: 47

IPS

## 1. General information

- 1.01 GIG:** Mediterranean  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Cyprus
- 1.05 Specification:** none
- 1.06 Method name:** *Specific Pollution-sensitivity Index*
- 1.07 Original name:** *Indice de Pollusensibilité Spécifique*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** n.a.

Physicochemical pressures (NO<sub>3</sub>, NH<sub>3</sub>, BOD, %DO)**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Physicochemical pressures (NO<sub>3</sub>, NH<sub>3</sub>, BOD, %DO) were tested against various indices including the IPS. However, due to the small data set no significant pressure-impact relationship could be detected.**1.10 Internet reference:****1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

CEMAGREF, 1982. Etude des methodes biologiques d'appréciation quantitative de la qualité des eaux. Rapport Q.E. Lyon - A.F. Bassin Rhone-Mediterranee-Corse. 218pp. Μοντεσάντου, Β., 2008. Αξιολόγηση της Οικολογικής Ποιότητας των ρέοντων υδάτων της Κύπρου με βιολογικούς δείκτες Διάτομα (Φυτοβένθος) - Εφαρμογή της Οδηγίας Πλάσιο για τα Ύδατα (2000/60/ΕΕ). Τελική Έκθεση. Αρ. Σύμβασης 22/2007.

**1.13 Method developed by**

Dr. Varvara Montesantou  
bmontes@biol.uoa.gr  
National and Kapodistrian University of Athens

**1.14 Method reported by**

Iakovos Tziortzis  
itziortzis@wdd.moa.gov.cy  
Water Development Department - Ministry of Agriculture, Natural Resources and Environment

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

CEN 13946, 2003. Water quality. Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers. European Committee of Standardization 2003. CEN 14407: 2004 Water quality. Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters. European Committee of Standardization 2004.

**2.02 Short description**

A minimum of 5 cobbles (total area about 100cm<sup>2</sup>) are randomly selected from areas of running water deeper than 10cm, well lighted (if possible). The upper part of the stones is sampled with a hard toothbrush for epilithic diatoms, rinsed with distilled water and the sample is preserved using formaldehyde 4%, in small plastic bottles.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge**2.04 Sampling/survey device:** Brush**2.05 Specification:** Toothbrush**2.06 Sampled/surveyed habitat:** Single habitat(s)  
Hard bottom (Cobbles - Mesolithal preferred)**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant**2.08 Sampling/survey month(s):** February - March**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

5 replicates

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**A total of 100cm<sup>2</sup> of hard bottom surface

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** n.a.

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

Two drops of the sample are used to prepare two permanent slides.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Relative abundance

in relation to n.a.

Percentage of each species' individuals in relation to total number of individuals

**Unit** Percentage

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

IPS metrics: Relative abundance of each species, Pollution sensitivity of each species (5 classes of sensitivity), Indicator value or stenocoe degree of each species (3 classes)

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites

**3.07 Reference site characterisation**

**Number of sites:** 8 (4 sites each for R-M4 and R-M5 type)

**Geographical coverage:** Central, southern and western part of the island

**Location of sites:** Upstream parts of Pyrgos, Limnitis, Vasilikos, Ayia (R-M5) and Kargotis, Gialia, Xeros rivers (R-M4)

**Data time period:** April 2007-May 2008

**Criteria:**

Diatoms community structure and IPS values were the main criterion. pressure data (Land Use, Hydromorphological and Physicochemical) were also used and their intensity and impact was evaluated using experts judgment, following the guidelines of MedGIG's template.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

Possible reference sites were selected based on diatoms community structure and IPS values and were screened for pressures using experts judgment. Sites with minimum pressures were then selected as Reference sites. The H/G boundary was set as the 25th percentile of STAR ICMi values at reference sites. The G/M boundary was set as H/G boundary\*0.75. The M/P boundary was set as H/G boundary\*0.5. The P/B boundary was set as H/G boundary\*0.25.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 222

PT-PB-RI

## 1. General information

**1.01 GIG:** Mediterranean  
Small mid-altitude Mediterranean streams (R-M1); Medium lowland Mediterranean streams (R-M2)  
**1.02 Category:** and Small, lowland, temporary (R-M5)

**1.03 BQE:** Benthic Diatoms

**1.04 Country:** Portugal

**1.05 Specification:** Not applicable to Very Large Rivers (>10000 km<sup>2</sup>)

**1.06 Method name:** *Rivers Biological Quality Assessment Method - Diatoms*

**1.07 Original name:** *Método de Avaliação da Qualidade Biológica de Rios - Diatomáceas*

**1.08 Status: Method is/will be used in** First RBMP (2009)

**1.09 Detected pressure(s):** Acidification, Eutrophication, Flow modification, Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Relation between diatom indices and several pressure variables was examined (type specific). Best Spearman Correlation Coefficients were mainly obtained for Nutrients Parameters (ranging from 0,3 and 0,6). Results were statistically significant except for types with a low number of sites.

**1.10 Internet reference:** [http://dqa.inag.pt/dqa2002/port/docs\\_apoio/nacionais.html](http://dqa.inag.pt/dqa2002/port/docs_apoio/nacionais.html)

**1.11 Pertinent literature of mandatory character:**

National sampling protocol: Inag, I.P., 2008. Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Directiva Quadro da Água - Protocolo de amostragem e análise para o fitobentos - diatomáceas. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água, I. P. (available online). Based on CEN Standards: EN 13946 (2003) and EN 14407 (2004). National Ecological Status Classification Guidelines: INAG, I.P., 2009. Critérios para a Classificação do Estado das Massas de Água Superficiais- Rios e Albufeiras. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água, I. P. (available online).

**1.12 Scientific literature:**

CEMAGREF, 1982. Etude des méthodes biologiques d'appréciation quantitative de la qualité des eaux. Rapport Q. E. Lyon. Agence de l'Eau Rhone-Mediterranee-Corse-Cemagref. Lyon. Descy, J.P. & M. Coste, 1991. A test of methods for assessing water quality based on diatoms. Verh. Internat. Verein. Limnol. 24: 2112-2116. Ferreira, J., J.M. Bernardo & M.H. Alves, 2008. Exercício de intercalibração em rios no âmbito da Directiva-Quadro da Água. Acta do 9º Congresso da Água, Lisboa.

**1.13 Method developed by**

Not applicable. Sampling and analysis procedures are based on CEN Standards EN 13946 (2003) and EN 14407 (2004). Quality evaluation methods are based on available indices. The chosen indices were tested within a national wide project promoted by the Water

n.a.

**1.14 Method reported by**

João Ferreira, Salomé Almeida

joao.ferreira@inag.pt / salmeida@ua.pt

Water Institute (Instituto da Água, I.P.) / University of Aveiro

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

National protocol: Inag, I.P., 2008. Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Directiva Quadro da Água - Protocolo de amostragem e análise para o fitobentos - diatomáceas. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água, I. P. (available online). Based on CEN Standards: EN 13946 (2003) and 14407 (2004).

**2.02 Short description**

Observation of field conditions before sampling takes place. In the sampling area look for riffle which contains stones. The area should preferably be unshaded, with water depth between 10 and 30 cm and current velocity between 10-50 cm/s. Five random stones should be chosen, stones with filamentous algae should be avoided. The stones are removed from the water and the upper surface of each stone is scraped using a toothbrush. The stones are washed after scraping is finished. The material which has been scraped is preserved in an identified plastic bottle using Lugol solution.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Brush

**2.05 Specification:** Toothbrush

**2.06 Sampled/surveyed habitat:** Single habitat(s)

Preferably hard substrate (stones occurring at sampling sites). If natural hard substrate is not

**2.07 Sampled/surveyed zones in areas with hard substrate:** as with hard substrate, but also sampled (walls, bridges, etc.). When hard bottom is

not available macrophytes can be sampled.

**2.08 Sampling/survey month(s):** Spring season, February to April in Southern Rivers and March to June in Northern Rivers.

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One sample per year.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1 replicate. Sampling is performed on 5 stones to guarantee the collection on a representative sample of the site.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Minimum of 100 cm<sup>2</sup>.

### **Sample processing**

**2.12 Minimum size of organisms sampled and processed:** Not Applicable

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

400 valves are analysed.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Relative abundance

**in relation to** n.a.

Relative abundance - the abundance of one species in relation to the abundance of the other species. About 400 valves are counted from each sample.

**Unit** Percentage of valves.

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

## **3. Data evaluation**

### **Evaluation**

**3.01 List of biological metrics**

Specific Pollution Index (IPS. CEMAGREF, 1982) for Northern River Types and CEE Index (Descy & Coste, 1991) for Southern River Types.

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

### **Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** 78 Sites

**Geographical coverage:** Reference sites are representative of 7 diatom "river types" spread throughout the country.

**Location of sites:** n.a.

**Data time period:** Data from 2004 and 2005.

**Criteria:**

In order to establish reference conditions the guidelines and pressure screening criteria provided by the Working Group 2.3 – REFCOND and described on CIS WFD Guidance Document N° 10 - Rivers and Lakes – Typology, Reference Conditions and Classification Systems were followed. The applied reference site identification methodology is integrative including spatial analysis, historical data analysis and expert judgment. Semi-quantitative analysis was used in order to assess the magnitude of 9 pressure variables (Land Use, Riparian Zone, Sediment Load, Hydrological Regime, Acidification and Toxicity, Morphological Condition, Organic Matter Contamination and Nutrient Enrichment, River Continuity) a procedure adapted from European Project FAME - Development, Evaluation and Implementation of a Fish-based Assessment Method for the Ecological Status of European Rivers. A Contribution to the Water Framework Directive (Contract EVK1-CT-2001-00094).

□

This procedure was applied according to the specificities of the different river types and lack of true reference sites in some river types lead to the selection of "best available sites". A final biological screening was also made in order to exclude sites with communities typical of degraded sites. Reference Conditions setting criteria will be updated in view of the work of the 2nd phase of the Intercalibration Exercise.

### **3.08 Reference community description**

Diatom reference community description was made using available reference sites for each of the 7 "diatom river types". A description of diatom reference community for each "river type" is only available in Portuguese, but not published yet.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

### **3.11 Boundary setting procedure**

No evident discontinuity was detected on the indexes response to the pressure gradient. High-Good classes boundary: 25th percentile of reference sites; the range below was divided in 4 equal classes; Good-Moderate =  $H/G \times 0.75$ ; Moderate-Poor =  $H/G \times 0.50$ ; Poor-Bad =  $H/G \times 0.25$ .

**3.12 "Good status" community:** Not available yet.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 48

STAR ICMI

## 1. General information

**1.01 GIG:** Mediterranean  
n.a.

**1.02 Category:** Rivers

**1.03 BQE:** Benthic Invertebrates

**1.04 Country:** Cyprus

**1.05 Specification:** none

**1.06 Method name:** *STAR Intercalibration Common Metric Index*

**1.07 Original name:** *STAR Intercalibration Common Metric Index*

**1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)

**1.09 Detected pressure(s):** Catchment land use, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Data from 30 stations in 8 rivers were screened to examine the relationship between pressures and macroinvertebrate communities. The relationship between six pressure indices and invertebrate communities was significant ( $R^2$  ranging between 0.23 and 0.6)

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

AQEM consortium, 2002. Manual for the application of the AQEM method. A comprehensive method to assess European streams using macroinvertebrates, developed for the purpose of the Water Framework Directive. Version 05/2002. Buffagni, A. & J.L. Kemp, 2002. Looking beyond the shores of the United Kingdom: Addenda for the application of River Habitat Survey in South European rivers. *J. Limnol* 61 (2): 199-214. Buffagni, A., M. Campitello & S. Erba, 2005. Il river Habitat Survey Sub Europeo: principi e schede di applicazione. *Notiziario dei metodi Analitici Ist. Ric. Acque* Luglio. IRSA-CNR, 2007. *Notiziario dei Metodi Analitici*, Marzo 2007.

**1.13 Method developed by**

Dr. Andrea Buffagni

buffagni@irsa.cnr.it

Water Research Institute, CNR-IRSA, Italy

**1.14 Method reported by**

Iakovos Tziortzis

itzortzis@wdd.moa.gov.cy

Water Development Department - Ministry of Agriculture, Natural Resources and Environment

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

AQEM consortium, 2002. Manual for the application of the AQEM method. A comprehensive method to assess European streams using macroinvertebrates, developed for the purpose of the Water Framework Directive. Version 05/2002. STAR Project, 2002: The AQEM sampling method to be applied in STAR.

**2.02 Short description**

Multi-habitat sampling designed for sampling major habitats in proportion to their presence within a sampling reach is carried out. A sample consists of 16 "sampling units" taken from all habitat types at the sampling site with a share of at least 5 % coverage. A "sampling unit" is a stationary sampling performed by positioning the surber sampler and disturbing the substrate in a quadratic area that equals the frame-size upstream of the net (0.25 x 0.25 m). Sediments must be disturbed to a depth of 10cm (where possible) depending on substrate compactness.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Surber or Hess sampler

**2.05 Specification:** Surber sampler (0.25x0.25m)

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** February to March and April to May/June

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One (However, usually ecological quality classification is based on the average of two sampling occasions per year)

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

16 replicates covering a total of 1m<sup>2</sup> (one per each stream microhabitat >5% coverage)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Sum of 16 spatial replicates (0.0625 m<sup>2</sup> \* 16 = 1m<sup>2</sup> of stream bottom)

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 500µm (mesh size of sampler)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Sample is subsampled until at least 700 individuals are analysed. If sample is < 700 individuals, no sub-sampling is performed.
- 2.14 Level of taxonomical identification:** Family
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per one square-metre
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
- ASPT - Log10(sel\_EPTD) [Log10 (sum of Heptageniidae, Ephemeridae, Leptophlebiidae, Brachycentridae, Goeridae, Polycentropodidae, Limnephilidae, Odontoceridae, Dolichopodidae, Stratyomidae, Dixidae, Empididae, Athericidae, Nemouridae)] - 1-GOLD 1 [Relative abundance of Gastropoda, Oligochaeta, Diptera] - Total number of Families - Number of EPT families [Sum of Ephemeroptera, Plecoptera, Trichoptera] - Shannon-Wiener diversity index
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**  
**Number of sites:** 4 sites (8 samples)  
**Geographical coverage:** Central and Western part of the island in Troodos mountains  
**Location of sites:** Upstream parts of Kargotis, Xeros and Gialia rivers  
**Data time period:** November 2005 - March 2006  
**Criteria:**  
Followed the REFCOND Guidance criteria based on pressure criteria. The absence of pressures had to be illustrated and this was done by using methods and indices such as SH\_RHS, LRD (Lentic-lotic River Descriptor), HMS (Habitat Modification Score), HQA (Habitat Quality Assessment), LIM ( Livello inquinamento macrodescrittori - Pollution Macroconstituents Level), IFF (Index of Fluvial Functioning), LUI (Land Use Index) from CORINE and CARAVAGGIO
- 3.08 Reference community description**  
High diversity, High number of families, High number of EPT families, High values of EPTD+1
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure**  
Pressures were quantified using various indices and tested positively for correlation with STAR ICMi through a gradient of pressures covering sites from high to bad status. The H/G boundary was set as the 25th percentile of STAR ICMi values at reference sites. The G/M boundary was set as H/G boundary\*0.75. The M/P boundary was set as H/G boundary\*0.5. The P/B boundary was set as H/G boundary\*0.25
- 3.12 "Good status" community:** All metrics of STAR ICMi show slight deviation from Reference sites values.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

The ecological soundness of the method applied for setting the boundaries was validated by checking the distribution of the WDF-compliant metrics composing the STAR ICMi as a function of the proposed classification. Each individual metric of the index was checked as a function of the proposed classification and was validated. It was concluded that the achieved confidence and precision in the classification of the quality element is considered to be adequate.

**3.14 Comments:**

none

ID: 216

PT-BI-RI

## 1. General information

- 1.01 GIG:** Mediterranean  
Small mid-altitude Mediterranean streams (R-M1); Medium lowland Mediterranean streams (R-M2)
- 1.02 Category:** and Small, lowland, temporary (R-M5)
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Portugal
- 1.05 Specification:** Not applicable to Very Large Rivers (>10000 km<sup>2</sup>)
- 1.06 Method name:** *Rivers Biological Quality Assessment Method - Benthic Invertebrates*
- 1.07 Original name:** *Método de Avaliação da Qualidade Biológica de Rios - Invertebrados Bentónicos*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Acidification, Eutrophication, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter, Riparian habitat alteration

### *Has the pressure-impact-relationship been tested?*

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Relation between benthic invertebrates indices and several pressure variables was examined (type specific). Best Spearman Correlation Coefficients were mainly obtained for general degradation (ranging from 0,3 and 0,6). Results were statistically significant except for types with a low number of sites.

- 1.10 Internet reference:** [http://dqa.inag.pt/dqa2002/port/docs\\_apoio/nacionais.html](http://dqa.inag.pt/dqa2002/port/docs_apoio/nacionais.html)
- 1.11 Pertinent literature of mandatory character:**  
National protocol: INAG, I.P., 2008. Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Directiva Quadro da Água - Protocolo de amostragem e análise para os Macroinvertebrados Bentónicos. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água, I. P. (available online). Based on CEN Standards: EN 27828 (1994). National Ecological Status Classification Guidelines: INAG, I.P., 2009. Critérios para a Classificação do Estado das Massas de Água Superficiais – Rios e Albufeiras. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água, I. P. (available online).
- 1.12 Scientific literature:**  
Ferreira, J., J.M. Bernardo & M.H. Alves, 2008. Exercício de intercalibração em rios no âmbito da Directiva-Quadro da Água. Acta do 9º Congresso da Água, Lisboa.; Further information can also be found in Annex 2.4.1E from Intercalibration Technical Report - Rivers.
- 1.13 Method developed by**  
Not applicable. Sampling and analysis procedures are mostly based on CEN Standard EN 27828 (1994). Quality evaluation methods were developed and tested within the Mediterranean GIG. The chosen indices were tested within a national wide project promoted by
- 1.14 Method reported by**  
João Ferreira, Maria João Feio  
  
joao.ferreira@inag.pt / mjf@ci.uc.pt  
Water Institute (Instituto da Água, I.P) / University of Coimbra,  
Institute of Marine Research - CIC
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
National protocol: INAG, I.P., 2008. Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Directiva Quadro da Água - Protocolo de amostragem e análise para os Macroinvertebrados Bentónicos. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água, I. P. (available online). Based on CEN Standards: EN 27828 (1994).
- 2.02 Short description**  
Multi-habitat sampling designed for sampling major habitats in proportion to their presence within a sampling reach is carried out. A sample consists of 6 "sampling units" taken from all habitat types. A "sampling unit" is a one meter long kick or sweep sample performed by positioning the net and disturbing the substrate within the frame width (0.25 m).
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** Hand net with 250mm wide frame and 500 micrometer mesh size.
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** Spring season, February to April in Southern Rivers and March to June in Northern Rivers.
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**

One sample per year.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1 replicate. Sampling is performed on 6 "sampling units".

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

1.5m<sup>2</sup>

**Sample processing****2.12 Minimum size of organisms sampled and processed:** 500 micrometer**2.13 Sample treatment:** Organisms of the complete sample are identified.

Usually the complete sample is analysed, however the fractions between 0.5 and 2mm may be sub-sampled by area but at least 700 individuals should be identified.

**2.14 Level of taxonomical identification:** Family, Other

Oligochaeta to class level and Acari to order level.

**2.15 Record of abundance:** Relative abundance

in relation to Area

Unit Number of individuals per 1,5m<sup>2</sup>.

**2.16 Quantification of biomass:** n.a.**2.17 Other biological data:** none**2.18 Special cases, exceptions, additions:** none**2.19 Comments**

none

**3. Data evaluation****Evaluation****3.01 List of biological metrics**

Índice Português de Invertebrados Norte - IPTIN (Portuguese Invertebrate Index North) for Northern River Types and Índice Português de Invertebrados Sul - IPTIS (Portuguese Invertebrate Index South) for Southern River Types. See Annex 2.4.1e from Intercalibration Technical Report - Rivers and J. Ferreira, J.M. Bernardo, M.H. Alves (2008). Exercício de intercalibração em rios no âmbito da Directiva-Quadro da Água. Acta do 9º Congresso da Água, Lisboa.

**3.02 Does the metric selection differ between types of water bodies?** Yes**3.03 Combination rule for multi-metrics:** Weighted average metric scores**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

**Reference conditions****3.05 Scope of reference conditions:** Surface water type-specific**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions

**3.07 Reference site characterisation****Number of sites:** 134 Sites**Geographical coverage:** Reference sites are representative of 11 river types spread throughout the country.**Location of sites:** n.a.**Data time period:** Data from 2004 and 2005.**Criteria:**

In order to establish reference conditions the guidelines and pressure screening criteria provided by the Working Group 2.3 – REFCOND and described on CIS WFD Guidance Document N° 10 - Rivers and Lakes – Typology, Reference Conditions and Classification Systems were followed. The applied reference site identification methodology is integrative including spatial analysis, historical data analysis and expert judgment. Semi-quantitative analysis was used in order to assess the magnitude of 9 pressure variables (Land Use, Riparian Zone, Sediment Load, Hydrological Regime, Acidification and Toxicity, Morphological Condition, Organic Matter Contamination and Nutrient Enrichment, River Continuity) a procedure adapted from European Project FAME - Development, Evaluation and Implementation of a Fish-based Assessment Method for the Ecological Status of European Rivers. A Contribution to the Water Framework Directive (Contract EVK1-CT-2001-00094).

☐

This procedure was applied according to the specificities of the different river types and lack of true reference sites in some river types lead to the selection of "best available sites". A final biological screening was also made in order to exclude sites with communities typical of degraded sites. Reference Conditions setting criteria will be updated in view of the work of the

2nd phase of the Intercalibration Exercise.

**3.08 Reference community description**

Benthic Invertebrate reference community description was made using available reference sites for each of the 11 river types. A description of benthic invertebrate reference community for each "river type" is only available in Portuguese, but not published yet.

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

No evident discontinuity was detected on the indexes response to the pressure gradient. High-Good classes boundary: 25th percentile of reference sites; the range below was divided in 4 equal classes; Good-Moderate =  $H/G \times 0.75$ ; Moderate-Poor =  $H/G \times 0.50$ ; Poor-Bad =  $H/G \times 0.25$ .

**3.12 "Good status" community:** n.a.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 247

IPS

## 1. General information

- 1.01 GIG:** Northern  
No type approach was used in intercalibration
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Finland
- 1.05 Specification:**
- 1.06 Method name:** *Indice de Polluo-Sensibilité Spécifique (Specific Pollution sensitivity Index SPI)*
- 1.07 Original name:** *IPS-indeksi*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Eloranta, P. & Soininen, J. 2002. Ecological status of some Finnish rivers evaluated using benthic diatom communities. J. Appl. Phycol. 14: 1–7.
- 1.10 Internet reference:** [www.ymparisto.fi](http://www.ymparisto.fi)
- 1.11 Pertinent literature of mandatory character:**  
Standard SFS-EN 13946. 2003. Water quality - Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers. 13 pp.  
Standard SFS-EN 14407. 2004. Water quality. Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters. 12 pp.  
Eloranta, P., Karjalainen, S.M. & Vuori, K-M. 2007. Diatom communities in classification and monitoring ecological status of rivers – guidance to methods. Ympäristöopas. North Ostrobothnia Regional Environment Centre. 58 p. (in Finnish)
- 1.12 Scientific literature:**  
Mykrä, H, Aroviita, J., Hämäläinen, H., Karjalainen, S.M., Visuri, M., Riihimäki, J., Miettinen, J. & Vuori, K-M. 2009. Validity of a single a priori river typology for reference conditions of boreal macroinvertebrates and diatoms. Fundamental and Applied Limnology 175, 269-280.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Pertti Eloranta and Janne Soininen<br>pertti.eloranta@elisanet.fi<br>Department of Limnology and Environmental<br>Protection/Limnology, University of Helsinki | <b>1.14 Method reported by</b><br>Satu Maaria Karjalainen<br>satu.maaria.karjalainen@ymparisto.fi<br>Finnish Environment Institute (SYKE) |
|---|---|
- 1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Hellsten, H., Järvinen, M., Karjalainen, S. M., Meissner, K., Mykrä, H. & Vuori, K.-M. 2009. Jokien ja järvien biologinen seuranta: näytteenotosta tiedon tallentamiseen. Finnish Environment Institute.
- 2.02 Short description**  
Five to ten cobbles are sampled and brushed. Diatoms are removed from the brush into water which is bottled and preserved with ethanol.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Brush
- 2.05 Specification:**
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
epilithic substrates
- 2.07 Sampled/surveyed zones in areas with tidal influence:**
- 2.08 Sampling/survey month(s):** September to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Composite sample consisting diatoms from 5-10 cobbles brushed is used for each site
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
approximate sampled area from five to ten cobbles 0.03-0.07 m<sup>2</sup> from surveyed area of approx. 20 metres of rapid

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** organisms in all sizes are sampled and processed further and identified and counted
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
A part of the well-shaken sample is processed further into slide preparation from which 400 frustules/valves are counted.

- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** n.a.  
400 frustules/valves counted  
**Unit** Number of individuals / 400 frustules/valves
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:**
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
IPS
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**
- Number of sites:** Not specified in Eloranta, P. & Soininen, J. 2002. Ecological status of some Finnish rivers evaluated using benthic diatom communities. J. Appl. Phycol. 14: 1–7.
- Geographical coverage:** Not specified in Eloranta, P. & Soininen, J. 2002. Ecological status of some Finnish rivers evaluated using benthic diatom communities. J. Appl. Phycol. 14: 1–7.
- Location of sites:** Not specified in Eloranta, P. & Soininen, J. 2002. Ecological status of some Finnish rivers evaluated using benthic diatom communities. J. Appl. Phycol. 14: 1–7.
- Data time period:** Data has been collected between 1970 and 2000
- Criteria:**  
High quality rivers with more or less natural state (NS-HQ): very little human activity in the drainage basin (Eloranta & Soininen 2002).
- 3.08 Reference community description**  
No description of reference communities, only abiotic conditions are considered in reference site selection. (See Eloranta, P. & Soininen, J. 2002. Ecological status of some Finnish rivers evaluated using benthic diatom communities. J. Appl. Phycol. 14: 1–7.)
- 3.09 Results expressed as EQR?** No classification with IPS

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Using discontinuities in the relationship of anthropogenic pressure and the biological response.
- 3.11 Boundary setting procedure**  
The rivers studied were classified to five classes according to the degree of human impacts in the drainage basin in general or near the sampling station. Rivers with more or less natural state of very low degree of human impacts showed IPS values > 16, whereas those with slight human impact had the IPS from 14 to 16. The index values decreased markedly with increasing strength of human impact. Based on the results, the following limit values for IPS for evaluation of ecological water quality classes were proposed. ☐High quality IPS>17☐Good quality IPS 15-17☐Moderate quality 12-15☐Poor quality 9-12☐Bad quality <9 ☐(Eloranta & Soininen 2002)
- 3.12 "Good status" community:** No description.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**

The utility of the river typology used in Finland has been tested with diatom communities, but it did not seem to be correct for diatom communities. Variation in the diatom communities was better explained when typology with more detailed regional stratification was used. Metrics Type-specific taxa and Percent Model Affinity (PMA) were compared with IPS index.

ID: 146

FI-BI-RI

## 1. General information

- 1.01 GIG:** Northern  
RN-3
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Finland
- 1.05 Specification:** none
- 1.06 Method name:** *Finnish multimetric index*
- 1.07 Original name:** *Pohjaeläinindeksi*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation

The method is stressor nonspecific

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

- 1.10 Internet reference:** <http://www.ymparisto.fi>

**1.11 Pertinent literature of mandatory character:**

Anon, 2009. Pintavesien ekologisen luokittelun vertailuolot ja luokan määrittäminen. Finnish Environment Institute, Finnish Game and Fisheries Research Institute. [Hämäläinen, H., J. Aroviita, E. Koskenniemi, A. Bonde & J. Kotanen, 2007. Suomen jokien tyypittelyn kehittäminen ja pohjaeläimiin perustuva ekologinen luokittelu. Länsi-Suomen ympäristökeskuksen raportteja 4/2007. 66 s.](#)

**1.12 Scientific literature:**

Aroviita, J., E. Koskenniemi, J. Kotanen & H. Hämäläinen, 2008. A priori typology-based prediction of benthic macroinvertebrate fauna for ecological classification of rivers. *Environmental Management* 42: 894-906. [Aroviita, J., H. Mykrä, T. Muotka & H. Hämäläinen, 2009. Influence of geographical extent on typology- and model-based assessments of taxonomic completeness of river macroinvertebrates. \*Freshwater Biology\* 54: 1774-1787. \[Mykrä, H., J. Aroviita, H. Hämäläinen, S.M. Karjalainen, M. Visuri, J. Riihimäki, J. Miettinen & K.M. Vuori, 2009. Validity of a single a priori river typology for reference conditions of boreal macroinvertebrates and diatoms. \\*Fundamental and Applied Limnology\\* \\(in press\\).\]\(#\)](#)

**1.13 Method developed by**

Heikki Mykrä  
heikki.mykra@ymparisto.fi  
Finnish Environment Institute (SYKE)

**1.14 Method reported by**

Heikki Mykrä  
heikki.mykra@ymparisto.fi  
Finnish Environment Institute (SYKE)

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Hellsten, H., M. Järvinen, S.M. Karjalainen, K. Meissner, H. Mykrä & K.-M. Vuori, 2009. Jokien ja järvien biologinen seuranta: näytteenotosta tiedon tallentamiseen. Finnish Environment Institute.

**2.02 Short description**

Six or nine replicate samples are taken (6 samples from rivers with catchment area <1000 km<sup>2</sup> and 9 samples from rivers with catchments >1000 km<sup>2</sup>. Sampling effort is divided equally to three different habitat types: boulders, pebble/gravel, and slow-flowing river margins.

- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Stratified sampling/surveying

- 2.04 Sampling/survey device:** Hand net

- 2.05 Specification:** Kick net

- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

- 2.08 Sampling/survey month(s):** September to October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Composite sample consisting from 6 to 9 30 second subsamples is used for each site

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

1.8 - 2.7 square-meters

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 500 µm

- 2.13 Sample treatment:** Organisms of the complete sample are identified.

With very large samples subsampling can be used. Proportion (e.g. 1/3) of the material is sorted (using a tray with equal sized grids) and number of individuals is then extrapolated (multiplied) back to original sample size.

- 2.14 Level of taxonomical identification:** Family, Genus, Species/species groups  
EPTC taxa are identified mostly to species or genus level. Family level is generally used for Diptera. Oligochaeta are not identified.
- 2.15 Record of abundance:** Individual counts  
**in relation to** n.a.  
per sample  
**Unit** Density / sample
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Occurrence of type-specific taxa, occurrence of type-specific EPT families, Percent Model Affinity. Type specific taxa or EPT families are taxa that occur in at least 40 % of reference sites in a given river type. Expected value for these metrics is the mean number of observed type specific taxa at reference sites in each particular type (see Aroviita et al. 2008). For calculation of PMA, see Novak, M.A. & Bode, R.W., 1992. Percent model affinity: a new measure of macroinvertebrate community composition. Journal of the North American Benthological Society 11:80–85. Reference site mean PMA is used as an E in calculation of EQR in each type.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
median quality class, metrics are first harmonized using a scoring procedure
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**  
**Number of sites:** 203  
**Geographical coverage:** Whole Finland  
**Location of sites:** n.a.  
**Data time period:** Data has been collected between 1986 and 2006  
**Criteria:**  
No point source pollution, percentage of Agriculture less than 15 % within catchment, no large clear cuts near reference sites, no obvious hydromorphological alteration.
- 3.08 Reference community description**  
No description of reference communities, only abiotic conditions are considered in reference site selection.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure**  
Pressure relationships has not been used in setting the class boundaries.
- 3.12 "Good status" community:** Only EQRs are used to define ecological quality classes. Good status is defined by the 25 percentage point of reference site EQR in each type.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

The utility of the river typology used in Finland has been tested in accounting natural variability of macroinvertebrate communities and the metric type-specific taxa used in national classification. The typology was compared to null models and best possible typology that was based on similarities of macroinvertebrate communities. Standard deviation of reference site ERQ was used as a measure of performance. The typology has also been compared to site-specific RIVPACS-type predictive model (see scientific literature).

**3.14 Comments:**

none

ID: 6

WFD-AWICsp

## 1. General information

- 1.01 GIG:** Northern  
n.a.
- 1.02 Category:** Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** United Kingdom
- 1.05 Specification:** none
- 1.06 Method name:** *WFD Acid Water Indicator Community species*
- 1.07 Original name:** *WFD Acid Water Indicator Community species*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Acidification

***Has the pressure-impact-relationship been tested?***

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Macroinvertebrate kick samples taken in spring from 67 rivers and matched to chemistry from the preceding two years. A minimum of four chemical samples had to be available. Only sites with mean of the lowest 2 values of pH <7, mean of the lowest 2 values of ANC <150 ueq/l and mean Ca < 4mg/l, were included. Linear regression of WFD-AWICsp v Cantrell ANC resulted in R-square =0.65, P = <0.001.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

No official documents as yet - these will be produced at the end of the project (December 09). However, internal reports have been produced and are available. [Murphy et al., 2009](#). Developing bio-diagnostic indices for assessing acidity in sensitive freshwaters. This is likely to be amended before being published. [McFarland, B.F., 2009](#). AWICsp re-testing report [RTT report](#) [McFarland, 2009](#). Development of a typology to assess acidification in UK rivers.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Ben McFarland & John Murphy  
ben.mcfarland@environment-agency.gov.uk  
Environment Agency

**1.14 Method reported by**

Ben McFarland  
ben.mcfarland@environment-agency.gov.uk  
Environment Agency, England & Wales

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

General guidelines for sampling and surveying are available in: United Kingdom Advisory Group, 2008. Uktag river assessment methods benthic invertebrate fauna river invertebrate classification tool (RICT). [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/rivers\\_invertebrates](http://www.wfduk.org/bio_assessment/bio_assessment/rivers_invertebrates) However - AWICsp requires only spring sampling and the classification sites independently from RICT.

**2.02 Short description**

To apply the method, benthic macro-invertebrates should be collected from shallow flowing waters by disturbing the substratum with the feet ("kick" sampling) upstream of a hand net (nominal mesh size: 1 mm) held vertically on the riverbed. All habitats in the chosen sampling site in the river should be sampled within a 3-minute period. In addition, a manual search, lasting one minute, should be performed and any invertebrates found attached to submerged plant stems, stones, logs or other solid surfaces should be removed and placed in the net.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge, Stratified sampling/surveying

**2.04 Sampling/survey device:** Hand net

**2.05 Specification:** pond net

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** Spring (March-June)

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Ideally 3 (1 sample in spring each year for 3 years). However, this is subject to resources.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

from 1 x 3 minute kick samples to 3 x 3 minute kick samples (subject to resources)

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 1mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Ephemeroptera - species ☐ Plecoptera - species ☐ Trichoptera - species
- 2.15 Record of abundance:** Abundance classes  
**in relation to** Time  
**Unit** log abundance per 3 minute kick sample where A = 1-9, B = 10-99, C = 100-999, D = 1000-9999
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
WFD-AWICsp is an abundance derived ASPT metric. Taxa are placed into 5 sensitivity classes (highly sensitive, sensitive, moderately tolerant, tolerant and highly tolerant) and scores depend on the log abundance of each taxa. WFD-AWICsp = sum of indicator scores / Sum of all taxa
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** 45  
**Geographical coverage:** Representative rivers throughout UK at risk from acidification  
**Location of sites:** Representative rivers throughout UK at risk from acidification  
**Data time period:** 5 years  
**Criteria:**  
Screening was by a two stage process. The first stage was a chemical screening. Using a minimum of 4 samples from the previous 2 years and taking the mean of the lowest 2 values for pH and ANC. Second stage used indicator taxa following Ormerod & Durance (2009). Each sample had to either have a minimum of 3 taxa, including at least 2 in group IV, or total of 5 from either groups. This second stage helped to screen out sites that suffer from episodic acid events which might have been missed during chemical sampling. Ormerod, S.J. & I. Durance, 2009. Restoration and recovery from acidification in upland Welsh streams over 25 years. *Journal of Applied Ecology* 46: 164-174.
- 3.08 Reference community description**  
Full reference community description not completed. However, Species typically found at Welsh reference sites but not as commonly (or in such high abundances) at Scottish sites are predominantly stonefly; *Leuctra inermis*, *Isoperla grammatica*, *Chloroperla torrentium* and *Brachyptera risi*. Non-humic Scottish reference sites typically have more mayfly; *Baetis rhodani*, *Alainites muticus*, *Rhithrogena semicolorata* and *Caenis rivulorum*. Sensitive species typical of Welsh reference sites driving the dissimilarities with both Scottish types are *Ecdyonurus* sp., *Chloroperla tripunctata*, *Heptagenia lateralis* and *Hydraena gracilis*. Species typically found at humic Scottish reference sites but not as commonly (or in such high abundances) at other Scottish sites are riffle beetles (Elmidae); *Elmis aenea*, *Oulimnius* sp. and, to a lesser extent, *Limnius volckmari*. The same can be true when compared to Welsh sites, although *L. volckmari* contributes more to the differences than either *E. aenea* or *Oulimnius*. This suggests the presence of high abundances of Elmidae are typical of naturally humic waters with low pH levels, but high ANC. Given these species are considered generally sensitive, they are likely to be good indicators of anthropogenic acidification at acid water sites. Other sensitive species typical of these sites are the caddisfly, *Lepidostoma hirtum* and *Hydropsyche siltalai*.

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.  
Using paired metrics that respond in different ways to the influence of the pressure  
Boundary setting due to be completed in mid-Dec 2009. One or all of three above may be used.

**3.11 Boundary setting procedure**

See above. It is not possible to complete this as yet.

**3.12 "Good status" community:** Expected to be lower abundances of some HS taxa.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 120

Fifi

## 1. General information

- 1.01 GIG:** Northern  
Common River Fish Intercalibration
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Finland
- 1.05 Specification:** none
- 1.06 Method name:** *Finnish River Fish Index*
- 1.07 Original name:** *Suomen jokikalaindeksi*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, General degradation, Habitat destruction
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Teppo Vehanen  
teppo.vehanen@rktl.fi  
Finnish Game and Fisheries Research Institute-
- 1.14 Method reported by**  
Teppo Vehanen  
teppo.vehanen@rktl.fi  
Finnish Game and Fisheries Research Institute-
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
Standardised electrofishing survey by wading
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Electrofishing gear
- 2.05 Specification:** EU approved electrofishing gear
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** July - November, water temp. above 5 C
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
1, analysis on number of sites is ongoing issue
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Minimum 1
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
numbers per square meter

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** No minimum size
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per 100 square meters
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** Length and weight of fish
- 2.18 Special cases, exceptions, additions:** none

## 2.19 Comments

none

## 3. Data evaluation

### Evaluation

#### 3.01 List of biological metrics

n.a.

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: Average metric scores

#### 3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

### Reference conditions

3.05 Scope of reference conditions: Surface water type-specific

#### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites

#### 3.07 Reference site characterisation

Number of sites: typically 20-50 per river type

Geographical coverage: whole Finland

Location of sites: whole Finland

Data time period: July - November, 1999 to present

#### Criteria:

No or very low amount of pressures, described in fish intercalibration

#### 3.08 Reference community description

Type -specific fish community

3.09 Results expressed as EQR? Yes

### Boundary setting

3.10 Setting of ecological status boundaries: High-good boundary derived from metric variability at near-natural reference sites

#### 3.11 Boundary setting procedure

n.a.

3.12 "Good status" community: n.a.

### Uncertainty

3.13 Consideration of uncertainty: No (to be done)

#### 3.14 Comments:

none

ID: 77

VIX

## 1. General information

- 1.01 GIG:** Northern  
Northern GIG; Siliceous mountain brooks (R-C3)
- 1.02 Category:** Rivers
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Sweden
- 1.05 Specification:** Only waterbodies situated below 800 m a. s. l. and drainage area larger than 3 km<sup>2</sup>.
- 1.06 Method name:** *Environmental quality criteria to determine the status of fish in running waters - development and application of VIX*
- 1.07 Original name:** *Bedömningsgrunder för fiskfaunans status i rinnande vatten - utveckling och tillämpning av VIX*
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** Acidification, Eutrophication, General degradation, Hydromorphological degradation
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).
- Fish data from 601 sites with known pressure and impact were examined to establish pressure-impact relationship between fish metrics and general impact, acidification, eutrophication, morphological and hydrological impact and connectivity. Different types of impact gave response in some metrics, in others not. Metrics that showed significant relationships with a certain impact was used to create side-indexes to the final index. The final index contains six fish metrics who could distinguish the degree of general human impact.
- 1.10 Internet reference:** [https://www.fiskeriverket.se/download/18.88bd54c111926b52898000688/Finfo+2007\\_5.pdf](https://www.fiskeriverket.se/download/18.88bd54c111926b52898000688/Finfo+2007_5.pdf)
- 1.11 Pertinent literature of mandatory character:**  
Beier, U., E. Degerman, B. Sers, B. Bergquist & M. Dahlberg, 2007. Bedömningsgrunder för fiskfaunans status i rinnande vatten – utveckling och tillämpning av VIX. Fiskeriverket Informerar 2007: 5, 59 sidor. Published on Swedish Board of Fisheries website ([www.fiskeriverket.se](http://www.fiskeriverket.se)).
- 1.12 Scientific literature:**  
Beier, U., E. Degerman, B. Sers, B. Bergquist & M. Dahlberg, 2007. Environmental quality criteria to determine the status of fish in running waters- development and application of VIX. Fiskeriverket Informerar 2007: 5-59.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Ulrika Beier, Erik Degerman, Berit Sers, Björn Bergquist, Magnus Dahlberg<br>ulrika.beier@fiskeriverket.se<br>Swedish Board of Fisheries, Institute of Freshwater Research | <b>1.14 Method reported by</b><br>Magnus Dahlberg<br>magnus.dahlberg@fiskeriverket.se<br>Swedish Board of Fisheries, Institute of Freshwater Research |
|---|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
CEN, 2003. Water quality - sampling of fish with electricity. EN 14011:2003. Comité Européen de Normalisation.
- 2.02 Short description**  
The strategy is to sample a defined area of the river. The selection of sites shall be representative of habitats within the watershed and suitable for electric fishing. The sampling of a particular site is carried out at the same time of year (august-september). Sampling is performed once a year by 3 runs of electric fishing on each site.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Electrofishing gear
- 2.05 Specification:** Electrofishing gear (isolated wading boots, power unit, control box, electrofishing staff, catching net)
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** July-October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3-30 sites depending on the size of the river.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Total area at least 300 m<sup>2</sup>

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** Young-of-the year fish

- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per one-hundred square meter
- 2.16 Quantification of biomass:** n.a.  
Weight of individual specimens (optional), otherwise estimated biomasses calculated by using length
- 2.17 Other biological data:** Length of individual specimens
- 2.18 Special cases, exceptions, additions:** Weight of individual specimens (optional)
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
1) Abundance of salmon and trout, 2) Proportion of salmonid species reproducing, 3) Proportion of tolerant species, 4) Proportion of intolerant species, 5) Proportion of lithophilic individuals and 6) Proportion of tolerant individuals.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** 601 Swedish sites  
**Geographical coverage:** Sites are spread all over the country, containing sites with different types of impact.  
**Location of sites:** Sites are spread all over the country  
**Data time period:** The latest electric-fishing occasion for each site.  
**Criteria:**  
Non or minor deviation from site-specific reference values.
- 3.08 Reference community description**  
The set of metrics presented in C-1 are used as indicators of species composition, abundance, age-structure and occurrence of species sensitive to impact. References are defined as sites with non or minor deviation from site-specific reference values.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
Focus on the boundary between good and moderate status (See C-15)
- 3.11 Boundary setting procedure**  
To apply the index, theoretical expected values for each metric are calculated using multivariate regression incorporating relevant environmental variables (transformed values). The residuals between expected values and observed values are transformed in two steps. First, residuals are transformed to z-values by dividing the residual with the standard deviation of the residuals for each metric. The z-values are transformed to P-values, which are probabilities for the observed value to represent impacted conditions, adjusted for the direction of the expected change in the metric with increased impact (the lower the P-value, the higher probability that the site is impacted). The index consists of the mean of these P-values. The main focus was to find the clearest possible separation between impacted and unimpacted sites, i.e. the border between good and moderate status according to the Water Framework Directive. The boundary between good and moderate status was chosen where the probabilities of making type-I and type-II errors were equal, i.e. the same risk of classifying an impacted site (preclassified impact 3-5) as unimpacted (preclassified impact 1-2) or vice versa. The borders for status classes of the index values are: class 1 (high status)  $\geq 0.749$ , class 2 (good)  $\geq 0.467$ , class 3 (moderate)  $\geq 0.274$ , class 4 (poor)  $\geq 0.081$ ,

and class 5 (bad) <0.081.

**3.12 "Good status" community:** The border between good and moderate status was chosen where the probabilities of making type-I and type-II errors were equal, i.e. the same risk of classifying an impacted site (preclassified impact 3-5) as unimpacted (preclassified impact 1-2) or vice versa.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

A model for expected variation in form of standard deviation (SD) was created by multiple linear regression (N=336, R<sup>2</sup>=0.2, P<0.001). Observed SD for sites which were electrofished at least 3 times and had non or minor impact of acidification, eutrophication, morphology or hydromorphology was used in the model. The model used the same environmental variables as for estimations of reference values.

**3.14 Comments:**

none

ID: 243

QAELS

## 1. General information

- 1.01 GIG:** Mediterranean
- 1.02 Category:** Lakes, Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Spain
- 1.05 Specification:** Index tested in Catalunya (NE Spain) applied at three water body types (Temporary freshwaters; Permanent freshwaters; Brackish waters)
- 1.06 Method name:** **Water Quality of Lentic Shallow Water Ecosystems**
- 1.07 Original name:** *Qualitat de l'Aigua d'Ecosistemes Lenitics Soms*. Ref: Boix D, Gascón S, Sala J, Martinoy M, Gifre J, Quintana XD (2005) A new index of water quality assessment in Mediterranean wetlands based on crustacean and insect assemblages: the case of Catalunya (NE Iberian peninsula). *Aquat Conserv-Mar Freshw Ecosyst* 15:635-651
- 1.08 Status: Method is/has been used in:** Water Quality of Lentic Shallow Water Ecosystems (2009)
- 1.09 Detected pressure(s):** Eutrophication, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Method previously developed as described in Boix et al. 2005 has been recently tested using 100 samples of different water body types under eutrophication gradient. Some improvements has been incorporate using TRIx index (Vollenweider et al. 1998) as a measure of eutrophication pressure. After which the new QAELS and TRIx index were significantly correlated (Spearman correlations ranging from 0.2 to 0.6 depending on water body types).
- 1.10 Internet reference:** [http://aca-web.gencat.cat/aca/appmanager/aca/aca?\\_nfpb=true&\\_pageLabel=P1206254461208200588613](http://aca-web.gencat.cat/aca/appmanager/aca/aca?_nfpb=true&_pageLabel=P1206254461208200588613)
- 1.11 Pertinent literature of mandatory character:**  
Agència Catalana de l'Aigua (2006) ECOZO Protocol d'avaluació de l'estat ecològic de les zones humides, Agència Catalana de l'Aigua, Barcelona.
- 1.12 Scientific literature:**  
Boix D, Gascón S, Sala J, Martinoy M, Gifre J, Quintana XD (2005) A new index of water quality assessment in Mediterranean wetlands based on crustacean and insect assemblages: the case of Catalunya (NE Iberian peninsula). *Aquat Conserv-Mar Freshw Ecosyst* 15:635-651
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Dani Boix<br>dani.boix@udg.edu<br>Institute of Aquatic Ecology, University of Girona | <b>1.14 Method reported by</b><br>Xavier D. Quintana Pou<br>xavier.quintana@udg.edu<br>Institute of Aquatic Ecology, University of Girona |
|---|---|
- 1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Agència Catalana de l'Aigua (2006) ECOZO Protocol d'avaluació de l'estat ecològic de les zones humides, Agència Catalana de l'Aigua, Barcelona.
- 2.02 Short description**  
Invertebrate sampling was performed using a 20 cm diameter dip-net (mesh size: 250 mm). At each wetland, three sweeps per visit were carried out along transects. Each sweep consisted of 20 dip-net 'pushes' in rapid sequence, to cover all the different habitats in the littoral zone of the wetland. Samples were preserved in 4%.
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** Hand net (250 µm - 20 cm diameter)
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:**
- 2.08 Sampling/survey month(s):** Late winter and spring
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Two samples every year (minimum)
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1 sample = 20 dip net pushes
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Sum of 20 dip net pushes (semiquantitative method with a sampling volume of 30L aprox. per dip net= 0.6 cubic-meters per sample))

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 250 µm sampled
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

A subsample was only used to the organisms from the first sweep were used to estimate the index, based on relative abundances of microcrustaceans, whereas all sweeps were used to calculate the index based on taxon richness.

- 2.14 Level of taxonomical identification:** Genus, Other  
Crustaceans and adults of coleoptera and heteroptera, to genus level. Family level for insects' larval, pupae and nymph stadia.
- 2.15 Record of abundance:** Relative abundance  
relative abundance for species with sensitivity coefficient and presence of the rest (for the assesment of taxa richness)  
**in relation to** Volume  
relative abundance measured as captures per unit effort (CPUE=1 dip net)  
**Unit** Number of individuals per CPUE
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:**
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
ACCO index= relative abundance of taxa with oligosaprobic valence  
RIC index= taxa richness of crustaceans and insects  
 $QAELS=(ACCO+1)*\log_{10}(RIC+1)$
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** n.a.  
 $QAELS=(ACCO+1)*\log_{10}(RIC+1)$
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** Brackish waters (4); temporary freshwaters (4); Permanent freshwaters (1)  
**Geographical coverage:** Catalunya (North East of Spain)  
**Location of sites:** Catalunya (North East of Spain) Park natural dels Aiguamolls de l'Empordà (brackish waters), Paratge de l'Albera (temporary freshwaters) , Parc Natural de la Zona volcànica d'el Garrotxa (Permanent freshwaters)  
**Data time period:** Historical data from 1996 (brackish waters) and the rest some disperse historical data from 2003.  
**Criteria:**  
Refcond Guidance
- 3.08 Reference community description**  
Not reference community description yet
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites  
boundary setting established with percentil distributions according to REFCOND Guidance
- 3.11 Boundary setting procedure**  
High boundary QAELS values higher than percentil 90  
Good boundary QAELS values between percentil 90 and 75  
Moderate boundary QAELS values between percentil 75 and 50  
Poor boundary QAELS values between percentil 50 and 25  
Bad boundary QAELS values smaller tha percentil 25
- 3.12 "Good status" community:** Not produced

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

100 permutations were done for each water body type to obtain a most robust oligosaprobic valence for each genus. Each permutation was done extracting randomly 5% of samples. The oligosaprobic valence of each taxa was then calculated as the weighted mean of the values obtained from the permutations.

**3.14 Comments:**

ID: 46

EE-BI-RL

## 1. General information

- 1.01 GIG:** Central-Baltic  
n.a.
- 1.02 Category:** Lakes, Rivers
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Estonia
- 1.05 Specification:** none
- 1.06 Method name:** *Estimation of freshwater quality using macroinvertebrates*
- 1.07 Original name:** *Estimation of freshwater quality using macroinvertebrates*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Riparian habitat alteration

**Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

General degradation that primarily includes the other pressures shown above. The testing is in progress.

- 1.10 Internet reference:** <https://www.riigiteataja.ee/ert/act.jsp?id=13210253&replstring=33>
- 1.11 Pertinent literature of mandatory character:**  
Pinnaveekogumite moodustamise kord ja nende pinnaveekogumite nimestik, mille seisundiklass tuleb määrata, pinnaveekogumite seisundiklassid ja seisundiklassidele vastavad kvaliteedinäitajate väärtused ning seisundiklasside määramise kord, 2009. Keskkonnaministri 28. juuli 2009. a. määrus nr 44 (RTL, 06.08.2009, 64, 941).

**1.12 Scientific literature:**

Timm, H., 2003. Typology and classification of freshwaters in Estonia: preliminary results using shallow-water macroinvertebrates. In Ruoppa, M., P. Heinonen, A. Pilke, S. Rekolainen, H. Toivonen & H. Vuoristo (eds), How to assess and monitor ecological quality in freshwaters. TemaNord 547: 164-169. Timm, H., 2005. Benthic invertebrates as a tool to classify ecological status of inland waters. Estonian experiences. In Lääne, A. & P. Heinonen (eds), Presentations of three training seminars about Quality Assurance (QA), Biological methods of Water Framework Directive and Waste water sampling techniques. Suomen ympäristökeskuksen moniste, Helsinki 328: 89-94. Timm, H. & E. Mälton, 2006. Littoral macroinvertebrates in large lakes: can they tell us something about the status of lake? - European Large Lakes Symposium 2006. Ecosystem changes and their ecological and socioeconomic impacts. Programme and abstracts. Tartu, Estonia: 54-55. Timm, H., K. Mardi & T. Möls, 2008. Macroinvertebrates in Estonian streams: the effect of habitat, season and sampling effort on some common metrics of biological quality. Estonian Journal of Ecology 57 (1): 37-57. Timm, H. & T. Möls, 2008. Do shallow-water macroinvertebrate assemblages correspond to physico-chemical habitats of streams and lakes? Verh. Internat. Verein. Limnol 31 (1): 138-140. Wasson, J.-G., B. Villeneuve, A. Iital, J. Murray-Bligh, M. Dobiasova, S. Bacikova, H. Timm, H. Pella, N. Mengin & A. Chandesris. Large-scale relationships between basin and riparian land cover and ecological status of European rivers: examples with invertebrate indices from France, Estonia, Slovakia and United Kingdom. Freshwater Biology (accepted).

**1.13 Method developed by**

Henn Timm  
henn.timm@emu.ee  
Estonian University of Life Sciences, Centre for Limnology

**1.14 Method reported by**

Henn Timm  
henn.timm@emu.ee  
Estonian University of Life Sciences, Centre for Limnology

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
From each locality: 1) Five 1-m long kick-sample replications from the most typical habitat 2) qualitative sample from all available habitats
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** Handnet with 25 cm edge length, 0.5 mm mesh size
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** April - May, or September - November
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per year (in optimal time)
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
5
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

1.25 m<sup>2</sup>**Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Not specified but > 300 as a rule
- 2.13 Sample treatment:** Organisms of the complete sample are identified.  
Subsampling is used only to estimate the abundance of dominants. The decision which is a dominant is made separately for each replication. No subsampling is used for qualitative sample.
- 2.14 Level of taxonomical identification:** Family, Species/species groups  
Oligochaeta, Diptera (most), Pisidium, Hydrachnidia etc. that need higher magnification, are identified on group level.
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Per sample and per square-metre
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

**3. Data evaluation****Evaluation**

- 3.01 List of biological metrics**  
Multimetric index, based on five metric: 1) total taxa richness 2) EPT taxa richness 3) Shannon diversity 4) ASPT index 5) Danish Stream Fauna index (in streams only) 6) Swedish Acidity (index (in lakes only). In several cases (very large lakes and rivers), some indices may be pointless.
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores  
After testing two approaches: the "one-out, all out", and "mean quality class" that both provided inappropriate results, a particular formula was developed.
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

**Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** For different waterbody types, habitats and quality indices, 3-60 sites were available  
**Geographical coverage:** Estonia  
**Location of sites:** Estonia  
**Data time period:** 2000-2006  
**Criteria:**  
According to Wallin M., Wiederholm, T. & Johnson R., 2003. Guidance on establishing reference conditions and ecological status class boundaries for inland surface waters. CIS Working Group 2.3 – REFCOND, 7th version
- 3.08 Reference community description**  
Near-natural community of shallow-water macroinvertebrates (littoral in lakes)
- 3.09 Results expressed as EQR?** Yes

**Boundary setting**

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** Each single index yields either five balls (high quality), four balls (good quality), two balls (moderate quality), or zero balls (poor or bad quality). Good status is defined as the sum of

balls ranging 18-22 (derived from five indices), or 14-17 (derived from four indices).

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 36

SI-PB-LA

## 1. General information

- 1.01 GIG:** Alpine  
Alpine GIG; L-AL3
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Slovenia
- 1.05 Specification:** none
- 1.06 Method name:** *Ecological status assessment system for lakes using phytobenthos*
- 1.07 Original name:** *Vrednotenje ekološkega stanja jezer s fitobentosom*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [http://www.mop.gov.si/si/delovna\\_podrocja/direktorat\\_za\\_okolje/sektor\\_za\\_vode/ekolosko\\_stanje\\_povrsinskih\\_vod\\_a/](http://www.mop.gov.si/si/delovna_podrocja/direktorat_za_okolje/sektor_za_vode/ekolosko_stanje_povrsinskih_vod_a/)
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- |  |  |
|--|--|
| <b>1.13 Method developed by</b><br>Gorazd Kosi<br>gorazd.kosi@nib.si<br>Nationale Institute of Biology | <b>1.14 Method reported by</b><br>Gorazd Kosi<br>gorazd.kosi@nib.si<br>National Institute of Biology |
|--|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Kosi, G. & M. Bricelj, 2006. Metodologija vzorčenja in laboratorijske obdelave fitobentosa v jezerih v skladu z zahtevami vodne direktive (Direktiva 2000/60/ES), Nacionalni inštitut za biologijo, 11 str.
- 2.02 Short description**  
Brushing and splashing of different substrates collected from different habitats. Organisms from all substrates represent a sample.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Brush
- 2.05 Specification:** Brush
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** June-September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
three samples per lake
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
500 valves per sample are counted.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Relative abundance  
in relation to n.a.  
**Unit** Number of individuals of 500 counted valves.

- 2.16 Quantification of biomass:** n.a.  
**2.17 Other biological data:** none  
**2.18 Special cases, exceptions, additions:** none  
**2.19 Comments:**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Trophic index (TI = Sum of (Indicator Taxa Abundance \* Trophic value\* Indicator weight) / Indicator Taxa Abundance\* Indicator weight),  
**3.02 Does the metric selection differ between types of water bodies?** No  
**3.03 Combination rule for multi-metrics:** Worst metric score  
**3.04 From which biological data are the metrics calculated?**  
Data from single spatial replicate

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific  
**3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge  
**3.07 Reference site characterisation**  
**Number of sites:** 10 sites in one lake  
**Geographical coverage:** Alps  
**Location of sites:** Bohinjnsko jezero  
**Data time period:** 2006-2007  
**Criteria:**  
n.a.  
**3.08 Reference community description**  
n.a.  
**3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites  
**3.11 Boundary setting procedure**  
n.a.  
**3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)  
**3.14 Comments:**  
none

ID: 238

## 1. General information

- 1.01 GIG:** Alpine  
Alpine GIG; L-AL3
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Slovenia
- 1.05 Specification:** Methods are type specific
- 1.06 Method name:** *Ecological status assessment system for rivers using benthic invertebrates*
- 1.07 Original name:** *Vrednotenje ekološkega stanja jezer z bentoškimi nevretenčarji*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Hydromorphological degradation
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Pressure impact relationship was tested As hydromorphological pressure variable was used a Lakeshore modification index (LMI).
- 1.10 Internet reference:** [http://www.mop.gov.si/si/delovna\\_podrocja/direktorat\\_z\\_okolje/sektor\\_zavode/ekolosko\\_stanje\\_povrsinskih\\_vod\\_a/](http://www.mop.gov.si/si/delovna_podrocja/direktorat_z_okolje/sektor_zavode/ekolosko_stanje_povrsinskih_vod_a/)
- 1.11 Pertinent literature of mandatory character:**
- 1.12 Scientific literature:**
- 1.13 Method developed by**  
Gorazd Urbanič  
gorazd.urbanic@izvrs.si  
Institute for water of the Republic of Slovenia
- 1.14 Method reported by**  
Gorazd Urbanič  
gorazd.urbanic@izvrs.si  
Institute for water of the Republic of Slovenia

### 1.15 Comments

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Urbanič G., Tavzes B., Ambrožič Š., Pavlin M., Sever M. 2006. Metodologija vzorčenja in laboratorijske obdelave bentoških nevretenčarjev v jezerih v skladu z zahtevami Vodne direktive (Direktiva 2000/60/ES). Biotehniška fakulteta, Oddelek za biologijo, 94. str.
- 2.02 Short description**  
Multi-habitat sampling designed for sampling major habitats in proportion to their presence within a sampling reach is carried out. A sample consists of 10 "sampling units" taken from all habitat types at the sampling site with a share of at least 10 % coverage. A "sampling unit" is a stationary sampling performed by positioning the net and disturbing the substrate in a quadratic area that equals the frame-size upstream of the net (0.25 x 0.25 m). Sediments must be disturbed to a depth of 15-20 cm (where possible) depending on substrate compactness.
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** Hand net, Surber or Hess sampler
- 2.05 Specification:**
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:**
- 2.08 Sampling/survey month(s):** July-August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
10 replicates (one per stream microhabitat >10% coverage)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Sum of 10 spatial replicates à 0.0625 square-metres = 0.625 square-metres of stream bottom in total

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 500 µm (mesh-size of hand net)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Family, Genus, Species/species groups  
Mostly species/genus, Chironomidae (subfamily), Tubificidae, some Brachycera (family)
- 2.15 Record of abundance:** Individual counts  
in relation to Area

Unit Number of individuals per 0,625 square meter

2.16 Quantification of biomass: n.a.

2.17 Other biological data:

2.18 Special cases, exceptions, additions: none

2.19 Comments

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

Lakeshore hydromorphology index (LHM = Weighted average of three metrics (Littoral fauna index, Number of taxa, Margalef diversity index)

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: Weighted average metric scores

##### 3.04 From which biological data are the metrics calculated?

Data from single spatial replicate

#### Reference conditions

3.05 Scope of reference conditions: Site-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites

##### 3.07 Reference site characterisation

Number of sites: 11

Geographical coverage: Alps

Location of sites:

Data time period: 2006-2007

Criteria:

n.a.

##### 3.08 Reference community description

n.a.

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: Using paired metrics that respond in different ways to the influence of the pressure

##### 3.11 Boundary setting procedure

n.a.

3.12 "Good status" community:

#### Uncertainty

3.13 Consideration of uncertainty: No (to be done)

3.14 Comments:

ID: 195

AT-FI-LA

## 1. General information

**1.01 GIG:** Alpine**1.02 Category:** Lakes**1.03 BQE:** Fish Fauna**1.04 Country:** Austria**1.05 Specification:****1.06 Method name:** *Assessment of fish fauna in lakes***1.07 Original name:** *n.a.***1.08 Status: Method is/will be used in** *n.a.***1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:****1.11 Pertinent literature of mandatory character:**<http://wisa.lebensministerium.at/article/articleview/74897/1/27032/.....B1> Leitfaden Seen - Qualitätselement Fische (PDF 739,93 kB).**1.12 Scientific literature:**

Gassner, H. & J. Wamzenböck, 2007. Application of population size structure indices to Austrian whitefish (*Coregonus lavaretus*) stocks exploited by anglers. *Archiv für Hydrobiologie, Spec. Issues Advanc. Limnol.* 60: 377-384.

Gassner, H., J. Wanzenböck & G. Tischler, 2003. Ecological Integrity Assessment of Lakes Using Fish Communities – Suggestions of new Metrics developed in two Austrian prealpine Lakes. *International Review of Hydrobiology* 88: 635-652.

Gassner, H., J. Wanzenböck, D. Zick, G. Tischler & B. Pamminer-Lahnsteiner, 2005. Development of a fish based lake typology for natural Austrian Lakes > 50 ha based on the reconstructed historical fish communities. *International Review of Hydrobiology* 90: 422-432.

Wanzenböck, J., H. Gassner, B. Lahnsteiner, Y. Hassen, G. Hauseder, C. Doblander & G. Köck, 2002. Ecological integrity assessment of lakes using fish communities: An example from Lake Traunsee exposed to intensive fishing and to effluents from soda-industry. *Water, Air, and Soil pollution: Focus 2*: 227-248.

Zick, D., H. Gassner, J. Wamzenböck, P. Filzmoser, B. Pamminer-Lahnsteiner & G. Tischler, 2006. Increased human population: Major driver of fish decline in lakes. *European Commission, DG Environment News Alert Service, Issue 32*.

Zick, D., H. Gassner, M. Rinnerthaler & P. Jäger, 2007. Application of population size structure indices to arctic Charr *Salvelinus alpinus* (L.) in Alpine lakes in Austria. *Ecology of Freshwater Fish* 16: 54-63.

Zick, D., H. Gassner, P. Filzmoser, J. Wanzenböck, B. Lahnsteiner & G. Tischler, 2006. Changes in the fish species composition of all Austrian lakes > 50 ha during the last 150 years. *Fisheries Management and Ecology* 13: 1-9.

**1.13 Method developed by**

Hubert Gassner

hubert.gassner@baw.at

Federal Agency for Water Management, Institute for Water Ecology, Fisheries and Lake Research

**1.14 Method reported by**

Hubert Gassner

hubert.gassner@baw.at

Federal Agency for Water Management, Institute for Water Ecology, Fisheries and Lake Research

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**<http://wisa.lebensministerium.at/article/articleview/74897/1/27032/.....B1> Leitfaden Seen - Qualitätselement Fische (PDF 739,93 kB).**2.02 Short description**

1.) Gill netting is conducted between July and September by using NORDIC gillnets, according to the CEN standard EN-14 757. Corresponding to the relatively high depths of the Austrian lakes (max. 190 m) it is necessary to sample the whole water column (= also the deep water region) by gillnets. The pelagic nets are set only at the deepest part of the lake when the lake area is < 5 km<sup>2</sup>. If the lake area is between 5 and 10 km<sup>2</sup> the pelagic nets are additionally set at a second sampling station and lakes > 10 km<sup>2</sup> are sampled on 3 pelagic sampling stations. 2.) The shoreline is sampled by electrofishing, whereas one sample site (sampling time: 15 minutes) per km<sup>2</sup> surface area or at least 4 sample sites for small lakes are examined. 3.) To get information on the overall fish biomass of a lake hydroacoustic surveys (Simrad EK 60; SONAR 5pro) are performed. Based on our experience surveys are carried out during night time on three occasions between July and December. One of these surveys is conducted parallel to the gill netting, the others are done between October and December.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying, Stratified sampling/s**2.04 Sampling/survey device:** Echo sounder, Electrofishing gear, Gill net  
additional data from fisheries**2.05 Specification:** none**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant**2.08 Sampling/survey month(s):** depends on the method used**2.09 Number of sampling/survey occasions (in time) to classify site or area**

n.a.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

depends on the method used; details see also <http://wisa.lebensministerium.at/article/articleview/74897/1/27032/> .....B1 Leitfaden Seen - Qualitätselement Fische (PDF 739,93 kB)).

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

n.a.

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:**

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Genus, Species/species groups

**2.15 Record of abundance:** Individual counts, Relative abundance  
overall abundance and overall biomass

**in relation to** Area

**Unit** fish/ha, kg/ha, fish /whole lake, tons/whole lake, fish per 12 hour and 100 m<sup>2</sup> net, kg per 12 hour and 100 m<sup>2</sup> net,

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** total length, age,

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

**3. Data evaluation**

**Evaluation**

**3.01 List of biological metrics**

is under development recent details see: <http://wisa.lebensministerium.at/article/articleview/74897/1/27032/> .....B1 Leitfaden Seen - Qualitätselement Fische (PDF 739,93 kB)).

**3.02 Does the metric selection differ between types of water bodies?**

**3.03 Combination rule for multi-metrics:** n.a.

**3.04 From which biological data are the metrics calculated?**

n.a.

**Reference conditions**

**3.05 Scope of reference conditions:**

**3.06 Key source(s) to derive reference conditions:**

Historical data

**3.07 Reference site characterisation**

**Number of sites:** all 43 natural Austrian Lakes

**Geographical coverage:** whole Austria

**Location of sites:** n.a.

**Data time period:** before approximately 1900

**Criteria:**

n.a.

**3.08 Reference community description**

details see <http://wisa.lebensministerium.at/article/articleview/74897/1/27032/> .....B1 Leitfaden Seen - Qualitätselement Fische (PDF 739,93 kB)).

**3.09 Results expressed as EQR?** No is under development and should be expressed as EQR

**Boundary setting**

**3.10 Setting of ecological status boundaries:** n.a.

is under development

**3.11 Boundary setting procedure**

is under development

**3.12 "Good status" community:** Is under development.

## **Uncertainty**

### **3.13 Consideration of uncertainty:**

#### **3.14 Comments:**

Overall there are 63 lakes > 50 ha (natural = 43; artificial = 20) in Austria, which have to be assessed according to the EU-WFD. The most important pressures in Austrian lakes are fisheries management, water level fluctuations and regulations, migration barriers, tourism (bathing, boating), shoreline degradation and in some rare cases still eutrophication. Depth: z<sub>max</sub> > 10 m: 60 lakes; z<sub>max</sub> < 10 m: 3 lakes. Ecoregion: 44 Alps, 8 Central Highlands, 6 Dinaric Western Balkans; 5 Hungarian Lowlands

Unfortunately, lakes with undisturbed, natural fish communities, which can be used as reference, are not available in Austria. Thus the near-natural fish species composition was reconstructed for all natural lakes using various historical documents and historical harvest records (see Zick et al. 2006). Cluster analyses (Jaccard's Coefficient) revealed four different natural lake types in Austria (arctic charr, minnow, bleak, pike-perch). For the artificial lakes cluster analyses using the current (stocked) fish species composition resulted in 3 different lake types (brown trout, bream, arctic charr) (see Gassner et al. 2005). For a fish-based assessment of the ecological status in our lakes a preliminary official national index had been developed based on Gassner et al. 2002. This preliminary assessment system is rather pragmatic and compares the reconstructed historical fish community and fish biomass (= reference condition) with the current situation. Based on the results of the so far investigated lakes (n = 14) the preliminary assessment system should be tested and probably adapted to get more realistic results. During this procedure it may be possible that some of the currently used metrics have to be replaced by others.

ID: 106

SI-FI-LA

## 1. General information

- 1.01 GIG:** Alpine  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Slovenia
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment of fish fauna in lakes*
- 1.07 Original name:** n.a.
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** n.a.
- Has the pressure-impact-relationship been tested?*  
0
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
0
- 1.14 Method reported by**  
Samo Podgornik  
samo.podgornik@zzrs.si  
Fsheries Research Institute of Slovenia
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
SIST EN 14757-2005: Water quality - sampling of fish with multi-mesh gill nets.
- 2.02 Short description**  
Sampling is performed by setting up, by lake area dependent, numbers of gill nets in the evening (between 6 and 8 p.m. and lifting in the morning (between 6 and 8). A standard nordic multi mesh gillnets are used. Additional sampling by electrofishing at the banks is performed.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Gill net
- 2.05 Specification:** nordic multi mesh gill nets
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** n.a.
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
n.a.
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
n.a.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to n.a.  
net area  
Unit number of individuals per panel, per mesh
- 2.16 Quantification of biomass:** n.a.

fresh weight of individuals by weighing

**2.17 Other biological data:** length of individual specimens

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**  
n.a.

**3.02 Does the metric selection differ between types of water bodies?** n.a.

**3.03 Combination rule for multi-metrics:** n.a.

**3.04 From which biological data are the metrics calculated?**  
n.a.

#### Reference conditions

**3.05 Scope of reference conditions:** n.a.

**3.06 Key source(s) to derive reference conditions:**  
n.a.

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** n.a.

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** n.a.

#### Boundary setting

**3.10 Setting of ecological status boundaries:** n.a.

**3.11 Boundary setting procedure**  
n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** n.a.

**3.14 Comments:**  
none

ID: 68

AIM Lakes

## 1. General information

- 1.01 GIG:** Alpine  
L-A13, L-A14
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Austria
- 1.05 Specification:** none
- 1.06 Method name:** *Austrian Index Macrophytes - Module 1 for Lakes*
- 1.07 Original name:** *Austrian Index Macrophytes - Module 1 for Lakes*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Riparian habitat alteration  
Main focus of Module 1 is on eutrophication and general degradation
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Ecological data from 482 transects out of 38 lakes with 9-90m mean depth were examined to establish pressure-impact relationship between macrophyte metrics and eutrophication gradient, water level fluctuation, degree of bank fixation. The relationship between macrophyte metrics and the mentioned pressures showed, typespecific, significant correlations.
- 1.10 Internet reference:** <http://wasser.lebensministerium.at/article/archive/5659/0> "Leitfaden für die Erhebung der biologischen Qualitätselemente"
- 1.11 Pertinent literature of mandatory character:**  
Pall, K. & V. Mayerhofer, 2008. Leitfaden zur Erhebung der biologischen Qualitätselemente, Teil B3 – Makrophyten. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft (Hrsg.), 62pp.
- 1.12 Scientific literature:**  
Pall, K. & V. Moser, 2009. Austrian Index Macrophytes (AIM). Module 1 for lakes: a Water Framework Directive compliant assessment system for lakes using aquatic macrophytes. *Hydrobiologia* 633: 83-104.
- 1.13 Method developed by**  
Karin Pall  
karin.pall@systema.at  
systema GmbH, Vienna, Austria
- 1.14 Method reported by**  
Karin Pall  
karin.pall@systema.at  
systema GmbH, Vienna, Austria
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
It combines a dGPS-supported echo-sounding of the entire littoral with a detailed mapping of selected transects by scuba diving. The transects are 25m wide, rectangular to the shoreline and reach from the long term mean water level to the lower limit of the macrophyte vegetation. The abundance of all in the different vegetation zones occurring species is estimated according to a five level scale.
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** n.a.  
echo sounding and scuba diving
- 2.05 Specification:** two channel echo-sounder with dGPS, scuba diving equipment
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** Mai to September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One survey per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
n.a.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
whole littoral area for echo-sounding, detailed mapping of 16 to 80 transects (25m wide) per lake (depending on lake size) by scuba diving

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** all visible plants of the regarded plant groups (charophytes, mosses, ferns, spermatophytes)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Abundance classes  
in relation to Area  
Unit 1=very rare, 2=rare, 3=common, 4=abundant, 5=very abundant, in masses
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** plant growth height, species specific
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Vegetation density, Vegetation limit, Characteristic zonation, Trophic indication, Species composition
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** 51 reference transects for the Austrian lakes  
**Geographical coverage:** Alpine region, perialpine region  
**Location of sites:** Alpine region, perialpine region  
**Data time period:** 1994 to 2003  
**Criteria:**  
LAKE: Trophic state: The lake has to be in the trophic basic state (total Phosphorus, Chlorophyll-a, Secchi-depth corresponding to the values defined for reference condition as fixed in Austrian law and agreed during intercalibration) pH, salinity: No deviation from reference conditions (Cl<sup>-</sup>-concentration and pH corresponding to the values defined for reference condition or high status as fixed in Austrian law) Hydrology: Artificial water level fluctuations must not be bigger than the natural range between the mean low water level and the mean high water level (comparison of long-term gage-data before and after regulation) TRANSECT (surrounding area with a radius of at least 500 m): Surrounding: No intensive agriculture or settlements Nutrient input: No direct local nutrient input or discharges Hydrology: No tributary Morphology: No (or insignificant) artificial modifications of the shoreline Other pressures: No recreation area, no other discernible pressures Vegetation: Undisturbed macrophyte vegetation, based on expert judgement
- 3.08 Reference community description**  
type specific!
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** n.a.  
The class boundaries for each metric were defined according to the normative definitions and interpretations of the WFD as given in the REFCOND Guidance (vgl. Pall & Moser, 2009).
- 3.11 Boundary setting procedure**  
The class boundaries for each metric were set according to the normative definitions and interpretations of the WFD as given in the REFCOND Guidance (vgl. Pall & Moser, 2009).

**3.12 "Good status" community:** Type specific!

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 82

SI-MA-LA

## 1. General information

- 1.01 GIG:** Alpine  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Slovenia
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment of macrophytes in lakes*
- 1.07 Original name:** n.a.
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Impact of alien species, Riparian habitat alteration  
*Has the pressure-impact-relationship been tested?*  
n.a.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
n.a.
- 1.14 Method reported by**  
Mateja Germ, Alenka Gaberščik  
mateja.germ@bf.uni-lj.si  
Biotechnical Faculty
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Germ, M. & A. Gaberscik, 2008. Metodologija vzorčenja makrofitov za vrednotenje ekološkega stanja jezer v skladu z Vodno direktivo (Direktiva 2000/60/ES). Ljubljana: Biotehniška fakulteta, Oddelek za biologijo.
- 2.02 Short description**  
Macrophytes and selected ecological parameters are sampled in transects, 2-6m width, coordinates taken by GPS. Transects reach from shore to the vegetation depth limit. Transects are rectangular to the shoreline and equally broad. Homogeneous littoral areas are selected. Transects are divided to different depth zones according to change of presence and abundance of different macrophyte species. On the certain transect in every depth zone the presence and abundance of macrophytes and their average height are detected. Survey is performed from the boat with the aid of echo sounder, underwater viewer, rake with hooks and extractable pole.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Rake
- 2.05 Specification:** Sampling is done from the boat. With the aid of echosounder, underwater viewer and rake with hooks and extractable pole.
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** normally from June to September, depending from local climate characteristics, preferably July and August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
If surface area of the lake is less than 0.5 km<sup>2</sup> - 1-6 transects. Surface area 0.5-2 km<sup>2</sup>, 4-8 transects. Surface area 2-5km<sup>2</sup>, 5-10 transects.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
3-6 transects per lake, 2-6m broad

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** macroscopic
- 2.13 Sample treatment:** Organisms of the complete sample are identified.

- 2.14 Level of taxonomical identification:** Genus, Other, Species/species groups  
Most macrophytes are determined to species level. If reproductive structures are missing on the basis the determination is done, the macrophytes are determined to the genus level (Callitriche, Sparganium, Charales). Filamentous algae is marked as "Filamentous algae".
- 2.15 Record of abundance:** Abundance classes  
in relation to Volume  
Unit relative unit
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** plant growth form; S - submersed, N - natant; E- emergent
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
n.a.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
n.a.

#### Reference conditions

- 3.05 Scope of reference conditions:** n.a.
- 3.06 Key source(s) to derive reference conditions:**  
n.a.
- 3.07 Reference site characterisation**  
Number of sites: n.a.  
Geographical coverage: n.a.  
Location of sites: n.a.  
Data time period: n.a.  
Criteria:  
n.a.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** No

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** n.a.
- 3.14 Comments:**  
none

ID: 34

AT-PP-LA

## 1. General information

- 1.01 GIG:** Alpine  
Alpine GIG; L-AL3 and L-AL4
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Austria
- 1.05 Specification:** only Alpine Region, separate assessment methods are applied for lakes in the Hungarian Plain and for reservoirs in the Central-
- 1.06 Method name:** **Guidance for the evaluation of the biological quality elements, part B2 – phytoplankton**
- 1.07 Original name:** *Leitfaden zur Erhebung der biologischen Qualitätselemente, Teil B2 – Phytoplankton*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Pressure = annual mean TP concentration (volume weighted or during spring circulation), impact metric = annual mean total biovolume.  
Regression equation see Fig. B-6 in Annex B, Part 5 of the Alpine GIG IC Technical Report (July 2007), N=640, r2=0.42, p<0.01
- 1.10 Internet reference:** <http://wasser.lebensministerium.at/article/articleview/52972/1/5659/>
- 1.11 Pertinent literature of mandatory character:**  
Wolfram, G. & M. Dokulil, 2009. Leitfaden zur Erhebung der biologischen Qualitätselemente, Teil B2 – Phytoplankton. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft.
- 1.12 Scientific literature:**  
Wolfram et al., 2009. Reference conditions and WFD compliant class boundaries for phytoplankton biomass and chlorophyll-a in Alpine lakes. *Hydrobiologia* 633: 45-58.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Georg Wolfram<br>georg.wolfram@dws-hydro-oekologie.at<br>DWS Hydro-Ökologie GmbH, Consulting Engineers for Hydro-Ecology and Landscaping | <b>1.14 Method reported by</b><br>Georg Wolfram<br>georg.wolfram@dws-hydro-oekologie.at<br>DWS Hydro-Ökologie GmbH, Consulting Engineers for Hydro-Ecology and Landscaping |
|---|--|
- 1.15 Comments**  
The literature cited above includes only parts of the national method: total biovolume reference values and class boundaries. The index on the taxonomic composition (Brettum index) and the combination of the metrics has not yet been published.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Wolfram, G. & M. Dokulil, 2009. Leitfaden zur Erhebung der biologischen Qualitätselemente, Teil B2 – Phytoplankton. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft.
- 2.02 Short description**  
Quantitative sampling of the epilimnion (usually fixed defined, e.g. 0-6 m in Carinthia, 0-11 m in most lakes in Salzburg) or the euphotic zone (e.g. Upper Austria, but max 20 m) using a water sampler at single sampling depths (combined to a mixed sample) or an integrating sampler such as the Schröder sampler. An unsieved subsample of 100 or 250 ml is used for later analysis in the lab. Sometimes, additional qualitative samples are taken using a 32 µm net for checking the taxonomic composition.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** Some institutions take samples from different depths for producing a mixed sample, some institutions use an integrating water sampler (e.g. Schröder sampler)
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
epilimnion or euphotic zone, above the deepest point of the lake
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** spring circulation (usually Mar/Apr), beginning of summer stratification (usually May/June), late summer stratification (usually Aug/Sep/Okt) and autumn circulation (usually Nov/Dec)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
4 sampling dates per year and 3 years in series (3-yrs-avg)
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1 sample (mixed or integrated over the epilimnion or the euphotic zone)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
100 or 250 mL glass bottle

## **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** no sieve is used, picoplankton is included (if present)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
see CEN standard on Utermöhl counting and the guidance in Wolfram & Dokulil (2009). Usually 1 chamber is counted at various magnifications. Large algae are counted in the whole chamber (100x), small algae in transects or fields at 200x and 400x (or 600x).
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Most taxa to species or genus as far as possible. Centrales are identified on genus (Cyclotella, Stephanodiscus) or order level (Centrales indet.) in some institutions. Some institutions determine Centrales on species level.
- 2.15 Record of abundance:** Individual counts  
**in relation to** Volume  
**Unit** Cells per mL. This information is only used for calculating the biovolume (= biomass), not as separate metric.
- 2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** Chl-a is measured, but not used as a metric in the assessment method for phytoplankton. The official method currently uses only biomass and a composition metric.
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
1) Annual mean total biovolume (arithmetic mean of biomass at single sampling dates), 2) Brettum index (= composition metric), calculated from the relative proportions of the annual mean biovolumes of the taxa in combination with taxon-specific trophic scores.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
Average of the normalised EQR values from annual mean total biovolume and Brettum index
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Data from single sampling/survey occasion in time  
biovolume metric calculated from single sampling dates, Brettum index calculated from annual mean biomass

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data, Modelling (extrapolating model results)  
The basis is a population of reference sites including historical data. Modelling and expert judgment was used additionally.
- 3.07 Reference site characterisation**  
**Number of sites:** L-AL3: 19 lakes (108 lake-years), L-AL4: 13 lakes (67 lake-years)  
**Geographical coverage:** Northern and Southern Alps; Germany, Slovenia and Austria  
**Location of sites:** AT: Carinthia, Salzkammergut region, Lunz; GE: Bavaria, SI: Triglav national park  
**Data time period:** historical data from the 1931-1938, recent data 1979-2005; all months  
**Criteria:**  
1) General reference criteria such as land use (>80–90% natural forest, wasteland, moors, meadows and pasture; no (or insignificant) intensive crops and vines, no (or insignificant) urbanisation and peri-urban areas, no deterioration of associated wetland areas, no (or insignificant) changes in the hydrological and sediment regime of the tributaries. No direct inflow of (treated or untreated) waste water. No (or insignificant) diffuse discharges. 2) General criteria less relevant for phytoplankton: No (or insignificant) change of the natural regime (regulation, artificial rise or fall, internal circulation, withdrawal). No (or insignificant) artificial modifications of the shore line. No loss of natural connectivity for fish (upstream and downstream). No introduction of fish where they were absent naturally (last decades). No fish-farming activities. No mass recreation (camping, swimming, rowing). No exotic or proliferating species (any plant or animal group). 3) Historical data (prior to major industrialisation, urbanisation and intensification of agriculture). Insignificant contribution of

anthropogenic nutrient load to total nutrient load. No deviation of the actual from the natural trophic state - Natural trophic state of L-AL3: oligotrophic (volume weighted annual mean TP  $\leq 8 \mu\text{g L}^{-1}$ ), natural trophic state of L-AL4: oligo-mesotrophic (volume weighted annual mean TP  $\leq 12 \mu\text{g L}^{-1}$ ). These TP values were derived from an extensive literature review on the response of phytoplankton to nutrient load in Alpine lakes since the IBP and OECD studies.

### 3.08 Reference community description

The high status in deep Alpine lakes is characterised by little spatial and temporal variability of phytoplankton biomass and taxonomic composition. Annual mean total biovolume is low (median biovolume:  $0.3 \text{ mm}^3 \text{ L}^{-1}$ ), transparency is correspondingly high (unless reduced by inorganic turbidity) and may reach values of 24 m (annual mean  $>10 \text{ m}$ ). The algal community usually comprises only a few nutrient-sensitive taxa (low taxa richness). A characteristic feature in the phytoplankton community of many deep Alpine lakes (L-AL3) is a strong dominance of *Cyclotella* species (e.g. *C. comensis*, *C. cyclopuncta*, *C. bodanica*). This fact is proved by monitoring data from reference sites, historical data (prior to intensive urbanisation) and palaeo-reconstructions. Typical accompanying taxa are *Ceratium hirundinella*, *Asterionella formosa*, various chrysoflagellates, cryptoflagellates and Chroococcales. Some of these taxa may also occur at higher trophic states, but usually form a significant part of the community in oligotrophic conditions. In moderately deep lakes (IC type L-AL4), variability and biovolume are slightly higher than in deep lakes (reference conditions = oligo-mesotrophic). The trophic gradient spanned by L-AL4 lakes is larger than in deep lakes, which makes this group more heterogeneous than the L-AL3 lake group. At the lower trophic end of L-AL4 lakes, biovolume and taxonomic composition are similar to those in deep lakes. At the upper trophic end, species richness may be significantly higher than in oligotrophic lakes. Also the proportion of nutrient tolerant taxa such as *Fragilaria crotonensis*, *Stephanodiscus* spp., *Tabellaria fenestrata* or *Planktothrix rubescens* may be slightly higher in L-AL4 lakes than in typical high status lakes of type L-AL3.

**3.09 Results expressed as EQR?** Yes For combining the metrics, the EQR values are converted to normalised EQR ( $0.8 = \text{H/G}$ ,  $0.6 = \text{G/M}$ , ...)

## **Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
High-good boundary derived from metric variability at near-natural reference sites

### 3.11 Boundary setting procedure

Boundaries were derived using the common GIG data set on reference sites. For each reference lake, the arithmetic mean of total bio volume data from single lake-years was calculated. From these data, the median was calculated and defined as reference value. The 95th percentile was defined as H/G boundary. The G/M boundary was set in compliance with the normative definitions of WFD and the Alpine GIG interpretation of the ecological classes for phytoplankton (see Table 2.1.5a. in the Technical Report from 2007). At good status, total bio volume is assumed to be slightly increased (2 to 3-fold) and the taxa composition is slightly altered. The latter corresponds to slight changes in taxa-composition metrics (German PTSI, Italian PTI, Austrian Brettum index). At moderate status, total bio volume is assumed to be significantly increased (4 to 6-fold) and sensitive taxa such as some *Cyclotella* species show a strong decline. This is supported by paleo-reconstruction in several lakes from AT, GE and IT. At moderate status other BQEs are already clearly affected (e.g., decrease of Charophytes, decrease of *Coregonus*). Following this conceptual description the values for the G/M boundary were finally derived by adopting values suggested by Nixdorf et al. (2005a), which were based on monitoring data (LAWA-index and total biovolume; LAWA 1999). The same class widths – applied to different H/G boundaries as starting points – were used for lake type L-AL3 and L-AL4. Equidistant class widths (on a logarithmic basis) are applied to both Alpine IC lake types for setting the class boundaries M/P and P/B.

**3.12 "Good status" community:** See C-11 and C-15.

## **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

It could be shown that uncertainty increased at low sampling frequency and was reduced at higher numbers of sampling dates. While the number of sampling dates per year were not changed, the classification is now done on a three-years average.

**3.14 Comments:**

none

ID: 28

IT-PP-LA

## 1. General information

- 1.01 GIG:** Alpine  
Alpine GIG; L-AL3 and L-AL4
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Italy
- 1.05 Specification:** Natural lakes in the Alpine ecoregion
- 1.06 Method name:** *Phytoplankton Assessment Method for the Ecological status of Lakes*
- 1.07 Original name:** *Indici fitoplanctonici per la valutazione della qualità ecologica dei laghi*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Correlation between the TP concentration (usually volume weighted annual mean or spring overturn) and the PT<sub>lot</sub> of 438 lakes were examined with a correlation coefficient R=0,748

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Indici per la valutazione della qualità ecologica dei laghi. report CNR-ISE, 2/09. In press.

**1.12 Scientific literature:**

Salmaso, N., G. Morabito, F. Buzzi, L. Garibaldi, M. Simona & R. Mosello, 2006. Phytoplankton as an indicator of the water quality of the deep lakes south of the Alps. *Hydrobiologia* 563: 167-187. [DOI](#) Wolfram, 2009. Reference conditions and WFD compliant class boundaries for phytoplankton biomass and chlorophyll-a in Alpine lakes. *Hydrobiologia* 633: 45-58.

**1.13 Method developed by**

Fabio Buzzi  
f.buzzi@arpalombardia.it  
ARPA LOMBARDIA Dipartimento di Lecco

**1.14 Method reported by**

Fabio Buzzi  
f.buzzi@arpalombardia.it  
ARPA LOMBARDIA Dipartimento di Lecco

**1.15 Comments**

The literature cited above describe the use of the metrics total biovolume and chlorophyll (1), as well as the use of the composition metric PT<sub>Ispecies</sub> (phytoplankton trophic index). Literature describing the PT<sub>lot</sub> composition index has not yet been publi

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Buraschi, E., F. Buzzi, L. Garibaldi, A. Lugliè, E. Legnani, G. Morabito, A. Oggioni, S. Pozzi, N. Salmaso & G. Tartari, 2007. Protocollo per il campionamento di fitoplancton in ambiente lacustre. APAT e Ministero dell'Ambiente e della Tutela del Territorio e del Mare.

**2.02 Short description**

Quantitative integrated water sample from the euphotic zone = 2,5X Secchi depth- EN ISO7027(1999) is taken at the deepest point of the lake. When euphotic layer reaches the bottom, an integrated sample is taken from the surface to 1 m above the bottom. Samples are preserved and stored in the dark at room temperature. Qualitative samples are taken with the 10-25 µm mesh size plankton net from the surface to the depth of 20 m.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** IWS Integrating water sampler (436605 Hydrobios)

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
Water column – euphotic zone under the deepest point

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** January to March, April to May, first half of June, July to August, first half of September, October to December

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Minimum 6 samplings per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

One sample per sampling, integrated over the euphotic zone at the deepest point of the lake

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

1 to 2 liters of integrated water sample from the euphotic zone at the deepest point of the lake

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** picoplankton (0.2-2 µm)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
The sample is homogenised manually (turning the bottle upside down for 50 times). Then a sub-sample is placed in a sedimentation chamber. After sedimentation, identification and enumeration is carried out using inverted microscopy by Utermöhl- method . SI
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Most taxa are determined to the species level, rarely to genus .
- 2.15 Record of abundance:** Individual counts  
in relation to Volume  
**Unit** Abundance of phytoplankton is expressed as cells/ml, biomass as volume per litre (mm<sup>3</sup>•l<sup>-1</sup>)
- 2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Total phytoplankton biovolume (BV), chlorophyll a, composition metrics (phytoplankton indices PTIspecies and PTIot.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data
- 3.07 Reference site characterisation**
- Number of sites:** -AL3: 19 lakes (108 lake-years), L-AL4: 13 lakes (67 lake-years) (Boundary setting in Alpine lakes, compiled by G. Wolfram 2006)
- Geographical coverage:** Alpine region, lakes from AT,GE and SI
- Location of sites:** AT - Carinthia, Salzkammergut region, Lunz; GE- Bavaria,SI - Lake Bohinj , Triglav Natural Park
- Data time period:** historical data from the 1931-1938, recent data 1979-2005; all months, Georg Wolfram at al.,Boundary setting in Alpine lakes, 2006
- Criteria:**  
Reference sites among Alpine Lakes have been chosen on the base of historical data (at latest from the 1930ies), palaeoreconstruction and nutrient loading calculations: TP values for ref sites were derived from an extensive literature review on the response of phytoplankton to nutrient load in Alpine lakes since the IBP and OECD studies. • IC lake type L-AL3: Oligotrophic lakes (TP ≤8 µg L<sup>-1</sup>) • IC lake type L-AL4: Oligo- and oligo-mesotrophic (TP ≤12 µg L<sup>-1</sup>) • Insignificant contribution of anthropogenic to total nutrient loading, proved by nutrient lading calculations (CLC-Corine land cover , MEI-Morpho Edaphic Index, Equivalent population density)Reference: ALPINE GIG, Georg Wolfram at al.,Boundary setting in Alpine lakes 2006
- 3.08 Reference community description**  
Annual mean total biovolume is low (median biovolume: 0.3 mm<sup>3</sup> L<sup>-1</sup>). A characteristic feature of the phytoplankton community in many deep Alpine lakes (L-AL3) is a strong dominance of Cyclotella species ( Cyclotella comensis, Cyclotella bodanica, Cyclotella sp. (excl. ocellata, meneghiniana, radiosa)). Typical accompanying taxa besides Cyclotella are Ceratium hirundinella, Asterionella formosa, various chrysoflagellates, cryptoflagellates and Chroococcales. Some of these taxa may also occur at higher trophic states, but form a significant part of the community in oligotrophic conditions. In moderately deep lakes (L-AL4), variability and biovolume is slightly higher than in deep lakes (reference conditions = oligo-mesotrophic).
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

Alpine lakes –boundary setting, G. Wolfram 2006., Technical Report from 2007. Total bio volume was used to describe a continuum of impact (eutrophication). It is suggested to use the bio volume as basic metric and define the boundaries for other metrics accordingly either on the basis of box-whisker-plots (with bio volume as grouping variable) or on the basis of regression functions (with bio volume as independent variable). Reference values and the H/G boundaries for the Brettum index (AT) were derived using a combination of spatial approach and regression with total bio volume. The H/G boundary for the total bio volume was defined using the common GIG dataset. The median was suggested as reference value, the 95%-percentile was suggested as H/G boundary. Boundaries for the moderate and poor status are taken from Nixdorf et al. (2005a), derived from monitoring data (LAWA-index and total bio volume; LAWA 1999), following approximately an exponential function:  $\ln(y) = 0,9234x - 1,6417$   $x$  = class boundaries (H/G = 1, G/M = 2, ...)  $y$  = total bio volume [mm<sup>3</sup> L<sup>-1</sup>] F setting the boundaries below the good status in lake type L-AL4, the boundaries from L AL3 are taken, but moved to the corresponding next class. The P/B boundary is calculated from the upper equation.

**3.12 "Good status" community:** At good status total biovolume is assumed to be slightly increased (2 to 3-fold) and the taxa composition is slightly altered. The latter corresponds to slight changes in taxa-composition metrics. Still presence of Cyclotella species are still present but not dominant, other diatoms i.e. Fragilaria ulna var. angustissima and Asterionella formosa are more abundant. More abundant are also representatives of chrysophycean - Bitrichia sp., Dinobryon sp.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 112

SI-PP-LA

## 1. General information

- 1.01 GIG:** Alpine  
Alpine GIG; L-AL3 and L-AL4
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Slovenia
- 1.05 Specification:** Natural lakes (2) in Slovenia are located only in the Alpine ecoregion
- 1.06 Method name:** *Phytoplankton Assessment Method for the Ecological status of Lakes*
- 1.07 Original name:** *Metodologija vrednotenja ekološkega stanja jezer s fitoplanktonom*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).  
Slovenia adopted the AT Phytoplankton Assessment method for the Alpine lakes, developed by Dokulil (2001, 2003), Dokulil et al. (2005) and Wolfram et al. (2006). Total phosphorus (TP) concentration (volume weighted or during spring circulation) was used as a pressure parameter and total annual phytoplankton biovolume as impact metrics. Regression equation see Fig. B-6 in Annex B, Part 5 of the Alpine GIG IC Technical Report (July 2007), N=640, r<sup>2</sup>=0.42, p<0.01
- 1.10 Internet reference:** [http://www.mop.gov.si/fileadmin/mop.gov.si/pageuploads/podrocja/okolje/pdf/vode/ekolosko\\_stanje/metod\\_vredn\\_ekoloskega\\_st\\_jezer\\_fitoplanktonom.pdf](http://www.mop.gov.si/fileadmin/mop.gov.si/pageuploads/podrocja/okolje/pdf/vode/ekolosko_stanje/metod_vredn_ekoloskega_st_jezer_fitoplanktonom.pdf)  
[http://www.mop.gov.si/fileadmin/mop.gov.si/pageuploads/podrocja/okolje/pdf/vode/ekolosko\\_stanje/metod\\_vzorc](http://www.mop.gov.si/fileadmin/mop.gov.si/pageuploads/podrocja/okolje/pdf/vode/ekolosko_stanje/metod_vzorc)
- 1.11 Pertinent literature of mandatory character:**  
Wolfram, G. & M. Dokulil, 2009. Leitfaden zur Erhebung der biologischen Qualitätselemente, Teil B2 – Phytoplankton. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft.
- 1.12 Scientific literature:**  
Wolfram et al., 2009. Reference conditions and WFD compliant class boundaries for phytoplankton biomass and chlorophyll-a in Alpine lakes. *Hydrobiologia* 633: 45-58.
- 1.13 Method developed by**  
Slovenia adopted AT Phytoplankton Assessment Method for Alpine Lakes (Georg Wolfram). Responsible persons for adaptation: Spela Remec Rekar and Gorazd Urbanič.  
spela.remec-rekar@gov.si, gorazd.urbanic@bf.uni-lj.si  
Spela Remec Rekar, Environmental Agency of the Republic of Slovenia, Vojkova 1b, 1000 Ljubljana; Gorazd Urbanič, Institute for Water of the Republic of Slovenia, Hajdrihova 28, 1000 Ljubljana
- 1.14 Method reported by**  
Spela Remec Rekar  
spela.remec-rekar@gov.si  
Environmental Agency of the Republic of Slovenia, Hydrology and State of the Environment Office
- 1.15 Comments**  
The literature cited above includes only parts of the national method: total biovolume reference values and class boundaries. The index on the taxonomic composition (Brettum index) and the combination of the metrics has not yet been published.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Metodologija vzorčenja in laboratorijske obdelave vzorcev za vrednotenje ekološkega stanja jezer s fitoplanktonom. [http://www.mop.gov.si/fileadmin/mop.gov.si/pageuploads/podrocja/okolje/pdf/vode/ekolosko\\_stanje/metod\\_vzorc\\_lab\\_obd\\_vzorcev\\_vredn\\_ekoloskega\\_st\\_jezer\\_fitoplanktonom.pdf](http://www.mop.gov.si/fileadmin/mop.gov.si/pageuploads/podrocja/okolje/pdf/vode/ekolosko_stanje/metod_vzorc_lab_obd_vzorcev_vredn_ekoloskega_st_jezer_fitoplanktonom.pdf)
- 2.02 Short description**  
Quantitative integrated water sample from the euphotic zone = 2,5X Secchi depth- EN ISO7027(1999) is taken during stratification at the deepest point of the lake. During the mixing period integrated quantitative sample is taken from the surface to the depth of 20 m also at the deepest point of the lake. Samples are preserved and stored in the dark at 4-10°C. Qualitative samples are taken with the 10-25 µm mesh size plankton net from the surface to the depth of 20 m.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Water sampler  
10-25 µm pore size plankton net
- 2.05 Specification:** IWS Integrating water sampler (436605 Hydrobios)
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Water column – euphotic zone under the deepest point
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** March-April, June - July, August - September, October - November= once in all limnological periods
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**

Minimum 4 samplings per year, 3 years successively

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

One sample per sampling, integrated over the euphotic zone at the deepest point of the lake

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

250 ml of integrated water sample from the euphotic zone at the deepest point of the lake

### **Sample processing**

**2.12 Minimum size of organisms sampled and processed:** net phytoplankton - no sieve is used, picoplankton is included (if present)

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

The analysis starts with warming of quantitative sample to the room temperature and homogenisation of the sample after which a sub-sample is placed in a sedimentation chamber. After sedimentation identification and enumeration is carried out using inverted microscope.

**2.14 Level of taxonomical identification:** Genus, Species/species groups

Most taxa are determined to the species level, rarely to genus.

**2.15 Record of abundance:** Individual counts

Individual counts and measurements for biovolume determination

**in relation to** Volume

**Unit** Abundance of phytoplankton is expressed as biovolume per litre ( $\text{mm}^3 \cdot \text{l}^{-1}$ )

**2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique

Chlorophyll-a is a subsidiary parameter which is not included in the ecological state assessment but is regularly measured. Regularly measured beside biovolume

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

**3.01 List of biological metrics**

Total phytoplankton biovolume (BV) and Brettum index as composition metric. Brettum index is calculated in two steps. First common trophic class indexes are derived from relative biovolumes of single species and indicator species trophic class scores in 6 trophic classes. Brettum index is a ratio between sum of all 6 trophic class indexes weighted with specific weights of trophic classes and sum of all trophic class indexes.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

Average of the normalised EQR values for both metrics - total annual biovolume and Brettum index

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time  
Data from single sampling/survey occasion in time

Brettum index is calculated from annual mean biomass, BV from single sampling survey

### **Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Historical data, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** -AL3: 19 lakes (108 lake-years), L-AL4: 13 lakes (67 lake-years) (Boundary setting in Alpine lakes, compiled by G. Wolfram 2006)

**Geographical coverage:** Alpine region, lakes from AT, GE and SI

**Location of sites:** AT - Carinthia, Salzkammergut region, Lunz; GE- Bavaria, SI - Lake Bohinj, Triglav Natural Park

**Data time period:** historical data from the 1931-1938, recent data 1979-2005; all months, Georg Wolfram et al., Boundary setting in Alpine lakes, 2006

**Criteria:**

Reference sites among Alpine Lakes have been chosen on the base of historical data (at latest from the 1930ies),

palaeoreconstruction and nutrient loading calculations: TP values for ref sites were derived from an extensive literature review on the response of phytoplankton to nutrient load in Alpine lakes since the IBP and OECD studies. • IC lake type L-AL3: Oligotrophic lakes ( $TP \leq 8 \mu\text{g L}^{-1}$ ) • IC lake type L-AL4: Oligo- and oligo-mesotrophic ( $TP \leq 12 \mu\text{g L}^{-1}$ ) • Insignificant contribution of anthropogenic to total nutrient loading, proved by nutrient loading calculations (CLC-Corine land cover, MEI-Morpho Edaphic Index, Equivalent population density) Reference: ALPINE GIG, Georg Wolfram et al., Boundary setting in Alpine lakes 2006

### 3.08 Reference community description

Annual mean total biovolume is low (median biovolume:  $0.3 \text{ mm}^3 \text{ L}^{-1}$ ). A characteristic feature of the phytoplankton community in many deep Alpine lakes (L-AL3) is a strong dominance of *Cyclotella* species (*Cyclotella comensis*, *Cyclotella bodanica*, *Cyclotella* sp. (excl. *ocellata*, *meneghiniana*, *radiosa*)). Typical accompanying taxa besides *Cyclotella* are *Ceratium hirundinella*, *Asterionella formosa*, various chrysoflagellates, cryptoflagellates and Chroococcales. Some of these taxa may also occur at higher trophic states, but form a significant part of the community in oligotrophic conditions. In moderately deep lakes (L-AL4), variability and biovolume is slightly higher than in deep lakes (reference conditions = oligo-mesotrophic).

### 3.09 Results expressed as EQR? Yes

## **Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
High-good boundary derived from metric variability at near-natural reference sites

### 3.11 Boundary setting procedure

Alpine lakes –boundary setting, G. Wolfram 2006., Technical Report from 2007: Total biovolume was used to describe a continuum of impact (eutrophication). It is suggested to use the biovolume as basic metric and define the boundaries for other metrics accordingly either on the basis of box-whisker-plots (with biovolume as grouping variable) or on the basis of regression functions (with biovolume as independent variable). Reference values and the H/G boundaries for the Brettum index (AT) were derived using a combination of spatial approach and regression with total biovolume. The H/G boundary for the total biovolume was defined using the common GIG dataset. The median was suggested as reference value, the 95%-percentile was suggested as H/G boundary. Boundaries for the moderate and poor status are taken from Nixdorf et al. (2005a), derived from monitoring data (LAWA-index and total biovolume; LAWA 1999), following approximately an exponential function:  $\ln(y) = 0,9234x - 1,6417$   $x$  = class boundaries (H/G = 1, G/M = 2, ...)  $y$  = total biovolume [ $\text{mm}^3 \text{ L}^{-1}$ ] For setting the boundaries below the good status in lake type L-AL4, the boundaries from L-AL3 are taken, but moved to the corresponding next class. The P/B boundary is calculated from the upper equation.

**3.12 "Good status" community:** At good status total biovolume is assumed to be slightly increased (2 to 3-fold) and the taxa composition is slightly altered. The latter corresponds to slight changes in taxa-composition metrics. *Cyclotella* species are still present but not dominant, other diatoms i.e. *Fragillaria ulna* var. *angustissima* and *Asterionella formosa* are more abundant. More abundant are also representatives of chrysophyceans - *Bitrichia* sp., *Dindynobryon* sp.

## **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

### 3.14 Comments:

none

ID: 202

Phylib

## 1. General information

- 1.01 GIG:** Alpine, Central-Baltic  
L AL3, L AL4
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Diatoms, Macrophytes, Other Phytobenthos
- 1.04 Country:** Germany
- 1.05 Specification:**
- 1.06 Method name:** *German Assessment System for Macrophytes & Phytobenthos for the WFD*
- 1.07 Original name:** *Deutsches Bewertungsverfahren für Makrophyten & Phytobenthos nach EG-WRRL*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

The included trophic assessment systems for diatoms (Hofmann 1999, Schönfelder and Hofmann) are calibrated at TP-data. Referenz-Index Macrophytes is related MI (Melzer 87;  $R^2=0,83$ ) which is tested with TP.

- 1.10 Internet reference:** [http://www.lfu.bayern.de/wasser/forschung\\_und\\_projekte/phylib\\_deutsch/index.htm](http://www.lfu.bayern.de/wasser/forschung_und_projekte/phylib_deutsch/index.htm)

**1.11 Pertinent literature of mandatory character:**

LAWA-AO, 2006. RaKon Monitoring Teil B. Arbeitspapier III: Untersuchungsverfahren für biologische Qualitätskomponenten. Ständiger Ausschuss "Oberflächengewässer und Küstengewässer" der Bund/ Länder-Arbeitsgemeinschaft Wasser (LAWA-AO).

**1.12 Scientific literature:**

Schaumburg, J., U. Schmedtje, C. Schranz, B. Köpf, S. Schneider, P. Meilinger, D. Stelzer, G. Hofmann, A. Gutowski & J. Foerster, 2004. Erarbeitung eines ökologischen Bewertungsverfahrens für Fließgewässer und Seen im Teilbereich Makrophyten und Phytobenthos zur Umsetzung der EU-Wasserrahmenrichtlinie. – Bayerisches Landesamt für Wasserwirtschaft, Abschlußbericht an das Bundesministerium für Bildung und Forschung

(FKZ 0330033) und die Länderarbeitsgemeinschaft Wasser (Projekt Nr. O 11.03), 635. p., München. Schaumburg, J., C. Schranz, G. Hofmann, D. Stelzer, S. Schneider & U. Schmedtje, 2004. Macrophytes and phytobenthos as indicators of ecological status in German lakes – a contribution to the implementation of the Water Framework Directive. *Limnologica* 34: 302–311. Schaumburg, J., U. Schmedtje, C. Schranz, B. Köpf, S. Schneider, P. Meilinger, D. Stelzer, G. Hofmann, A. Gutowski & J. Foerster, 2005. Bewertungsverfahren Makrophyten & Phytobenthos, Fließgewässer- und Seenbewertung in Deutschland nach EGWRRL. – Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft, Heft 1/05: 24 p., München. Schaumburg, J., U. Schmedtje, C. Schranz, B. Köpf, S. Schneider, P. Meilinger, D. Stelzer, G. Hofmann, A. Gutowski & J. Foerster, 2005. Makrophyten und Phytobenthos in Flüssen und Seen – Das deutsche Bewertungsverfahren: Entwicklung, Praxistest und Ausblick. In Feld, R. & F. Sommerhäuser (eds), Typologie, Bewertung, Management von Oberflächengewässern, Stand der Forschung zur Umsetzung der EG-Wasserrahmenrichtlinie. *Limnologie aktuell*: Band 11: 63-75, Stuttgart. Stelzer, D., S. Schneider & A. Melzer, 2005.

Macrophyte based assessment of lakes - a contribution to the implementation of the European Water Framework Directive in Germany. In *Rev. Hydrobiol.* 90 (2): 223-237.

**1.13 Method developed by**

Jochen Schaumburg, Christine Schranz, Doris Stelzer, Gabriele Hofmann  
christine.schranz@lfu.bayern.de  
Bavarian Environment Agency LFU

**1.14 Method reported by**

Christine Schranz  
christine.schranz@lfu.bayern.de  
Bavarian Environment Agency LFU

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Schaumburg, J., C. Schranz, D. Stelzer & G. Hofmann, 2007. Action Instructions for the ecological Evaluation of Lakes for Implementation of the EU Water Framework Directive: Macrophytes and Phytobenthos.

**2.02 Short description**

All macrophytes of one transect are registered, determined at species-level and calculated the abundance of each taxon. A minimum of five cobbles are taken all over the transect, depth about 50 to 100 cm. The biofilm is taken from those cobbles with a spoon.

**2.03 Method to select the sampling/survey site or area:** n.a.

**2.04 Sampling/survey device:** Brush, Spoon  
diving or rake and aquascope

**2.05 Specification:** macrophytes: a rake with tines on two sides of the stick, weighted, fastened on a rope with marks each meter. Phytobenthos: spoon, sharpened on one side or toothbrush, cleaned solid after each sample.

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
the complete surveying-site from shore to the end of macrophyte expansion in the depth

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

- 2.08 Sampling/survey month(s):** summer, july until middle of august
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
specified above

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** ca. 2µm length
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Diatoms: after chemical oxidation of the material 500 objects of diatoms are determined and enumerated
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Abundance classes, Individual counts  
**in relation to** Area  
**Unit** abundance-class after Kohler 1987 and number of individuals
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Referenzindex:  $((\sum Q_{Ai} - \sum Q_{Ci}) / (\sum Q_{gi})) * 100$  RI = Referenzindex Q<sub>Ai</sub> = Quantität des i-ten Taxons aus Gruppe A Q<sub>Ci</sub> = Quantität des i-ten Taxons aus Gruppe C Q<sub>gi</sub> = Quantität des i-ten Taxons aller Gruppen n<sub>A</sub> = Gesamtzahl der Taxa aus Gruppe A n<sub>C</sub> = Gesamtzahl der Taxa aus Gruppe C n<sub>g</sub> = Gesamtzahl der Taxa aller Gruppen Total Quantity of several taxa depth of macrophyte-expansion Total quantity of macrophytes total abundance of aerophile benthic diatom-taxa Trophie-Index (Hofmann 1999) Trophieindex Schönfelder et al. Referenzartenquotient
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores, Mean quality class  
average for assess one site, mean quality class for assessing the waterbody
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data, Modelling (extrapolating model results)  
sediment-cores
- 3.07 Reference site characterisation**  
**Number of sites:** typespecific all undisturbed sites in which were available  
**Geographical coverage:** typespecific all undisturbed sites in which were available  
**Location of sites:** typespecific all undisturbed sites in which were available  
**Data time period:** summer and autumn, all data from reference.sites since 1990  
**Criteria:**  
The appropriate experts had to deliver reference conditions for the sites, in addition the chemical, physical and structural parameters had to show an undisturbed situation, also the environs of the sites.
- 3.08 Reference community description**  
The reference community should be dominated by the type specific defined species group "reference-species" A (macrophytes and phytobenthos-diatoms). E.g. macrophytes in alpine lakes with cobbles and rocks as a dominating sediment: mostly oligotrophic mosses, some characeae, only a few potamogeton-species and some others are in species group A.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

The boundaries were set at the zones of distinct changings of the biocoenosis (macrophytesn and diatoms), and depending on indicator species lists derived from nutrient dependent TI (diatoms).

**3.12 "Good status" community:** Typespecific reference species and tolerant species are still dominant, pressure indicators are rare. = slightly deviation from high status (normative definitions)

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 206

German PSI

## 1. General information

**1.01 GIG:** Alpine, Central-Baltic  
L CB1, L CB2, L AL3, L AL4

**1.02 Category:** Lakes

**1.03 BQE:** Phytoplankton

**1.04 Country:** Germany

**1.05 Specification:**

**1.06 Method name:** *German Phyto-Lake-Index*

**1.07 Original name:** *Phyto-See-Index*

**1.08 Status: Method is/will be used in** First RBMP (2009)

**1.09 Detected pressure(s):** Eutrophication

TP and LAWA-Index (1999) as a second eutrophication index (based on Vollenweider with parameters TP, Chla, Secchi depth)

### **Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

See Table 7-2; 7-4 in Mischke, Riedmüller, Hoehn & Nixdorf (2008a): Praxistest zur Bewertung.... In: Mischke, U. & B. Nixdorf (eds.), Gewässerreport (Nr. 10), BTUC-AR 2/2008, page 7-115- ISBN 978-3-940471-06-2. AL 3 (national type 4): Ecological data from 101 lake-years\* (lakes in the Alps; stratified) were examined to establish pressure-impact relationship between phytoplankton metrics and eutrophication gradient. The relationship between the three phytoplankton metrics (biomass; algal class; indicator taxa) and TP (seasonal means Apr-Nov) showed significant correlation (Spearman Correlation Coefficient ranging from 0.55 to 0.77). The multimetric PSI showed correlation (Spearman Correlation Coefficient) of 0.82 to TP. AL 4 (national type 2+3): Ecological data from 71 lake-years\* (lakes in the pre-Alps; stratified) were examined to establish pressure-impact relationship between phytoplankton metrics and eutrophication gradient. The relationship between the three phytoplankton metrics (biomass; algal class; indicator taxa) and TP (seasonal means Apr-Nov) showed significant correlation (Spearman Correlation Coefficient ranging from 0.70 to 0.85). The multimetric PSI showed correlation (Spearman Correlation Coefficient) of 0.82 to TP. CB 1 (national types 10.1 and 13): Ecological data from 119 lake-years\* (> 50 mg l<sup>-1</sup> CaCO<sub>3</sub> alkalinity and 3-15 m mean depth and retention time >1 and <10 years) were examined to establish pressure-impact relationship between phytoplankton metrics and eutrophication gradient. The relationship between the three phytoplankton metrics (biomass; algal class; indicator taxa) and TP (seasonal means Apr-Nov) showed significant correlation (Spearman Correlation Coefficient ranging from 0.42 to 0.82). The multimetric PSI showed correlation (Spearman Correlation Coefficient) of 0.67 for national lake type 10.1 (short retention time) and 0.82 for national lake type 13) and 0.90 to LAWA-Index (which includes chlorophyll a, TP and SD). CB 2 (national type 11.2): Ecological data from 74 lake-years\* (calcerous and <3m mean depth and retention time >0.1 and <1 years) were examined to establish pressure-impact relationship between phytoplankton metrics and eutrophication gradient. The relationship between the three phytoplankton metrics (biomass; algal class; indicator taxa) and TP (seasonal means Apr-Nov) showed significant correlation (Spearman Correlation Coefficient ranging from 0.28 to 0.51). The multimetric PSI showed correlation (Spearman Correlation Coefficient) of 0.48 to TP and 0.91 to LAWA-Index (which includes chlorophyll a, TP and SD). \* In case of a long-term data sets of one lake, then not more than 3 years were used!

**1.10 Internet reference:** <http://www.igb-berlin.de/~mischke>

### **1.11 Pertinent literature of mandatory character:**

Research report for LAWA project O 5.05. Mischke et al. 2008; Downloads on [http://www.laenderfinanzierungsprogramm.de/cms/WaBoAb\\_prod/WaBoAb/Vorhaben/LAWA/Vorhaben\\_des\\_Ausschusses\\_Oberflaechengewaesser\\_und\\_Kuestengewaeser\\_\(AO\)/biologische\\_Bewertungsverfahren\\_im\\_Rahmen\\_der\\_WRRL/index.jsp](http://www.laenderfinanzierungsprogramm.de/cms/WaBoAb_prod/WaBoAb/Vorhaben/LAWA/Vorhaben_des_Ausschusses_Oberflaechengewaesser_und_Kuestengewaeser_(AO)/biologische_Bewertungsverfahren_im_Rahmen_der_WRRL/index.jsp) First and not actual approach see:

LAWA-AO, 2006. RaKon Monitoring Teil B. Arbeitspapier III: Untersuchungsverfahren für biologische Qualitätskomponenten. Ständiger Ausschuss "Oberflächengewässer und Küstengewässer" der Bund/Länder-Arbeitsgemeinschaft Wasser (LAWA-AO).

### **1.12 Scientific literature:**

Mischke, U., U. Riedmüller, E. Hoehn, I. Schönfelder & B. Nixdorf, 2008. Description of the German system for phytoplankton-based assessment of lakes for implementation of the EU Water Framework Directive (WFD). In Mischke, U. & B. Nixdorf (eds), Gewässerreport (Nr. 10), BTUC-AR 2/2008, Eigenverlag BTU Cottbus, 117-146. <http://www.tu-cottbus.de/fakultaet4/de/gewaesserschutz/downloads/aktuelle-reihe.html> Document name: "2008\_ar\_10.pdf" Wolfram et al., 2009. Reference conditions and WFD compliant class boundaries for phytoplankton biomass and chlorophyll-a in Alpine lakes. *Hydrobiologia* 633: 45-58.

### **1.13 Method developed by**

Ute Mischke, Brigitte Nixdorf, Ursula Riedmüller, Eberhard Hoehn  
mischke@igb-berlin.de, nixdorf@tu-cottbus.de, lbh@gmx.de  
IGB Berlin, University of Cottbus (BTU) and LBH (Freiburg)

### **1.14 Method reported by**

Ute Mischke, Eberhard Hoehn, Ursula Riedmüller  
mischke@igb-berlin.de, lbh@gmx.de  
Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB Berlin) & Limnology Bureau Hoehn (LBH), Freiburg, Germany

### **1.15 Comments**

Method development reports (in German): Mischke, U., Riedmüller, U., Hoehn, E., Nixdorf, B., 2007: Praxistest Phytoplankton in Seen. (Results of the German national exercise of the first proposal) Endbericht zum LAWA – Projekt (O 5.05). Berlin, Freiburg, Bad Saarow, Oktober 2007. S. 114. Nixdorf, B., Mischke, U., Hoehn, E. & Riedmüller, U. (2006): Überarbeitete Fassung des Berichtes: Leitbildorientierte Bewertung von Seen anhand der Teilkomponente Phytoplankton im Rahmen der Umsetzung der EU-Wasserrahmenrichtlinie (First proposal of the German assessment method for lakes by phytoplankton", 190 S. Nur Internet-Version: <http://www.tu-cottbus.de/BTU/Fak4/Gewschu/downloads/projekte.htm>. Nixdorf, B., K. Knopf, E. Hoehn, & U. Mischke (2001): Phytoplankton monitoring, classification and assessment in German lakes and rivers: present state and problems. In: Karttunen, K.: Monitoring and assessment of ecological status of aquatic environments: TemaNord. Nordic council of ministers. 11-18.

## 2. Data acquisition

### Field sampling/surveying

#### 2.01 Sampling/Survey guidelines

Nixdorf, B., E. Hoehn, U. Riedmüller, U. Mischke, I. Schönfelder & M. Bahnwart, 2008. Anforderungen an Probeentnahme und Analyse der Phytoplanktonbiozönosen in Seen zur ökologischen Bewertung gemäß der EU-WRRL. In Mischke, U. & B. Nixdorf (eds), Gewässerreport (Nr. 10), BTUC-AR 2/2008, ISBN 978-3-940471-06-2, Eigenverlag BTU Cottbus, 147-184. <http://www.tu-cottbus.de/fakultaet4/de/gewaesserschutz/downloads/aktuelle-reihe.html> Document name: "2008\_ar\_10.pdf"

#### 2.02 Short description

At the deepest point of the lake one integrated sample is taken: in polymictic lakes sub-samples from all water depth in 0.5 or 1m steps and in stratified lakes when  $Z_{eu} < Z_{epi}$ , than from epilimnion zone and if  $Z_{eu} > Z_{epi}$ , than from the euphotic zone (=  $Z_{epi} = 2.5 \times \text{Secchi depth}$ ). The sampling is carried out monthly between (March-) April and October.

#### 2.03 Method to select the sampling/survey site or area: n.a.

#### 2.04 Sampling/survey device: Water sampler

#### 2.05 Specification: Integral sampler acc. Hydro-Bios, UWITEC or Schröder, tube-sampler

#### 2.06 Sampled/surveyed habitat: n.a.

in stratified lakes: "Epilimnion" (above thermocline) or in clear water lakes the "Euphotic

#### 2.07 Sampled/surveyed zones in areas with 5m Secchi depth" in polymictic lakes: whole water column (0.5m above sediment) - all integrated samples in 0.5m or 1m steps of each water layer

#### 2.08 Sampling/survey month(s): (March-) April to October

#### 2.09 Number of sampling/survey occasions (in time) to classify site or area

at least 6 times per season

#### 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area

1 (this will be proven in the uncertainty test in EU-WISER)

#### 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area

Mixed sample should be taken at least 2 Liters.

### Sample processing

#### 2.12 Minimum size of organisms sampled and processed: 2µm, (Picoplankton 1 µm)

#### 2.13 Sample treatment: Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

see Utermöhl-technique DIN EN 15204 (2006) at least 20 taxa and 400 objects and two magnifications at inverse microscope

#### 2.14 Level of taxonomical identification: Species/species groups

see special column in the German taxa list for phytoplankton, which describe the level of required taxa determination for 1600 taxa (as a download file and in printed version in Mischke, U., Kusber W.-H. & U. Riedmüller (2008): Auszüge aus der harmonisierten Taxaliste des Phytoplanktons mit einem Vorschlag zur verfahrensspezifischen Mindestbestimmungstiefe für die Bewertung von natürlichen Seen der Ökoregionen Alpen und norddeutsches Tiefland. In: Mischke, U. & B. Nixdorf (Hrsg.), Gewässerreport (Nr. 10), BTUC-AR 2/2008, ISBN 978-3-940471-06-2, Eigenverlag BTU Cottbus, 203-263.) Free download see: <http://www.tu-cottbus.de/fakultaet4/de/gewaesserschutz/downloads/aktuelle-reihe.html> Document name: "2008\_ar\_10.pdf" and digital Excel-sheet in [www.igb-berlin.de/~mischke](http://www.igb-berlin.de/~mischke) keyword: Harmonisierte Taxaliste Phytoplankton

#### 2.15 Record of abundance: Individual counts

in relation to Volume

Unit biovolume in mm<sup>3</sup>/litre = cm<sup>3</sup>/m<sup>3</sup> => Biomass mg/l

#### 2.16 Quantification of biomass: Chlorophyll-a concentration, Utermöhl technique

#### 2.17 Other biological data: none

#### 2.18 Special cases, exceptions, additions: none

#### 2.19 Comments

none

## 3. Data evaluation

### Evaluation

#### 3.01 List of biological metrics

see method description Mischke et al. 2008b engl version Biomass: Total biovolume of all phytoplankton taxa (seasonal mean) and chlorophyll a concentration (seasonal mean and maximum); The measured value is transformed to a parameter index value according to a lake type-specific function, which is determined by the class boundaries for the five status classes. The

parameter index value ranges from 0.5 to 5.5. Algal classes: Up to three assessment parameters are compared to their specific class boundaries and must be averaged to yield the metric "biomass" value. Depending on the lake type, biovolumes of cyanobacteria, chlorophytes and/or dinophytes and crypto-phytes are either summed or their proportion to total biovolume (chrysophytes, dinophytes) is calculated. All parameter values from the periods "July to October" or "April to October" are averaged. The mean value is transformed to a parameter index value by a lake type-specific function. The parameter index value ranges from 0.5 to 5.5. PTSI (Phytoplankton-Taxa-Seen-Index) First of all, the PTSI serves to classify the trophic status of lakes (oligotrophic to hypertrophic) based on their species composition. Secondly, the PTSI is applied by comparing its value with the preset trophic reference value of the appropriate lake type. The difference to the reference situation is first calculated and subsequently transformed to a PTSI quality value (EQ), which ranges from 0.5 to 5.5.

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Weighted average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time  
Data from single sampling/survey occasion in time

### **Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites  
and palaeo-limnological studies

**3.07 Reference site characterisation**

**Number of sites:** for L AL3 5 sites, L AL4 3 sites, additionally austrian Alpine lakes were used; L CB 1 = 7 sites; L CB2

**Geographical coverage:** Alps and Alpine forelands; Lowlands: L CB1 ok; L CB2 no GIG reference lakes in Germany; see Technical report IC Lakes

**Location of sites:** IC 1st round see Technical reports Lakes Phytoplankton: AlpGIG DE: Alpsee bei Füssen, Bannwaldsee, Eibsee, Königssee, Lustsee, Obersee, Tegernsee, Walchensee, Weitsee, Wörthsee; CB GIG: Wittwese; additionally other CB GIG lakes were used

**Data time period:**

**Criteria:**

Alp region: oligotrophic (AL 3) resp. oligo-mesotrophic lakes (AL 4), in high status by pre classification Central Baltic region: mesotrophic (CB1) resp. meso-eutrophic lakes (CB2), in high status by pre classification low in-lake eutrophication pressure: TP and chl<sub>a</sub> boundaries as derived in the GIGs and De.

**3.08 Reference community description**

Biomass and community of phytoplankton according trophic reference conditions: oligotrophic status L AL3, oligo-mesotrophic status L AL4; mesotrophic status L CB1; mesotrophic-eutrophic status L CB2 Metric biomass: parameters chlorophyll a, total biovolume und maximal value of chlorophyll a (for boundaries see method description in Mischke et al. 2008) Algal classes community: parameters different for each lake type (see method description in Mischke et al. 2008) Indicator species for reference status : see species with trophic score (TAW) < 1.3 in the significant indicator list: alps& prealps; stratified lowland lakes; polymictic lowland lakes (see method description in Mischke et al. 2008) L AL3 TAW <1.3: Cyclotella comensis, Stephanocostis chantaica, Ceratium cornutum, Botryococcus braunii, Chroococcus turgidus, Cymatopleura solea, Discostella glomerata, Chrysolykos skujae, Cyclotella bodanica, Diatoma vulgaris, Cyclotella cyclopuncta, Cyclotella delicatula, Bitrichia chodatii, Amphora ovalis, Gymnodinium uberrimum, Planctonema lauterbornii, Pseudopedinella erkensis, Gymnodinium lantzschii, Fragilaria danica, Nitzschia palea, Peridinium willei, Cyclotella comensis Typ pseudocomensis, Stephanodiscus binderanus, Peridinium umbonatum-Komplex, Fragilaria cyclopus, Synechococcus cedrorum, Tabellaria flocculosa, Tetraselmis cordiformis, Anabaena spiroides, Dinobryon divergens, Chroococcus limneticus, Chroococcus minutus L AL4 TAW <1.5: all as in LAL3 and additionally: Leptolyngbya tenuis, Fragilaria ulna var. ulna, Cyclotella meneghiniana, Chrysolykos planctonicus, Aulacoseira subarctica, Fragilaria capucina - Formenkreis, Aulacoseira islandica, Willea irregularis, Anabaena flos-aquae/solitaria, Cosmarium depressum, Planktothrix rubescens s.o.

**3.09 Results expressed as EQR?** Yes normalized EQR for all metrics along linear pressure scale

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.

**3.11 Boundary setting procedure**

The reference status of lake types are defined with a view to existing near-natural reference sites (listed in Nixdorf et al. 2005; Mischke et al. 2008), which were checked by paleo-limnological investigations, local expert judgement and total phosphorus

background boundaries according the modelling approach of the German LAWA Index (1999) based on the regressions found in the OECD study of Vollenweider. Land use data of the catchment area of the lakes were seldom available in Germany before 2007. In the case of the Alpine GIG Germany contributed 10 reference lakes. In CB GIG Germany could deliver only 3 reference sites for type LCB1 and none for LCB2, because population density not match the reference criteria. Reference sites of the whole GIG's, which were checked for land use data, were used to define TP and chlorophyll\_a criteria. These in-lake pressure criteria and boundaries were used in Germany to find further near-natural sites. Type-specific boundaries of chlorophyll a for H/G and G/M were first expressed as 0.5 step deviations from the reference status along the scale of the German LAWA-Index and a TP-derived Index. The preliminary class boundaries of biovolume and chlorophyll\_a were checked during the intercalibration process in the Alpine and CB GIG and were adopted if necessary to the common GIG boundaries of chlorophyll a. The trophic scores of the indicator species metric (PTSI) and the algal class metric were calibrated along the classifying trophic indices LAWA-Index/TP-Index. For assessment the PTSI is numerically compared with reference status.

- 3.12 "Good status" community:** At good status phytoplankton biomass and community were in trophic good conditions: mesotrophic1 status L AL3, mesotrophic2 status L AL4; mesotrophic2 status L CB1; eutrophic1 status L CB2. Example for L AP3: chlorophyll a remains below 3.7 µg/l in vegetation mean; max chl-a-value below 6.4 µg/l; total biovolume below 1mm<sup>3</sup>/l; the sum of chlorophytes & cryptophytes remains below 0.27mm<sup>3</sup>/l and of cyanobacteria below 1mm<sup>3</sup>/l in the vegetation mean; the proportion of dinophytes to total biovolume is more than 9.5%; the indicator species of the region (AVA) with trophic score <2.5 are clearly more abundant than species with trophic score below 2.5. For all IC lake types see method description Mischke et al. 2008

### **Uncertainty**

- 3.13 Consideration of uncertainty:** Yes

By long-term studies: Year to year changes were used to see if the index remains stable when pressure conditions remain the same.

- 3.14 Comments:**

in Alpine GIG metric PTSI and biomass are intercalibrated in CB GIG only parameter chlorophyll a is intercalibrated, but not the whole German biomass metric: total biovolume, chlorophyll a and max chl\_a value

ID: 237

LFI

## 1. General information

- 1.01 GIG:** Alpine, Mediterranean
- 1.02 Category:** Lakes
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Italy
- 1.05 Specification:** Only for Natural lakes
- 1.06 Method name:** *Lake Fish Index - Index for the assessment of quality of the fish communities in italian lakes*
- 1.07 Original name:** *Lake Fish Index - Indice per la valutazione dello stato di qualità della fauna ittica nei laghi italiani*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
CNR ISE Report 2/09. Indici per la valutazione della qualità ecologica dei laghi.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Pietro Volta  
p.volta@ise.cnr.it  
CNR-Institute of Ecosystem Study
- 1.14 Method reported by**  
Pietro Volta  
p.volta@ise.cnr.it  
CNR-Institute of Ecosystem Study
- 1.15 Comments**  
As fish fauna in italian lakes is highly impacted by anthropogenic pressures, it is impossible to find reference site and thus reference conditions. For this reason reference conditions for each lake typology were reconstructed on the base of historical data (presence/absence) on fish composition

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
CEN standards: sampling with multimesh gillnets and electricity.
- 2.02 Short description**  
Nets are put down from the evening until the following morning. Electrofishing is done by point abundance sampling along the perimeter of the lake, at least 120 points must be sampled.
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying, Stratified sampling/surveying
- 2.04 Sampling/survey device:** Electrofishing gear, Gill net
- 2.05 Specification:** Multimesh gillnets; Electrofisher > 4Kw
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** From 15th July to 15th October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Sampling is done once in a year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Every net for each depth strata is considered a replicate.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
The whole lake down to 70 m

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 1 cm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** n.a.  
Number of fish per effort (total effort- i.e. all nets + electrofishing)

**Unit** Number of individuals**2.16 Quantification of biomass:** n.a.

Fresh weight (g)

**2.17 Other biological data:** Length, age, sex**2.18 Special cases, exceptions, additions:** none**2.19 Comments**  
none**3. Data evaluation****Evaluation****3.01 List of biological metrics**

Metric 1: Relative Abundance of key species (NPUE) - Number of specimens captured in the standard sampling. It also considers informations coming from other surveys or fishing statistics. If the population of the key-species is in reference conditions but is sustained by stocking, the score of the metric must be decreased by one category. If there are more than one key species, the final score must be calculated as an average of each single score and eventually kept at the higher one.

SCORE Reference: >60 individuals : points 10  
7-60 individuals: points 8  
1-6 individuals: points 6  
Not captured in standard sampling but presence confirmed by informations from the last 5 years: Points 4  
Nor captured nor informations in the last 5 years: points 2

2. Population structure of the key species (Population Structure Density index - PSD) - Proportional Stock Density index PSD =  $(N_i \geq L_m) / (N_i \geq L_{stock}) * 100$   
Lstock =  $L_m - (L_{Trophy} - L_m) / 3$   
Lm = mean length at maturity

PSD = 35-65: reference - 10 points  
PSD 25-34; 66-75: 6 points  
PSD <25/>75: 2 points

If there is more than one key species, the score must be calculated as an average of all scores and eventually kept at the higher score.

3. Reproductive success of key- and type- specific fish species (presence/absence of juveniles 0+ 1+)

reference =>80%: 10 points  
80- 66%: 8 points  
65-51%: 6 points  
50-25%: 4 points  
<25%: 2 points

4. % reduction of key and type- specific fish species: % categories

reference: <25%: 10 points  
25-50%: 8 points  
51-65%: 6 points  
66-80%: 4 points  
>80%: 2 points

5. % presence of alien species: % categories

reference = <20%: 10 points  
20-40% = 8 points  
41-60% = 6 points  
61-80% = 4 points  
>80% = 2 points

Metric Score/Reference = EQR for each single metric

Finale EQR: simple average between EQR

Boundaries: 0.8 (High/Good); 0.6 (Good/moderate).....

**3.02 Does the metric selection differ between types of water bodies?** No**3.03 Combination rule for multi-metrics:** Average metric scores**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple spatial replicates

**Reference conditions****3.05 Scope of reference conditions:** Surface water type-specific**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data

**3.07 Reference site characterisation****Number of sites:** 50**Geographical coverage:** Italian natural lakes larger than 0.5km<sup>2</sup>**Location of sites:** All the Italian peninsula**Data time period:** Historical data before 1950**Criteria:**

It has been assumed that before 1950 pressures on fish fauna were very low in all Italian lakes. Therefore every lake was in "reference" conditions (or near natural), with its own specific fish species composition and well structured populations.

**3.08 Reference community description**

For each lake type a set of key species and type-specific fish species has been defined.

Type 1 deep lakes of Alpine Ecoregion (north west): well structured population of *Coregonus lavaretus* + *Alosa agone* + *Lota lota*; presence of young individuals of *Alburnus alburnus alborella*, *Leuciscus cephalus*, *Cyprinus carpio*, *Esox lucius*, *Perca fluviatilis*, *Scardinius erythrophthalmus*, *Tinca tinca*, *Rutilus erythrophthalmus*, *Salmo trutta*; low percentage (<20%) of alien species

Type 2 deep lakes of Alpine Ecoregion (north east) : well structured population *Esox lucius* + *Scardinius erythrophthalmus* + *Tinca tinca*; presence of young individuals of *Chondrostoma soetta*; *Leuciscus cephalus*; *Cyprinus carpio*; *Salmo trutta*; low % (<20%) of alien species.

Type 3: Shallow lake of Alpine Ecoregion: well structured population *Esox lucius* + *Scardinius erythrophthalmus* + *Tinca tinca*; presence of young individuals of *Alburnus alburnus alborella*, *Cyprinus carpio*; *Perca fluviatilis*

Type 4: high altitude lakes - Alpine Ecoregion: well structured population of *Phoxinus phoxinus*; presence of young individuals of *Salvelinus alpinus* and/or *Salmo trutta*; low % (>20%) of alien species

Type 5: deep lake of Mediterranean Ecoregion: well structured population of *Coregonus lavaretus* and at least one among *Alburnus alborella*, *Rutilus rubilio* and *Atherina boyeri*; presence of young individuals of *Perca fluviatilis*, *Leuciscus cephalus*, *Cyprinus carpio*, *Esox lucius*, *Scardinius erythrophthalmus*, *Tinca tinca*. Low percentage of alien species (<20%)

Type 6: shallow lake of MED :

well structured populations of *Esox lucius* + *Tinca tinca* + *Scardinius erythrophthalmus*; presence of young individuals of *Cyprinus carpio*, *Perca fluviatilis*, *Rutilus rubilio*, *Alburnus alburnus* *alboella*, *Atherina boyeri*; low percentage (<20%) of alien species

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

#### **3.11 Boundary setting procedure**

As the index does not consider each pressure but a general degradation, boundaries were set with equidistant division.

**3.12 "Good status" community:** More than 6 individuals of key species captured during sampling  
Population structure Index PSD >25;<75.  
Key and type specific species with reproductive success: > 65%  
Reduction of key and type specific fish species: <60%  
Presence of alien species: <60%

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

#### **3.14 Comments:**

none

ID: 203

PISIAD

## 1. General information

- 1.01 GIG:** Central-Baltic  
LCB1, LCB2
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish Region
- 1.06 Method name:** *Proportions of Impact-Sensitive and Impact-Associated Diatoms*
- 1.07 Original name:** *Procentuele abundantie van impact-sensitieve en impact-geassocieerde diatomeeën*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Acidification, Eutrophication, General degradation, Heavy metals, Hydromorphological degradation, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter salinity change
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
see Hendrickx & Denys (2005) for relations to chlorophyll a and TP in 202 ponds and small lakes
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. September 2009. Available in Dutch and English. Vlaamse Milieumaatschappij, Erembodegem, Belgium.
- 1.12 Scientific literature:**  
n.a.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Luc Denys<br>luc.denys@inbo.be<br>Research Institute for Nature and Forest | <b>1.14 Method reported by</b><br>Wim Gabriels<br>w.gabriels@vmm.be<br>Flemish Environment Agency |
|---|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
EN 13946:2003.
- 2.02 Short description**  
The order of preference for the substrate to be sampled is as follows: (1) living reed: Reed plants are cut with scissors. Only the zone about 10 cm below the water surface is collected; (2) other similar, living helophytes (monocotyls such as cattail (Typha), rushes (Scirpus, Juncus),...) are used in absence of reed; (3) stones: In absence of reed or other useful helphytes, stones are sampled. Five different stones that were found spread throughout the location are sampled. These stones are lifted from the water. With a (pocket) knife or sharpened spoon the epilithon is removed from the stones and stored in a container (60 – 100 ml) with a wide screw cap and extra closing lid; (4) artificial substrates are used in absence of all the above: preferably permanent, vandal-resistant constructions are chosen of inert material on which a biofilm can develop undisturbed during the whole year.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Scraper, Spoon
- 2.05 Specification:** Knife
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
epiphyton, or when this is not available, epilithon
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** june-september
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
at least 1
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3-9 per lake in function of variability of obtained EQRs
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
about 10 cm<sup>2</sup> epilithon or epiphyton

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** All valves observed in the microscope

- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
The sample is cleaned using oxidizing agents and homogenised and part of the sample is embedded in naphrax for identification with microscope. 500 valves are identified and counted.
- 2.14 Level of taxonomical identification:** Other, Species/species groups  
including subspecific taxa
- 2.15 Record of abundance:** Relative abundance  
**in relation to** n.a.  
number of valves  
**Unit** percentage, proportion
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Percentage of impact-associated diatoms (IAD); percentage of impact-sensitive diatoms (ISD)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
If IAD exceeds a predefined threshold, EQR gets a value between 0-0,60 based on a transformation of IAD; otherwise EQR gets a value between 0,60-1 based on a transformation of ISD.
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:**  
**Geographical coverage:**  
**Location of sites:** n.a.  
**Data time period:**  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
Reference conditions are characterised by a relatively low relative abundance of impact-associated diatoms and a relatively high relative abundance of impact-sensitive diatoms
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
Class boundaries based on IAD and ISD threshold values are based on expert judgement and comparison with historical data; they are transformed in such a way that equidistant division of the EQR gradient (boundaries at 0,8; 0,6; 0,4 and 0,2) is obtained
- 3.11 Boundary setting procedure**  
EQR gradient is assumed to represent a continuous trend with general degradation.
- 3.12 "Good status" community:** The EQR values at good status are characterised by a relatively low IAD and a ISD that is slightly reduced in comparison to reference.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 205

Diatom index TIJ

## 1. General information

**1.01 GIG:** Central-Baltic

**1.02 Category:** Lakes

**1.03 BQE:** Benthic Diatoms

**1.04 Country:** Poland

**1.05 Specification:**

**1.06 Method name:** *Assessment system for lakes using diatom phytobenthos*

**1.07 Original name:** *Ocena stanu ekologicznego jezior w oparciu o fitobentos okrzemkowy (Indeks Okrzemkowy TIJ)*

**1.08 Status: Method is/will be used in** Second RBMP (2015)

**1.09 Detected pressure(s):** Catchment land use, Eutrophication

*Has the pressure-impact-relationship been tested?*

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Joanna Picinska-Faltynowicz

joanna.faltynowicz@imgw.wroc.pl

Institute of Meteorology and Water Management, Wrocław  
Branch, Department of Ecology

**1.14 Method reported by**

Joanna Picinska-Faltynowicz

joanna.faltynowicz@imgw.wroc.pl

Institute of Meteorology and Water Management, Wrocław Branch,  
Department of Ecology

**1.15 Comments**

Trophic index for lakes is in preparation and the method will be ready till the end of March

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Picinska-Faltynowicz, J. & J. Blachuta, 2008. Zasady poboru i opracowania prób fitobentosu okrzemkowego z rzek i jezior. Przewodnik metodyczny. Wersja 2008.

**2.02 Short description**

A sample from one sampling site is composed of 5-6 sub-samples collected from different submerged macrophytes from a depth > 30 centimetres below water table, in a littoral zone adjacent to open waters and in places not affected by wave action.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Scraper

**2.05 Specification:** A knife

**2.06 Sampled/surveyed habitat:** Single habitat(s)

Surface of submerged macrophytes

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** August - October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

5-6 replicates constitute one sample from researched site

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

n.a.

### Sample processing

**2.12 Minimum size of organisms sampled and processed:**

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Relative abundance

**in relation to** n.a.

a total of 300-500 diatom valves counted per sample (in a permanent slide)

**Unit**

- 2.16 Quantification of biomass:** n.a.  
**2.17 Other biological data:** none  
**2.18 Special cases, exceptions, additions:** none  
**2.19 Comments:** none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Trophic Diatom Index for Lakes TIJ calculated using a weighted formula of Zelinka & Marvan (1961)@GR - module of reference taxa = a sum of relative abundances of these taxa; both metrics are in preparation
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:**  
**Geographical coverage:** Reference zones in natural and landscape parks of Central Plains, Baltic Province and Eastern Plains  
**Location of sites:** Drawieski Natural Park, Chojnicki, Drawski and Suwalski Landscape Parks  
**Data time period:** August-October 2006-2009  
**Criteria:**  
Absence of point pollution sources, sub-basin overgrown by natural forests, meadows and wetlands
- 3.08 Reference community description**  
Epiphytic diatom communities dominated by reference species, i.e. oligo-, meso- or eutrophilous depending on lake type
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
Ecological status boundaries are processing at present
- 3.11 Boundary setting procedure**  
In preparation
- 3.12 "Good status" community:** In preparation.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:** none

ID: 193

MMIF

## 1. General information

- 1.01 GIG:** Central-Baltic  
L-CB1, L-CB2
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish region
- 1.06 Method name:** **Multimetric Macroinvertebrate Index Flanders**
- 1.07 Original name:** *Multimetrische Macro-invertebratenindex Vlaanderen*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Heavy metals, Hydromorphological degradation, Impact of alien species, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter, Riparian habitat alteration

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. September 2009. Vlaamse Milieumaatschappij, Erembodegem, Belgium.

**1.12 Scientific literature:**

Gabriels, W., K. Lock, N. De Pauw & P.L.M. Goethals, 2010. Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). *Limnologica* (in press). DOI: 10.1016/j.limno.2009.10.001.

**1.13 Method developed by**

Wim Gabriels et al.  
w.gabriels@vmm.be  
Flemish Environment Agency

**1.14 Method reported by**

Wim Gabriels  
w.gabriels@vmm.be  
Flemish Environment Agency

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

NBN T92-402. Biological quality of watercourses. Determination of the Biotic Index based on aquatic macroinvertebrates.

**2.02 Short description**

With the handnet, a stretch of approximately 10-20 meters is sampled during 3-5 minutes. Sampling effort is proportionally distributed over all accessible aquatic habitats. This includes the bed substrate (stones, sand or mud), macrophytes (floating, submerged, emerged), immersed roots of overhanging trees and all other natural or artificial substrates, floating or submerged in the water. Each aquatic habitat is explored, either with the handnet or manually, in order to collect the highest possible diversity of macroinvertebrates. For this purpose, kick sampling is performed by vertically positioning the handnet on the bed and turning over bottom material located immediately upstream by foot or hand. In addition to the handnet sampling, animals are manually picked from stones, leaves or branches along the same stretch. If a site is too deep to be sampled with the handnet method, macroinvertebrates can alternatively be sampled using the so-called Belgian artificial substrates. These are composed of a plastic netting filled with medium-sized (4-8 cm) pieces of brick, with a total volume of approximately 5 L. Per sampling site, three substrates are placed in the water, anchored with a rope to a fixed point located on the bank. The substrates should not be placed in open water but along the banks: in protected sites among the vegetation near the surface, in unprotected sites, which are exposed to surface turbulence, in deeper water. After an exposure time of at least 3 weeks, the substrates are lifted from the water and transferred into a closed container.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Artificial substrate, Hand net  
Standard method is handnet; a

**2.05 Specification:** Handnet: standard handnet with 500 µm mesh size / Artificial substrates: a plastic netting filled with medium-sized (4-8 cm) pieces of brick, with a total volume of approximately 5 L

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** April - november

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

1

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

3

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Sampling duration of 3-5 minutes

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:** All animals retained after sieving with 500 µm mesh size

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Family, Genus, Other

Plathelminthes, Hirudinea, Mollusca, Hemiptera, Megaloptera, Odonata, Ephemeroptera, Plecoptera: genus; Polychaeta, Oligochaeta, Coleoptera, Trichoptera, Crustacea: family; Diptera (Chironomidae): group (thummi-plumosus or non thummi-plumosus); Diptera (other): family; Acari: presence (i.e. counted as one taxon)

**2.15 Record of abundance:** Individual counts

in relation to n.a.

Total sample

Unit number of individuals per sample

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

**Evaluation**

**3.01 List of biological metrics**

Total number of present taxa; number of EPT taxa; number of other sensitive taxa; Shannon-Wiener diversity index; mean tolerance score (the mean of the tolerance scores of all encountered taxa; the tolerance score is predefined for each taxon)

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

**Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge

**3.07 Reference site characterisation**

**Number of sites:**

**Geographical coverage:**

**Location of sites:** n.a.

**Data time period:**

**Criteria:**

n.a.

**3.08 Reference community description**

Reference conditions are assumed to correspond to an EQR value of 1, which is associated with expert-based type-specific metric values reflecting high taxa richness, sensitivity and diversity.

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** n.a.

Boundaries used for most river types (resulting from intercalibration exercise) are applied to lakes as well.

**3.11 Boundary setting procedure**

EQR gradient is assumed to represent a continuous correlation with general degradation.

**3.12 "Good status" community:** The EQR values at good status reflect metric values that are only slightly lower than at (expert-

based) reference state, hence the community can be characterised as only slightly different from reference in terms of taxa richness, sensitivity and diversity.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 138

KRW-maatlatten

## 1. General information

- 1.01 GIG:** Central-Baltic  
L-CB1, L-CB2
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Netherlands
- 1.05 Specification:** none
- 1.06 Method name:** *WFD-metrics for natural watertypes*
- 1.07 Original name:** *KRW-maatlatten voor natuurlijke watertypen*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Hydromorphological degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).  
The metric for invertebrates in lakes is validated for chemical pressures (n = 53 samples; Data for pressures in shallow fresh water lakes is scarce). High nutrient concentrations limited the metric score, but low nutrient concentration does not automatically result in a high metric score. Additionally a distinct relation between hydromorphological alteration and EQR was observed. Other pressures (maintenance, shipping, recreation) seemed to play an important role but the impact from these pressures could not be quantified.
- 1.10 Internet reference:** [http://themas.stowa.nl/thema/ecologische\\_beoordeling/krw-maatlatten.aspx?mld=7213&rid=817](http://themas.stowa.nl/thema/ecologische_beoordeling/krw-maatlatten.aspx?mld=7213&rid=817)
- 1.11 Pertinent literature of mandatory character:**  
Besluit Kwaliteitseisen en Monitoring Water (2009). Ministry of Housing, Spatial Planning and the Environment (presently under public consultation).
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
development by national expert group commissioned by STOWA, Bas van der Wal & RWS Waterdienst, Diederik van der Molen  
b.van.der.wal@stowa.nl  
STOWA Foundation for Applied Water Management Research & Rijkswaterstaat Waterdienst
- 1.14 Method reported by**  
Roel Knoben  
r.knoben@royalhaskoning.com  
Rijkswaterstaat Waterdienst
- 1.15 Comments**  
Description of KRW-maatlatten in Dutch.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) Quality Handbook Hydrobiology (in prep). 2009STOWA.
- 2.02 Short description**  
Multihabitat sampling in all habitats present in proportion to their presence. Active moving of handnet through vegetation and bottom substrates.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Corer, Grab, Hand net
- 2.05 Specification:** handnet 30\*15 cm. grab: Van Veen or Eckman Birge. core: boxcorer
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** march till 15 june
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
minimum one occasion per year (spring), but classification preferably averaged over three years.
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
one
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
lakes 5 m handnet = 1,5 m2

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 500 um
- 2.13 Sample treatment:** Organisms of the complete sample are identified.

only if some organisms occur in extreme high number, subsampling is done and total number is estimated.

**2.14 Level of taxonomical identification:** Species/species groups

Oligochaetes and Hydracarina may sometimes be determined at genus/family level.

**2.15 Record of abundance:** Individual counts

**in relation to** Area

**Unit** numbers in standard sample. (5 m handnet)

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

Biological WFD monitoring is performed by 26 regional water boards. Small differences may occur in sampling strategies etc.

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

lakes:  $EQR = \{ 200 * (KM\% / KMmax) + (100 - DN\%) + (KM\% + DP\%) \} / 400$  where  $KM\%$  = relative number of typical (for watertype) species in a sample  $KMmax$  - maximum achievable number of typical species under reference conditions  $DN$  = relative abundance of dominant negative species  $(DP + KM) =$  sum of relative abundances of dominant positive species and typical species  $\rightarrow$  Abundances are converted first to abundance (log) classes  $\rightarrow$  The metric for invertebrates in lakes is based on the littoral zone and not the pelagic or benthic zone.

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data, Least Disturbed Conditions  
no actual existing natural sites in lakes;

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** n.a.

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**

All lakes in The Netherlands are (very) high hydromorphological impacted, level fluctuation is completely controlled (less than 5 cm) and most of them are moderately to highly impacted by eutrophication. Too few lakes are assumed to meet the criteria of (almost) unimpacted

**3.08 Reference community description**

Regarding the metric: High status of lakes is characterized by a high abundance of dominant positive species and a high diversity and abundance of typical species. Dominant negative species are nearly absent. Furthermore a general description is given (in Dutch) in: STOWA (2009) Referenties en maatlaten voor natuurlijke watertypen. report 2007-32

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

The boundaries for the different EQR-classes (bad, poor, moderate, good and high) are set based on expert judgement and follow a more or less equal division of quality. The WFDi and its class-boundaries were validated by experts judging species lists from anonymous sites, using normative definitions. Validation was done based on existing data on shallow lakes from the Netherlands (Naardermeer, Randmeren, Vollenhovermeer and Wijchens Ven). In the validation of the method the response of the WFD-classes to pressures was tested. WFD-classes responded negatively to hydromorphologic pressure. Of the chemical pressures studied, EQR is most related to oxygen content. EQR and oxygen availability are positively correlated.

Influences of other chemical pressures considered (phosphate and nitrogen content) were less clear. Water bodies in the Netherlands are hydromorphologically altered, making physical pressure an important factor in assessment of Dutch water bodies.

- 3.12 "Good status" community:** Good status is characterized by a high diversity and abundance of typical species and an increasing abundance of dominant positive species. The abundance of dominant negative species is low.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** Yes

Precision and uncertainty is regarded in Van Herpen, van Tongeren, Knobben, Baggelaar, van Loon (2009). Quick scan precision and confidence of KRW assessment (in Dutch). This study resulted in a statistical method to assess the level of precision and confidence monitoring results and status classifications (including identifying outliers and estimates for missing values). The confidence of a status classification is expressed as the probability of exceeding a chemical limit value or the biological status classification moderate/good. Recommendations from this study are incorporated in the Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) (see question B.0). In the metric abundance is expressed in abundance classes to reduce the impact of extreme abundance of one species on the calculated EQR.

- 3.14 Comments:**

data for shallow lakes is scarce. It was difficult to derive type-specific metric so some type of lakes share the same metric.

ID: 197

IBI

## 1. General information

- 1.01 GIG:** Central-Baltic  
Lowland-Midland
- 1.02 Category:** Lakes
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish Region
- 1.06 Method name:** *Flemish Index of Biotic Integrity*
- 1.07 Original name:** *Vlaamse Index voor Biotische Integriteit*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Impact of alien species
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. September 2009. Vlaamse Milieumaatschappij, Erembodegem, Belgium.
- 1.12 Scientific literature:**  
Belpaire, C., R. Smolders, I. Vanden Auweele, D. Ercken, J. Breine, G. Van Thuyne & F. Ollevier, 2000. An Index of Biotic Integrity characterizing fish populations and the ecological quality of Flandrian waterbodies. *Hydrobiologia* 434 (1-3): 17-33.
- |  |  |
|--|--|
| <b>1.13 Method developed by</b>          | <b>1.14 Method reported by</b>           |
| Jan Breine                               | Jan Breine                               |
| jan.breine@inbo.be                       | jan.breine@inbo.be                       |
| Research Institute for Nature and Forest | Research Institute for Nature and Forest |
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
100 m electric fishing along the banks, fykes are placed randomly for a period of 24-48 h (remark: method has yet to be standardised)
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Electrofishing gear, Fyke net, Gill net, Seine netting
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** March - November
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
1 per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
depends on site : 3/ha
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
100 m electric and 24 - 48 h fykes

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 1 mm but all fish are processed (weighed and measured)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to Area  
Unit kg/ha
- 2.16 Quantification of biomass:** n.a.

balance

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
method needs modifications

### 3. Data evaluation

#### **Evaluation**

**3.01 List of biological metrics**  
see Belpaire et al., 2000

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

Data from single spatial replicate

#### **Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Historical data

**3.07 Reference site characterisation**

**Number of sites:**

**Geographical coverage:**

**Location of sites:** n.a.

**Data time period:**

**Criteria:**

n.a.

**3.08 Reference community description**

Reference conditions are assumed to correspond to an EQR value of 1, which is associated with expert-based type-specific metric values reflecting high taxa richness, sensitivity and diversity.

**3.09 Results expressed as EQR?** Yes

#### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.

**3.11 Boundary setting procedure**

**3.12 "Good status" community:** The EQR values at good status reflect metric values that are only slightly lower than at (expert-based) reference state, hence the community can be characterised as only slightly different from reference in terms of taxa richness, sensitivity and diversity.

#### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 239

LaFiEstA EQR3,5

## 1. General information

- 1.01 GIG:** Central-Baltic
- 1.02 Category:** Lakes
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Estonia
- 1.05 Specification:** up to now on the data of 14 lakes estimated in 2009
- 1.06 Method name:** *Lake Fish in Estonia. Assessment EQR 3,5*
- 1.07 Original name:** *Järvekalad Eestis. Hindamine. EQR 3,5*
- 1.08 Status: Method is/will be used in** neither first nor second RBMP
- 1.09 Detected pressure(s):** Eutrophication  
over-catch

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

- 1.10 Internet reference:** n.a.

- 1.11 Pertinent literature of mandatory character:**

- 1.12 Scientific literature:**

- 1.13 Method developed by**

n.a.

Teet.Krause@emu.ee, Anu.Palm@emu.ee

Centre for Limnology, Institute of Agricultural and Environmental  
Sciences, Estonian University of Life Sciences

- 1.14 Method reported by**

Teet Krause

Teet.Krause@emu.ee

Centre for Limnology, Institute of Agricultural and Environmental  
Sciences, Estonian University of Life Sciences

- 1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**

EN 14757:2005 Water-quality - Sampling of fish with multimesh gillnets

- 2.02 Short description**

Around 8p.m. gill-nets are launched from a boat in a line Nordic and common type nets interlaced in certain order. In addition to depth measurements, coordinates are taken at the start and end of the sample-line by GPS. 12 hours later (8a.m.) nets are taken out into the boat, if possible, every fish is removed from the net and sorted by net and mesh-size before specification and measurements (weight to 0.1 g, total length to 1 mm) individually at place (otherwise transported directly to lab for measurements). Piscivorous species are examined for food items and sex. Scales, operculum or cleithrum are/is removed for age determinations.

- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying

- 2.04 Sampling/survey device:** Gill net

- 2.05 Specification:** Nordic-type multimesh gill-net 1,5 x 30 m, benthic and pelagial; Nordic-type multimesh gill-net 6 x 30 m pelagial

- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

- 2.07 Sampled/surveyed zones in areas with tidal influence:**

- 2.08 Sampling/survey month(s):** July-October

- 2.09 Number of sampling/survey occasions (in time) to classify site or area**

one

- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

At least 5 per sampling

- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

for 12 hours at least 150 m

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 35 mm (TL), 0,3 g (TW)

- 2.13 Sample treatment:** Organisms of the complete sample are identified.

- 2.14 Level of taxonomical identification:** Species/species groups

- 2.15 Record of abundance:** n.a.

Length, weight, age, sex for piscivorous species

**in relation to** n.a.

- per gill-net  
**Unit** individuals per gill-net (NPUE)
- 2.16 Quantification of biomass:** n.a.  
 weight per gill-net (WPUE)
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
 none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
 Number of individuals per net (NPUE), Total weight of a catch (WPUE); percentage of non-piscivorous individuals (weight) in a catch (KI); share of each species (weight) in catch to calculate Simpson Dw; presence of endangered species; number of age-classes in a sample,
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
 Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
 Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
 All 12 mesh-sizes capture individuals; at least 10 year-classes are represented. Log (10) NPUE/KI per index of shoreline complexity < 1. Simpson Dw per log(10) lake area < 1. At least one endangered species inhabits the site.
- 3.08 Reference community description**  
 There are not any undisturbed fish communities in Estonia, 'least disturbed sites' are the best available choice - even those are angled at least.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
 1 - reference; 0,9 - very good; 0,5-0,8 - good; <0,5 poor
- 3.12 "Good status" community:** At least 6 mesh-sizes capture individuals; at least 5 year-classes are represented. Log (10) NPUE/KI per index of shoreline complexity is between 1 and 2. Simpson Dw per log(10) lake area is between 1 and 2.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)  
 no
- 3.14 Comments:**  
 none

ID: 196

DE-FI-LA

## 1. General information

**1.01 GIG:** Central-Baltic

**1.02 Category:** Lakes

**1.03 BQE:** Fish Fauna

**1.04 Country:** Germany

**1.05 Specification:** not applied, under development

**1.06 Method name:** *Assessment of fish fauna in lakes*

**1.07 Original name:** *n.a.*

**1.08 Status: Method is/will be used in** *n.a.*

**1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction  
general degradation

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Mass screening of Spearman CC was used to identify significant indicators for eutrophication, shoreline degradation and in-lake use, no final selection of metrics by now

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

*n.a.*

**1.12 Scientific literature:**

*n.a.*

**1.13 Method developed by**

David Ritterbusch, Uwe Braemick

david.ritterbusch@ifb-potsdam.de

Institute of Inland Fisheries e.V. Potsdam Sacrow

**1.14 Method reported by**

David Ritterbusch

david.ritterbusch@ifb-potsdam.de

Institute of Inland Fisheries e.V. - Potsdam Sacrow

**1.15 Comments**

Assessment system is under development in cooperation with MS of the CB GIG. Not finished and not finally reviewed or accepted by experts and/or officials on a national level. Unclear if and when the method will be used.

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

CEN 14011@CEN 14757@CEN 14962.

**2.02 Short description**

random stratified multimesh gillnets, fishing with electricity in most cases

**2.03 Method to select the sampling/survey site or area:** Random sampling/surveying, Stratified sampling/surveying

**2.04 Sampling/survey device:** Electrofishing gear, Gill net

**2.05 Specification:** see CEN 14757, CEN 14011

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** May-October (water temperature > 15°C)

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

not validated until now

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

depends on lake size and depth (see CEN descriptions)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

*n.a.*

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** ?

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

**in relation to** *n.a.*

effort (area of nets/night)

Unit Number/weight of individuals per square-meter net per night

- 2.16 Quantification of biomass: n.a.  
fishes are weighted
- 2.17 Other biological data: length
- 2.18 Special cases, exceptions, additions: none
- 2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics  
not present
- 3.02 Does the metric selection differ between types of water bodies? Yes
- 3.03 Combination rule for multi-metrics: n.a.
- 3.04 From which biological data are the metrics calculated?  
Aggregated data from multiple sampling/survey occasions in time  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:
- 3.06 Key source(s) to derive reference conditions:  
n.a.
- 3.07 Reference site characterisation  
Number of sites:  
Geographical coverage:  
Location of sites: n.a.  
Data time period:  
Criteria:  
n.a.
- 3.08 Reference community description  
n.a.
- 3.09 Results expressed as EQR? Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries: n.a.
- 3.11 Boundary setting procedure
- 3.12 "Good status" community: n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty: No (to be done)
- 3.14 Comments:  
none

ID: 140

KRW-maatlatten

## 1. General information

- 1.01 GIG:** Central-Baltic  
L-CB1, L-CB2
- 1.02 Category:** Lakes
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Netherlands
- 1.05 Specification:** none
- 1.06 Method name:** *WFD-metrics for natural watertypes*
- 1.07 Original name:** *KRW-maatlatten voor natuurlijke watertypen*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Acidification, Eutrophication, General degradation, Hydromorphological degradation, Riparian habitat alteration  
under water vegetation
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).  
ordination for fish communities related to pressures resulting of clusters of environmental variables (ranging from n = 4 to n = 38). Isolation of lakes in relation to number of species was assessed (n = 4 to n = 40)
- 1.10 Internet reference:** [http://themas.stowa.nl/thema/ecologische\\_boordeling/krw-maatlatten.aspx?mid=7213&rid=817](http://themas.stowa.nl/thema/ecologische_boordeling/krw-maatlatten.aspx?mid=7213&rid=817)
- 1.11 Pertinent literature of mandatory character:**  
Evers, C.H.M., H. de Mars, A.J.M. van den Broek, R. Buskens, M. Klinge & N. Jaarsma, 2005. Validatie en verdere operationalisering van de concept KRW-maatlatten voor de natuurlijke rivier- en meertypen. Consortium (Royal Haskoning, Taken Landschapsplanning, Witteveen+Bos) in opdracht van RWS-RIZA. [Jaarsma, N., M. Klinge & R. Pot \(eds\), 2007. Achtergronddocument Referenties en Maatlatten Vissen ten behoeve van de Kaderrichtlijn Water. STOWA, Utrecht.](#) [Van der Molen, D.T. & R. Pot \(eds\), 2007. Referenties en maatlatten voor natuurlijk watertypen voor de Kaderrichtlijn Water. STOWA 2007-32; RWS-WD 2007-018.](#)
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
development by national expert group commissioned by  
STOWA, Bas van der Wal & RWS Waterdienst, Diederik van der Molen  
b.van.der.wal@stowa.nl  
STOWA Foundation for Applied Water Management Research & Rijkswaterstaat Waterdienst
- 1.14 Method reported by**  
Roel Knobens  
r.knobens@royalhaskoning.com  
Rijkswaterstaat Waterdienst
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Klinge, M., G. Hensens, A. Brenninkmeijer & L. Nagelkerke, 2003. STOWA Handboek Visstandbemonstering. Voorbereiding, bemonstering en beoordeling. STOWA, Utrecht.
- 2.02 Short description**  
Electrofishing for riparian zone by wading in streams/rivers <= 3 m; by boat in streams/rivers > 3m. Fishing at daytime. Open water fished with seine or trawl by using boats. Seine fishing by daytime; trawl fishing by night time. Trawl fishing only in large lakes. Remark: not one, but many organisations responsible (regional waterboards, state-managed waters). Sampling is mostly performed by consultancy firms.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Beam trawl, Electrofishing gear, Seine netting  
codend nets
- 2.05 Specification:** backpack, barge or boat-mounted electrofishing models depending on sampling conditions;  
one or two anode with size 0.5 m; current type: (P)DC; beam trawl/codends and seine netting
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** from mid July till september for large lakes; small lakes entire year except march - july
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
at least 2 samples

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

depend on size lakes. 10-100 ha: 10-20% riparian zone with electrofishing; 10-35% with seine; 2-10% with trawl.

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:**

Mesh size anode electrofishing gear: 8 mm ;  
codend/trawl/seine: > 40 mm;

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts, Relative abundance

**in relation to** Area

**Unit** number per ha

**2.16 Quantification of biomass:** n.a.

weight of fishes to determine relative abundance based on biomass

**2.17 Other biological data:** Length (either fork length or total length); According to the guidelines (answer B01) total length of fishes should be recorded.

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

**3. Data evaluation**

**Evaluation**

**3.01 List of biological metrics**

Number of species and relative abundance (in % biomass) of bream, perch+roach, phytophilic species and tolerant specie to low oxygen levels. EQR = average of the 5 metrics. Age composition is only part of the assessment in one type of lake (large deep buffered lake): % of eel and pikeperch > minimal size mentioned in the fishery regulations

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores, Weighted average metric scores

lakes excluding metric for age composition: average. other: weighted average

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple spatial replicates

**Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** 0; there are no reference sites in the Netherlands.

**Geographical coverage:** datas from lakes in the Netherlands and lakes in Danube delta and Poland/Russia

**Location of sites:** over 80 different lakes

**Data time period:** n.a.

**Criteria:**

No reference sites available in the Netherlands.

**3.08 Reference community description**

Based on expert judgement. reference values depend on water type specific hydromorphological characteristics .Important are water level fluctuation, size of the water, isolation and trophic level. Reference is species rich, with low abundance of bream and high abundance of roach-perch and phytophilic fish. Level of dominance depends on water type

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites

**3.11 Boundary setting procedure**

Expert judgement. Boundaries based on shifts in fish communities in relation to pressures. GM boundary relates to disappearance of habitat for spawning of and juvenile phytophilic fish. MP boundary relates to shift from macrophyto to phytoplankton dominated system.

**3.12 "Good status" community:** Based on expert judgement. Lower dominance compared to reference situation. The level of

dominance varies per river type.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 240

LFI

## 1. General information

- 1.01 GIG:** Central-Baltic
- 1.02 Category:** Lakes
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Poland
- 1.05 Specification:** on all lakes taken into account a fishery management was carried on
- 1.06 Method name:** *Lake Fish Index*
- 1.07 Original name:** *Ocena stanu ekologicznego jezior na podstawie ichtiofauny -Jeziorny Indeks Rybny*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation  
TSI Carlsonska, wskaźniki SOJJ (Water purity classes and Category susceptibility for degradation)
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** does not exist
- 1.11 Pertinent literature of mandatory character:**
- 1.12 Scientific literature:**
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Witold Białokoz, Łucjan Chybowski, Arkadiusz Wołos, Tomasz Czerwiński, Hanna Draszkwicz-Mioduszevska<br>r.furgal@gios.gov.pl<br>Inland Fisheries Institute | <b>1.14 Method reported by</b><br>Renata Furgal<br>r.furgal@gios.gov.pl<br>Department of Monitoring and Environmental Information, Chief Inspectorate for Environmental Protection |
|---|--|
- 1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
Materials collected, archived and actualized by Inland Fishery Institute for over 50 years were used for valuation and calculation of ecological status of lakes on the basis of ichthyofauna. Data consist of fish species composition, catches and their changes. All materials, together with environmental data are unique and the only in large-scale of such kind materials in Poland.
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** Electrofishing gear, Fyke net, Gill net, Seine netting
- 2.05 Specification:**
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:**
- 2.08 Sampling/survey month(s):** whole year
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
for several years and during the whole year commercial catches
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
data covering 15 years of whole-year catches were used
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
data covering 15 years of whole-year catches were used for whole lake area

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:**
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Relative abundance  
in relation to Area  
Unit kg/ha
- 2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:****2.18 Special cases, exceptions, additions:** none**2.19 Comments****3. Data evaluation****Evaluation****3.01 List of biological metrics**

L – large M – medium S – small UN – undersized Roach M [% of total catches] Roach S [% of total catches] Total roach (M+S) [% of total catches] % of roach M in total roach [% of total catches] White bream [% of total catches] Sparling [% of total catches] Bleak [% of total catches] Trash fish [% of total catches] Total catches [% of total catches] Salmonids (whitefish+vendace+peled [% of total catches] Cyprinids (all cyprinids+others+trash fish) [% of total catches] Predators (pikeperch+pike+perch) [% of total catches] Littoral (pike+tench) [% of total catches] Cyprinids L (bream L + roach M) [% of total catches] Cyprinids S (bream M+S+UN+roach S+others+ trash fish) [% of total catches]

**3.02 Does the metric selection differ between types of water bodies?** Yes**3.03 Combination rule for multi-metrics:** Average metric scores  
mathematic models**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple spatial replicates

**Reference conditions****3.05 Scope of reference conditions:** Surface water type-specific**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data

**3.07 Reference site characterisation****Number of sites:** 288**Geographical coverage:** Great Masurian Lake System in northeastern Poland, western mesoregion of the Eastern Baltic Lake District located across the Eastern Baltic-Belarusian Lowland**Location of sites:** Great Masurian Lake System in northeastern Poland/western mesoregion of the Eastern Baltic Lake District located across the Eastern Baltic-Belarusian Lowland**Data time period:** First 15 years of observations (usually 1951-1965) Reference was defined as the mean of the metrics in the catches from 1950-1964**Criteria:**

No or only very minor, evidence of distortion in lake community before industry and intensify agriculture.

**3.08 Reference community description**

Fish communities comparable to those being type-specific for lakes (i.e. for preindustrial and corp intensification period)

**3.09 Results expressed as EQR?** No points, range from 0,00 to 1,00**Boundary setting****3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
High-good boundary derived from metric variability at near-natural reference sites**3.11 Boundary setting procedure**

Boundaries were established by expert judgement/methods on the basis of definitions of states from the WFD annex.

**3.12 "Good status" community:** There are small changes of the share of species and functional groups comparing to the reference states, specific for a given type of water. Many years' continuity of catches species and their groups indicates continuity of reproduction and recruitment.**Uncertainty****3.13 Consideration of uncertainty:** No (to be done)**3.14 Comments:**

1. We are currently planning a monitoring of fish based methods with CEN standard (mainly realized by gill net) on lakes with no data and assessment method - LFI will be applied to on all lakes taken into account a fishery management was carried on.  
2. Commercial catches are the basement for our method because: - are collected for many years, - regularly carried on, many times per year  
3. Mainly succession of particular species or group of species during changes of environment were used to the estimation of lakes transformation degree in Poland. Changes in collected commercial catches data indicate on visible negative changes in lakes environment.

ID: 124

FL-MA-RI

## 1. General information

- 1.01 GIG:** Central-Baltic  
LCB1, LCB2
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Belgium (Flanders)
- 1.05 Specification:** Flemish region
- 1.06 Method name:** *Flemish macrophyte assessment system*
- 1.07 Original name:** *Vlaams macrofytenbeoordelingssysteem*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Acidification, Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter, Riparian habitat alteration  
salinity change

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

see Leyssen et al. (2005) for relations to chlorophyll ( $r = -0.24$ ,  $p = 0.001$ ) and TP ( $r = -0.21$ ,  $p = 0.004$ ) in 186 ponds and small lakes

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. September 2009. Vlaamse Milieumaatschappij, Erembodegem, Belgium.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Luc Denys  
luc.denys@inbo.be  
Research Institute for Nature and Forest

**1.14 Method reported by**

Wim Gabriels  
w.gabriels@vmm.be  
Flemish Environment Agency

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

n.a.

**2.02 Short description**

A survey of the entire lake is carried out. The lake is divided into several, more or less homogeneous, segments according to adjacent vegetation and land use, morphological structure and vegetation. The species occurring in the water zone and in the riparian zone are listed separately. The vegetation survey consists of a recording made at the bank (riparian and water survey) and, if possible, by additional transect surveys. The survey is based on observations along the bank, as well as wading through the water, while submerged vegetation is collected with a rake. For each species observed per segment the covering is recorded based on a simplified Tansley-scale. Additionally, the submerged vegetation development is recorded using a four-class cover scale.

**2.03 Method to select the sampling/survey site or area:** n.a.

**2.04 Sampling/survey device:** Grapnel, Rake

**2.05 Specification:** a rake is used for submerged vegetation from the shore; a rake or grapnel are used from a row or motor boat

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** june-september

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

at least 1

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

n.a.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

entire lake up to a maximum depth of 2-4 m (depending on lake type)

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** all macrophytes present except for mosses
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Other, Species/species groups  
species level except for filamentous algae: genus or unspecified
- 2.15 Record of abundance:** n.a.  
Modified Tansley scale for individual taxa (rare/occasional/frequent/low-abundant/abundant/co-dominant/dominant); presence/absence for growth forms; ECOFRAME-like scale for submerged plant abundance
- in relation to** n.a.  
surface-weighted average of segments with homogeneous vegetation
- Unit** Modified Tansley scale, presence/absence; ordinal
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** observed growth forms for specified taxa
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Type specificity (the abundance-weighted mean of all (predefined, species-specific) type specificity values of all present species); disturbance (the abundance-weighted mean of all (predefined, species-specific) disturbance values of all present species); growth forms (a type specific evaluation of number of present growth forms); submerged vegetation development (based on a four-class cover scale)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Worst metric score
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
Reference conditions are characterised by high proportions of type-specific species, low proportions of species associated with disturbance, presence of most growth forms associated with the lake type in question, and a high submerged vegetation development
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
EQR gradient is assumed to represent a continuous trend with general degradation.
- 3.12 "Good status" community:** The EQR values at good status reflect metric values that are only slightly lower than at (expert-based) reference state, hence the community can be characterised as only slightly different

from reference in terms of taxa richness, sensitivity and diversity.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 233

SLAM (Small Lakes Assessment by Macrophytes)

## 1. General information

- 1.01 GIG:** Central-Baltic
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Estonia
- 1.05 Specification:**
- 1.06 Method name:** *Assessment of status of lakes on the basis of macrophytes*
- 1.07 Original name:** *Järvede seisundi hindamine suurtaimestiku alusel*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?*
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
  
helle.maemets@emu.ee
- 1.14 Method reported by**  
Helle Mäemets  
helle.maemets@emu.ee  
Centre for Limnology, Institute for Agricultural and Environmental Sciences, Estonian University of Life Sciences

**1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Traditional investigation method used on the small Estonian lakes since the 1950s.
- 2.02 Short description**  
Circling on boat around the lake, mainly along the border between the floating-leaved and submerged plants. Depth measurements for different vegetation zones on the transects. All angiosperms and macroalgae and their relative abundance registered. Compiling of the vegetation schemes.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Dredge, Grapnel, Rake
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Study of the whole littoral area (or main part of them).
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** June 15 - September 10
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Once per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
n.a.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
As much as possible

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:**
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Family, Species/species groups  
Green filamentous algae and some mosses on the family level, others on species level.
- 2.15 Record of abundance:** Relative abundance  
**in relation to** n.a.  
among ecological group, e.g. submerged plants  
**Unit** relative abundance of taxon per lake

- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** plant growth form
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments:**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**
1. Depth limit of submerged plants
  2. Main hydrophyte groups in order of importance
  3. Relative abundance (1-5 points) of Potamogeton perfoliatus and P. lucens, Isoetes, Lobelia, Myriophyllum alterniflorum, ceratophyllids, lemniids, Chara aspera, Ch. tomentosa, Cladium mariscus
  4. Abundance of large filamentous algae (1-5 points; non-relative)
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Mean quality class
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**
- Number of sites:** 1-3 sites per type
- Geographical coverage:** Estonia
- Location of sites:** n.a.
- Data time period:** Mostly 60 years, in some cases 100 years.
- Criteria:**  
n.a.
- 3.08 Reference community description**  
Depending on the lake type are prevailing charids, elodeids or isoetids, i.e. growth conditions near the bottom are good. Among primary producers prevail slowly growing small-sized species preferring open sandy bottom.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.
- 3.11 Boundary setting procedure**  
Good-moderate boundary: In most cases - relative abundance of the indicators of reference conditions falls <3, relative abundance of the species characteristic for impacted status grows >1-2. Depth limit (used in deeper lakes) near 3 m. All other borders proportional. For "very bad" examples are not available.
- 3.12 "Good status" community:** Parameter values characteristic for the reference conditions are weakened.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 60

L-RI

## 1. General information

- 1.01 GIG:** Central-Baltic  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Lithuania
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment of lakes using modified German Reference Index*
- 1.07 Original name:** *Ežerų būklės vertinimas pagal modifikuotą Vokietijos etaloninį indeksą*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Significant at  $p < 0,05$  negative correlation (-0,62) were estimated between average value of L-RI and summer TP for 9 alkaline lakes LCB-1.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Zofija Sinkevičienė  
zofijasin@gmail.com  
Institute of Botany
- 1.14 Method reported by**  
Jelena Titova  
j.titova@aaa.am.lt  
Lithuanian Environmental Protection Agency
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Schaumburg, J., C.H. Schranz, D. Stelzer & G. Hofmann, 2007. Action Instructions for the ecological Evaluation of Lakes for Implementation of the EU Water Framework Directive Makrophytes and Phytobenthos. Bavarian Water Management Agency, Munich. Stelzer, D., S. Schneider & A. Melzer, 2005. Macrophyte-based assessment of lakes – a contribution to the implementation of the Water Framework Directive in Germany. Intern. Rev. Hydrobiol. 90 (2): 223-237.
- 2.02 Short description**  
Macrophytes was sampled in perpendicular to shoreline transects divided into 0–1 m, 1–2 m, 2–4 m and >4 m depth zones. At least three samples of macrophytes were taken from each depth zone (totally 3x4 per transect). The abundance of species was estimated according 5 degree scale: 1 = very rare, 2 = rare, 3 = common, 4 = frequent and 5 = very frequent. The minimal number of transects determined according to the lake area size-class (Keskitalo, Salonen, 1993).
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying, Stratified sampling/s
- 2.04 Sampling/survey device:** Grapnel  
Aquascope
- 2.05 Specification:** A device consisting of several hooks for grasping and holding with a rope. Aquascope (Under water viewer).
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** July and August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per vegetation season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
n.a.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 2-3 mm (Lemna spp.)
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Genus, Other, Species/species groups

Species/species groups level – Magnoliophyta, Equisetophyta, Lycopodiophyta, Polypodiophyta, Charophyta ☐ Genus – Bryophyta☐ Other – Macroalgae

**2.15 Record of abundance:** Abundance classes

**in relation to** n.a.

**Unit** Score (1 - very rare, 2 - rare, 3 - common, 4 - frequent, 5 - very frequent)

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** plant growth form

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

Score (1 - very rare, 2 - rare, 3 - common, 4 - frequent, 5 - very frequent), for calculation transformed to plant quantity = score<sup>3</sup> (1, 8, 27, 64, 125).

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Reference Index calculated according to Lithuanian list of indicator species (A – sensitive, C – insensitive and B – ☐ indifferent taxa) and named L-RI. ☐Depth limit (m) of vegetation (additional criteria)☐Formula for calculation of reference index according Stelzer et al., 2005☐RI = Reference Index☐Q<sub>Ai</sub> = Quantity of the i-th taxon of species group A☐Q<sub>Bi</sub> = Quantity of the i-th taxon of species group B☐Q<sub>Gi</sub> = Quantity of the i-th taxon of all groups☐n<sub>A</sub> = Total number of taxa in group A☐n<sub>C</sub> = Total number of taxa in group C☐ng = Total number of taxa in all groups☐☐☐ Quantity = abundance<sup>3</sup>

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

n.a.

Index is calculated for each transect and calculation is based on list of taxa and its abundance, estimated at different depth zones.

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** 7 sites (transects) in 3 lakes

**Geographical coverage:** Least impacted lakes or its sites situated in protected areas of Lithuania

**Location of sites:** Lake Germantas in Western part, lake Baltys in South - Western part, lake Alnis in Eastern part of Lithuania

**Data time period:** 1953 - 1970 historical data, 1993 - data of monitoring.

**Criteria:**

The absence or minimal human impact in the site or in all catchment area. The macrophyte community correspond with description of reference community description. Diversity of macrophyte species correspond with diversity of substrates. Low quantity of nutrients. Unaltered morphology and hydrology.

**3.08 Reference community description**

In high alkalinity lakes cover of submerged vegetation with dominant Chara spp. is well developed. Sensitive submerged species are very abundant and dominant. Occurrence of tolerant and indifferent species is insignificant. The belt of helophytes and floating leaved plant not developed or very badly developed.

**3.09 Results expressed as EQR?** No

#### Boundary setting

**3.10 Setting of ecological status boundaries:** n.a.

Preliminary ecological status boundaries estimated for German RI were used

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** Cover of Chara spp. in high alkalinity lakes is well developed and sensitive species have higher abundance than tolerant species, but are decreasing and replaced by tolerant and indifferent species.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 142

KRW-maatlatten

## 1. General information

- 1.01 GIG:** Central-Baltic  
L-CB1 en L-CB2
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Netherlands
- 1.05 Specification:** none
- 1.06 Method name:** *WFD-metrics for natural watertypes*
- 1.07 Original name:** *KRW-maatlatten voor natuurlijke watertypen*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, General degradation, Hydromorphological degradation, Pollution by organic matter, Riparian habitat alteration
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- The metric on species composition is correlating quite well with eutrophication indicating parameters (TP, Chf-a and Secchi depth). Most clear is that the maximum value of EQR species composition is reduced at higher levels of phosphorus
- 1.10 Internet reference:** [http://themas.stowa.nl/thema/ecologische\\_beoordeling/krw-maatlatten.aspx?mld=7213&rid=817](http://themas.stowa.nl/thema/ecologische_beoordeling/krw-maatlatten.aspx?mld=7213&rid=817)
- 1.11 Pertinent literature of mandatory character:**  
Besluit Kwaliteitseisen en Monitoring Water, 2009. Ministry of Housing, Spatial Planning and the Environment (presently under public consultation).
- 1.12 Scientific literature:**  
n.a.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>development by national expert group commissioned by<br>STOWA, Bas van der Wal & RWS Waterdienst, Diederik van der Molen<br>b.van.der.wal@stowa.nl<br>STOWA Foundation for Applied Water Management Research &<br>Rijkswaterstaat Waterdienst | <b>1.14 Method reported by</b><br>Roelf Pot<br><br>roelfpot@wxs.nl<br>Rijkswaterstaat Waterdienst |
|--|---|
- 1.15 Comments**  
Description of KRWmaatlatten in Dutch.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
STOWA, 2009. Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009)
- 2.02 Short description**  
Cover estimation of all present species in (minimum 3) classes in survey plot (100 - 10000 m<sup>2</sup>, depending on water type); cover estimate of 5 growth forms in percentage in the same area (1 of the growth forms being filamentous algae, which are in fact phytobenthos); estimate of the percentage well developed riparian vegetation of the whole waterbody
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Stratified sampling/surveying
- 2.04 Sampling/survey device:** Rake  
non-destructive survey
- 2.05 Specification:** visual recognition of species and estimate of cover; assisted by boat; rake is additionally used
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** june- august
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
per waterbody: 6 in small lakes, 20 in very large lakes
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
6 - 20 surveys, size between 100 and 10000 m<sup>2</sup>

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 1 cm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Other, Species/species groups

**2.15 Record of abundance:** Abundance classes, Percent coverage  
**in relation to** Area

**Unit** Abundance class (related to percentage cover) for every species; Percentage cover for growth forms.

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

Biological WFD monitoring is performed by 26 regional water boards. Small differences may occur in sampling strategies etc.

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

1. Weighted number of characteristic species, weight 1-4 depending on species indication value, species abundance and species consistence over 6 - 20 sampled stretches. EQR = total score/expected score in reference. 2. Deviation of growth form cover from expected cover in reference in suitable area. EQR derived from class boundaries 3. final EQR = (EQR species + (mean of EQRs growth forms) ) / 2

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple spatial replicates

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data, Least Disturbed Conditions  
no actual existing natural sites in lakes; spatial references from foreign countries

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** Western and Central, temperate Europe

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**

All lakes in The Netherlands are (very) high hydromorphologically impacted, level fluctuation is completely controlled (less than 5 cm) and most of them are moderately to highly impacted by eutrophication. Too few lakes are assumed to meet the criteria of (almost) unimpacted.

**3.08 Reference community description**

High status of lakes is characterized by a high variety of species, growing at diverse habitats and continues depth gradient near shoreline. Pressure tolerant species are present but only in low abundance and a few sites; total cover of vegetation is moderate or low and type-specific. Furthermore a general description is given (in Dutch) in: STOWA (2009) Referenties en maatlaten voor natuurlijke watertypen. report 2007-32

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

The reference score for the sum of the scores of the species is derived from frequency data in the national vegetation database on well developed plant communities in The Netherlands (Schaminée et al.) , which is considered a good estimate for the probability of finding the species in a fixed amount of samples. The fraction of species at G/M and H/G are estimated with expert judgment and adjustment may be needed because of too low number of reference sites. Final adjustment of the reference scores are based on intercalibration results.

**3.12 "Good status" community:** Good status of lakes is characterized by a variety of species, growing at several habitats and existing gradient. Pressure tolerant species are present, but occur only in low abundance. Total cover of vegetation is moderate and type-specific.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

Precision and uncertainty is regarded in Van Herpen, van Tongeren, Knoben, Baggelaar, van Loon (2009). Quick scan precision and confidence of KRW assessment (in Dutch). This study resulted in a statistical method to assess the level of precision and confidence monitoring results and status classifications (including identifying outliers and estimates for missing values). The confidence of a status classification is expressed as the probability of exceeding a chemical limit value or the biological status classification moderate/good. Recommendations from this study are incorporated in the Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009).

**3.14 Comments:**

none

ID: 44

ESMI

## 1. General information

- 1.01 GIG:** Central-Baltic  
LCB1, LCB2
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes  
elodeides (charophytes - macroalgae, bryophytes and flowery plant), nymphaeides, helophytes
- 1.04 Country:** Poland
- 1.05 Specification:** none
- 1.06 Method name:** *Macrophyte-based indication method for lakes - Ecological Status Macrophyte Index*
- 1.07 Original name:** *Metoda makrofityindykacji jezior*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic matter, Riparian habitat alteration

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Biological and environmental data from 47 stratified lakes and 40 non-stratified lakes (all highly alkaline; >1meq/l, >25 mgCa/l) were examined to establish pressure-impact relationship between macrophyte metrics and eutrophication gradient. The relationship between macrophyte index - ESMI (Ecological Status Macrophyte Index) and TP, TN, SD, chlorophyll "a" concentration (spring, summer and mean values) showed significant correlation (Monte Carlo permutation test, p<0,005)

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Ciecierska, H., A. Kolada, H. Soszka & M. Golub, 2005- 06. Methodological Aspects of Macrophyte-Based Biological Monitoring of Lakes – a Pilot Study. In Koda, A., H. Soszak, M. Golub, H. Ciecierska, K. Szoszkiewicz, J. Zbierska, S.Z. Jusik & T. Zgola (eds), Methodological Aspects of Macrophyte. Based Biological Monitoring of Surface Waters – a Pilot Study, stage I – November 2005, stage II – 24 November 2006. Ministry of the Environment, Warsaw.

**1.12 Scientific literature:**

Ciecierska, H., 2008. Makrofity jako wskaźniki stanu ekologicznego jezior [Macrophyte-based indices of the ecological state of lakes]. Dissertations and Monographs 139. University of Warmia and Mazury, Olsztyn.

**1.13 Method developed by**

Hanna Ciecierska, Agnieszka Kolada  
makrof@uwm.edu.pl, akolada@ios.edu.pl  
Department of Botany and Nature Protection, University of Warmia and Mazury in Olsztyn; Department of Freshwater Assessment Methods and Monitoring, Institute of Environmental Protection in Warsaw

**1.14 Method reported by**

Hanna Ciecierska, Agnieszka Kolada  
makrof@uwm.edu.pl; akolada@ios.edu.pl  
Department of Botany and Nature Protection, University of Warmia and Mazury in Olsztyn; Department of Freshwater Assessment Methods and Monitoring, Institute of Environmental Protection in Warsaw

**1.15 Comments**

A paper describing the proposed method will be published in an international scientific journal

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Kolada, A. & H. Ciecierska, 2009. Wytyczne do prowadzenia badań terenowych makrofity w jeziorach oraz do sposobu zestawiania i przetwarzania danych [Guidelines for a study of macrophyte communities in lakes and for data compilation and processing], Department of Freshwater Assessment Methods and Monitoring. Institute of Environmental Protection, Warsaw.

**2.02 Short description**

The following data is collected for each transect: \* all plant communities (not species - only the occurrence of a predominant species over a surface area of at least 1 m<sup>2</sup> with 25% coverage is considered a community - phytosociological approach) in which vegetation abundance is measured using the quantitative Braun-Blanquet scale (1951), \*\* maximum vegetation depth, \*\*\* vegetation cover (%)

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Grapnel

**2.05 Specification:** A grapnel on a scaled rope, dense enough to enable reaching submerged macrophytes

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** mid-June - mid-September (optimally July-August)

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One lake is examined once every 6 years, once in a year during a vegetation season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

One transect is one survey area, of the width of 30 m and the length from shoreline to maximum colonisation depth

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Data from all transects is then recalculated as average to provide a basis for the assessment of the ecological status of a lake

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:** no limit

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Other

Communities of the following ecological groups: charophytes, elodeids, nymphaeids and helophytes

**2.15 Record of abundance:** Percent coverage

**in relation to** Area

**Unit** The cover of each plant community in B-B point scale; afterwards recalculated to absolute area occupied by each plant community in m<sup>2</sup> /ha

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** No of syntaxa, syntaxonomic composition, abundance in B-B scale, maximum colonisation depth, overall vegetation %cover within a transect

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

**3. Data evaluation****Evaluation****3.01 List of biological metrics**

Measure of taxonomic composition – phytocenotic diversity index H (Shannon-Weaver index based on a syntaxa level) and maximum phytocenotic diversity index Hmax (lnS, where S - no of syntaxa); Measure of abundance - colonization index Z (the proportion of a total area occupied by macrophytes and area of phytolittoral where water is shallower than 2,5 m) The ecological status of lakes is assessed based on the values of the multimetric Ecological State Macrophyte Index - ESMI (combination of H, Hmax and Z; exponential function)

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

All metrics combined in one multimetric formula

**3.04 From which biological data are the metrics calculated?**

n.a.

Data from all transects surveyed recalculated to the whole lake level; all metrics calculated for a lake

**Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** All lakelands in Poland (lowland, CB-GIG); reference lakes more or less evenly distributed but some areas less affected and more reference rich (NE Poland)

**Location of sites:** All lakelands in Poland but most lakes situated in NE part

**Data time period:** Contemporary data (existing lakes surveyed in 2000-2006)

**Criteria:**

Mainly pressure criteria - no evidence of pressure (on sources of pollution, no urban and agricultural areas, forests dominating, no tourist pressure); the vegetation composition and spatial structure correspond to the description of non-disturbed community.

**3.08 Reference community description**

Highly alkaline, lowland lakes: vegetation well developed, dense and extensive Chara-meadows dominating, in deep lakes high maximum colonisation depth (>3-4 m, even 5m and more), in shallow lakes high %cover of bottom area (~100%); rush vegetation developed only to a small or at least moderate extent (not dominating).

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

H/G boundary set as median of ESMI values of reference sites; remaining boundaries set by division of ESMI gradient between H/G and the lowest ESMI value recorded in the dataset in logarithmic scale; class boundaries set for shallow and deep lakes separat

**3.11 Boundary setting procedure**

H/G boundary set as median of ESMI values of reference sites; remaining boundaries set by division of ESMI gradient between H/G and the lowest ESMI value recorded in the dataset in logarithmic scale; class boundaries set for shallow and deep lakes separately (type-specific class boundaries). No pressure specific!

**3.12 "Good status" community:** Highly alkaline, lowland lakes: Vegetation is still well developed, Chara-meadows are not dominating but still exist (or welcome). Dense and extensive submerged vegetation is dominating (vascular plants communities). In deep lakes maximum colonisation depth is not lower than 2.5m. Rush vegetation developed only to a moderate extent (still not dominating).

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 63

FL-PP-LA

## 1. General information

**1.01 GIG:** Central-Baltic  
LCB1, LCB2, LCB3

**1.02 Category:** Lakes

**1.03 BQE:** Phytoplankton

**1.04 Country:** Belgium (Flanders)

**1.05 Specification:** Flemish region

**1.06 Method name:** *Flemish phytoplankton assessment method for lakes*

**1.07 Original name:** *Vlaamse fytoplankton beoordelingsmethode voor meren*

**1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)

**1.09 Detected pressure(s):** Eutrophication

*Has the pressure-impact-relationship been tested?*

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

VMM, 2009. Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. Vlaamse Milieumaatschappij, Erembodegem, Belgium.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Jeroen Van Wichelen  
jeroen.vanwichelen@UGent.be  
Ghent University

**1.14 Method reported by**

Jeroen Van Wichelen  
jeroen.vanwichelen@UGent.be  
Ghent University

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

n.a.

**2.02 Short description**

In small lakes (<5 ha) water is collected in a large container from 8 random locations scattered across the lake using a boat. In large lakes (> 5 ha) 16 random sites are sampled. In shallow lakes, it is sufficient to take each time a sample of the entire water with a tube sampler (a plastic 2-meter-long tube), ensuring that the soil and submerged vegetation is not touched to avoid contamination. One should also remain at a sufficient distance from the bank in order to avoid contamination with typical littoral species. In deep lakes, at each point the entire circulating upper layer (epilimnion) is sampled. From the surface to the metalimnion, every meter, or every two meters in case of a very extensive epilimnion, a sample is taken using a Niskin bottle. The depth to which sampling should be done, is determined by the measurement of a vertical temperature and/or oxygen profile. When no data on the average depth of the lake is available, as many depth measurements as possible can be made during the transportation between two points. At a central point (or where the lake is at its deepest) using a multimeter the temperature, oxygen content, conductivity, acidity, the Secchi depth and ideally also the depth (in deep lakes) of the entire water column is measured with an interval of 50 cm. On the basis of the depth profile of the temperature, the thermocline to be determined up to where the biota should be sampled. During transport between two points, the container should always be closed with a lid. After water is collected at all locations, subsamples are taken from the large container for microscopic and pigment analysis. The water should be thoroughly stirred in advance in order to homogenize floating organisms.

**2.03 Method to select the sampling/survey site or area:** Random sampling/surveying

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Tube sampler for shallow lakes / Niskin bottle for taking samples at several depths throughout the epilimnion for deeper lakes

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
Surface water / epilimnion

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** April-september

**2.09 Number of sampling/survey occasions (in time) to classify site or area**  
at least one occasion per month during the growing season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
8 for small lakes (< 5 ha); 16 for larger lakes (> 5 ha)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Total volume sampled (prior to subsampling) is (bucket volume) x (3-5 samples per occasion) x (6 months) x (number of monthly samples; at least one)

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:** All cells in the sample, including picocyanobacteria

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Subsamples are taken from a thoroughly homogenised sample

**2.14 Level of taxonomical identification:** Genus, Species/species groups  
To the species level where possible, otherwise genus

**2.15 Record of abundance:** n.a.  
counts of individuals or, where applicable, colonies  
**in relation to** Volume

**Unit** biomass per volume

**2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

**3. Data evaluation**

**Evaluation**

**3.01 List of biological metrics**  
Biomass (chlorophyll a); relative proportion of cyanobacteria

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Worst metric score

**3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

**Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**  
Expert knowledge

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** n.a.

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**  
n.a.

**3.08 Reference community description**  
Reference conditions are characterised by a relatively low biomass per volume, and the absence of cyanobacterial blooms

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** n.a.  
Biomass metric class boundaries are taken from intercalibration exercise;  
boundaries for proportion of cyanobacteria is based on expert judgement

**3.11 Boundary setting procedure**  
EQR gradient is assumed to represent a continuous trend with general degradation.

**3.12 "Good status" community:** The EQR values at good status are characterised by metric values that are only slightly lower than at (expert-based) reference state, hence a slightly increased biomass per volume, and a slight increase of cyanobacteria are possible.

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**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 223

WL-PP-LA

## 1. General information

- 1.01 GIG:** Central-Baltic  
Lakes in Wallonia are storage basins and classified as HMWB .
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Belgium (Wallonia)
- 1.05 Specification:**
- 1.06 Method name:** *Assessment of phytoplankton in reservoirs*
- 1.07 Original name:** *n.a.*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Acidification, Eutrophication, Habitat destruction, Pollution by organic compounds (e.g. DDT, PCB),  
Pollution by organic matter
- Has the pressure-impact-relationship been tested?**
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Sarmiento, H. & J.-P. Descy, 2008. Use of marker pigments and functional groups for assessing the status of phytoplankton assemblages in lakes. J. Appl. Phycol. 20: 1001-1011.
- 1.12 Scientific literature:**  
Sarmiento, H. & J.-P. Descy, 2008. Use of marker pigments and functional groups for assessing the status of phytoplankton assemblages in lakes. J. Appl. Phycol. 20: 1001-1011.
- |  |  |
|--|--|
| <b>1.13 Method developed by</b><br>Sarmiento, H. & Descy, JP<br>jean-pierre.descy@fundp.ac.be<br>Facultés Notre Dame de la Paix - Namur (Belgium). | <b>1.14 Method reported by</b><br>Keulen Christine<br>Christine.Keulen@spw.wallonie.be<br>Service Public de Wallonie -DEMNA -5030 Gembloux (Belgium) |
|--|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Sarmiento, H. & J.-P. Descy, 2008. Use of marker pigments and functional groups for assessing the status of phytoplankton assemblages in lakes. J. Appl. Phycol. 20: 1001-1011.
- 2.02 Short description**  
Samples are collected at different depths in the water column (from surface to bottom, every 2.5 m in the deep lakes and every 1 m in the shallow lakes) with a 3 L Van Dorn bottle. Samples for examination by microscopy were immediately fixed with Lugol's solution and concentrated by settling. These concentrates were further preserved with neutral formaldehyde (2–4% final concentration) for long-term storage in the dark. At least one sample per lake and per month was selected from the vertical profile for a rapid screening with the inverted microscope (Leica DM IL with phase-contrast) and, whenever necessary, species identification with a standard microscope (Zeiss Axioskop equipped with an AxioCam digital camera). The choice of samples for identification by microscopy was made taking into account the vertical biomass profiles from the marker pigment analysis, for instance to identify taxa in phytoplankton developing at particular depths. Identifications were based on specialised taxonomic literature. Samples for Chl a and secondary pigment analysis followed a procedure described in Descy et al. (2000): a water volume was filtered on Macherey-Nägel (Düren, Germany) GF/3 filters until filter-clogging. Pigment extraction was carried out in 8 ml 90 % HPLC grade acetone. After two 15 min sonications separated by an overnight period at 4°C in the dark, HPLC analysis was carried out using the Wright et al. (1991) gradient elution method. Calibration was made using commercial external standards (DHI, Denmark). Carotenoids not present in the standard were quantified against fucoxanthin, using as relative response the ratio of the specific absorbance coefficients at 440 nm (Jeffrey et al., 1997) in methanol. Identification of pigments was checked against a library of pigment spectra, obtained by diode array acquisition of chromatograms from pure pigment solutions and from acetone extracts of pure cultures of algae. Chromatograms processing was done with the Waters Empower software. Abundances of algal taxa were determined from HPLC algal pigment measurements using CHEMTAX, a matrix factorisation program, which estimates the contribution of each specified phytoplankton pigment class to the total chl a concentration in a water sample, (Mackey et al., 1996).
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** *n.a.*  
Van Dorn bottle , 3 L
- 2.05 Specification:** 0.7µm
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** March to october

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

8 /survey site

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

on average 3 L \* 5 depths

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:**

**2.13 Sample treatment:** n.a.

N/A

**2.14 Level of taxonomical identification:** Genus, Species/species groups

**2.15 Record of abundance:** n.a.

Contribution of phytoplankton groups to chlorophyll a

in relation to Volume

Unit µg chlorophyll a L-1

**2.16 Quantification of biomass:** n.a.

determination of chlorophyll a biomass by HPLC pigment analysis followed by processing pigment concentrations with CHEMTAX

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

### 3. Data evaluation

**Evaluation**

**3.01 List of biological metrics**

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

Worst quality class (on ne calcule pas différentes métriques, mais on situe dans une classe de qualité)

**3.04 From which biological data are the metrics calculated?**

n.a.

Phytoplankton functional group classification according to Reynolds et al. (2002)

**Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

n.a.

not relevant because all the "lakes" in Wallonia are HMWB

**3.07 Reference site characterisation**

**Number of sites:** not relevant because all the "lakes" in Wallonia are HMWB

**Geographical coverage:** not relevant because all the "lakes" in Wallonia are HMWB

**Location of sites:** not relevant because all the "lakes" in Wallonia are HMWB

**Data time period:** not relevant because all the "lakes" in Wallonia are HMWB

**Criteria:**

not relevant because all the "lakes" in Wallonia are HMWB

**3.08 Reference community description**

not relevant because all the "lakes" in Wallonia are HMWB

**3.09 Results expressed as EQR?** No

**Boundary setting**

**3.10 Setting of ecological status boundaries:** n.a.

**3.11 Boundary setting procedure**

**3.12 "Good status" community:** n.a.

---

### **Uncertainty**

**3.13 Consideration of uncertainty:**

**3.14 Comments:**

none

ID: 50

DK-PP-LA

## 1. General information

- 1.01 GIG:** Central-Baltic  
L-CB1 and L-CB2
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Denmark
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment system for lakes using Chlorophyll-a*
- 1.07 Original name:** *Klorofyl a vurderingsindeks for søer*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

The method has been adopted from the intercalibration exercise. Chlorophyll-a method from high alkalinity (> 0.2 meq/l) very shallow lakes (< 3 m mean depth, 1409 lake years) and shallow lakes (> 3 m mean depth, 690 lake years) were examined to establish pressure-impact relationship between chlorophyll a metrics and eutrophication gradient TP (summer mean) showing a significant correlation

**1.10 Internet reference:****1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Central Baltic GIG

Danish Ministry of the Environment, Agency for Spatial and Environmental Planning

**1.14 Method reported by**

Ivan Karottki

ibk@blst.dk

Danish Ministry of the Environment, Agency for Spatial and Environmental Planning

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

n.a.

**2.02 Short description**

Look: <http://www2.dmu.dk/Pub/TA25.pdf> (in Danish)

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying**2.04 Sampling/survey device:** Water sampler**2.05 Specification:** none**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant**2.08 Sampling/survey month(s):** 1st. April - 30th September + one sample in November**2.09 Number of sampling/survey occasions (in time) to classify site or area**

min. 7 samplings per season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

-

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

n.a.

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** n.a.**2.13 Sample treatment:** n.a.

-

**2.14 Level of taxonomical identification:** n.a.**2.15 Record of abundance:** n.a.

-

in relation to Volume

Unit chlorophyll a (ug/l)

2.16 Quantification of biomass: Chlorophyll-a concentration

2.17 Other biological data: none

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

3.01 List of biological metrics

Only Chlorofyll-a is used as metrics

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: Not relevant

3.04 From which biological data are the metrics calculated?

Aggregated data from multiple spatial replicates

#### Reference conditions

3.05 Scope of reference conditions: n.a.

3.06 Key source(s) to derive reference conditions:

n.a.

The reference condition is derived from the Intercalibration result

3.07 Reference site characterisation

Number of sites: -

Geographical coverage: -

Location of sites: n.a.

Data time period: -

Criteria:

-

3.08 Reference community description

-

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: Boundaries taken over from the intercalibration exercise

3.11 Boundary setting procedure

see intercalibration result

3.12 "Good status" community: n.a.

#### Uncertainty

3.13 Consideration of uncertainty: No (to be done)

3.14 Comments:

none

ID: 207

PPL

## 1. General information

- 1.01 GIG:** Central-Baltic  
LCB1, LCB2, LCB3
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Estonia
- 1.05 Specification:** All lakes except very large ones: L. Peipsi and L. Võrtsjärv
- 1.06 Method name:** *Assessment of status of lakes on the basis of phytoplankton*
- 1.07 Original name:** *Järvede seisundi hindamine fütoplanktoni alusel*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, General degradation, Hydromorphological degradation, Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

Regression analysis between phosphorus and biological characteristics, appr. 2000 samples, correlation coefficient should reveal strong connection (>0.7) on significant level ( $p < 0.05$ )

- 1.10 Internet reference:** <https://www.riigiteataja.ee/ert/act.jsp?id=85753>
- 1.11 Pertinent literature of mandatory character:**  
Pinnaveekogude veeklassid, veeklassidele vastavad kvaliteedinäitajate väärtused ning veeklasside määramise kord 1. Keskkonnaministri 22. juuni 2001. a määrus Nr. 33.
- 1.12 Scientific literature:**  
Ott, I., 2006. Some principles of ecological quality classification in Estonian lakes. In De Wit, H. & B.L. Skjelkvale (eds), Proceedings of the 21th meeting of the ICP waters programme Task Force in Tallinn, Estonia, October 17-19, 2005: 8-14. Ott, I., 2005. Phytoplankton as a tool to classify ecological status of lakes. Estonian experiences. In Lääne, A. & P. Heinonen (eds), Sampling. Presentations on three training seminars about quality assurance, biological methods of Water Framework Directive and Waste water sampling techniques: 48-56.

**1.13 Method developed by**

Ingmar Ott, Kairi Maileht  
ingmar.ott@emu.ee  
Estonian University of Life Sciences

**1.14 Method reported by**

Ingmar Ott  
ingmar.ott@emu.ee  
Estonian University of Life Sciences

**1.15 Comments**

These articles do not include final version of PPL method. Method is fully described in Central-Baltic GIG reports

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

CODEC 513, 2004. Water quali. Guidance standard for the routine analysis of phytoplankton abundance and composition using inverted microscopy (Utermöhl technique). CEN TC 230/WG 2/TG 3/N83. Jeffrey, S.W. & G.F. Humphrey, 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemie und Physiologie der Pflanzen 167: 191-194. Lorenzen, C.J., 1967. Determination of chlorophyll and pheopigments: Spectrophotometric equations. Limnol. Oceanogr. 12: 343- 346. Ott, I. & R. Laugaste, 1996. Fütoplanktoni koondindeks (FKI). Üldistus Eesti väikejärvede kohta. Eesti Keskkonnaministeeriumi Infoleht Nr. 3. Rott, E., N. Salmaso & E. Hoehn, 2007. Quality control of Utermöhl based phytoplankton biovolume estimates – an easy task or an Gordian knot. Hydrobiologia 578: 141-146.

**2.02 Short description**

Deepest point of the lake. Depending on stratification and depth, 1-3 samples per water column.

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying

**2.04 Sampling/survey device:** Plankton net, Water sampler

**2.05 Specification:** Apstein net, van Dorn sampler (2 L)

**2.06 Sampled/surveyed habitat:** n.a.

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** May, July, August, September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

4 times per vegetation period

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

n.a.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

n.a.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:**
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
If species cannot be achieved, genus level is used instead
- 2.15 Record of abundance:** Individual counts  
**in relation to** Volume  
**Unit**
- 2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Phytoplankton compound quotient, Pielou's index of evenness, chlorophyll a concentration, description of communities
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** 1-3 sites per type  
**Geographical coverage:**  
**Location of sites:** n.a.  
**Data time period:** Historical data from vegetation period in different years  
**Criteria:**  
population density in the drainage area <10 pers./km<sup>2</sup>; no point pollution resources; 90% from land use of the drainage area should be natural or seminatural.
- 3.08 Reference community description**  
Biomass < 1 g/m<sup>3</sup>, chl a < 1 mg/m<sup>3</sup> in very alkaline lakes, <6 in other lakes, small number of species, no dominants, Large proportion of sensitive indicator species
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.

#### **3.11 Boundary setting procedure**

Boundary setting procedure was derived as follows: Chl a: Principles elaborated in Central-Baltic GIG were used in corresponding lake types for epilimnion. Water column chl a data are used as well in national lake types. H/G boundary was identified as weak shift from natural state. PPL EQR values are in range 0.57-0.64 depending on lake type. Still the most part of sensitive species form community. G/M boundary EQR values are 0.37-0.41. It is the break point where the share of tolerant and sensitive species is more or less equal. Ecological status can improve relatively rapidly. M/P boundary EQR values are 0.24-0.27. Some or one tolerant species prevail. P/B boundary EQR values are 0.14-0.15. This is the point between heavy water blooms and communities with high biomass where some tolerant species prevail. Sensitive species are

disappeared.

**3.12 "Good status" community:** At good stage no water blooms occur, abundance of taxonomical groups is balanced or only sensitive taxa prevail. Biomass is on moderate level.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 241

Part of biomass of Cyanobacteria (%)

## 1. General information

**1.01 GIG:** Central-Baltic**1.02 Category:** Lakes**1.03 BQE:** Phytoplankton**1.04 Country:** Lithuania**1.05 Specification:****1.06 Method name:** *Assessment method of lakes using part of biomass of Cyanobacteria (%)***1.07 Original name:** *Ežerų ekologinės būklės vertinimo metodas pagal mėsvabakterijų (Cyanobacteria) biomasės dalį nuo viso***1.08 Status:** Method is fully used in second RBMP (2015)**1.09 Detected pressure(s):** Eutrophication, General degradation**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Ecological data from 5 lakes (< 3 m mean depth) were examined to establish pressure - impact relationship between phytoplankton (part of biomass of Cyanobacteria) metrics and eutrophication gradient. The relationship for phytoplankton metrics and TP, TN (annual mean) did not show correlation. Ecological data from 11 lakes (3 - 9 m mean depth) were examined to establish pressure - impact relationship between phytoplankton (part of biomass of Cyanobacteria) metrics and eutrophication gradient. The relationship for phytoplankton metrics and TP, TN (annual mean) did not show correlation. Ecological data from 10 lakes (> 9 m mean depth) were examined to establish pressure - impact relationship between phytoplankton (part of biomass of Cyanobacteria) metrics and eutrophication gradient. The relationship for phytoplankton metrics and TP, TN (annual mean) did not show correlation.

**1.10 Internet reference:****1.11 Pertinent literature of mandatory character:**

LAND 53-2003 "Fitoplanktono tyrimo metodika paviršinio vandens telkiniuose". LST EN ISO 5667 – 3:2006. Vandens kokybė. Mėginių ėmimas. 3 dalis. Nurodymai, kaip konservuoti ir tvarkyti vandens mėginius (ISO 5667 – 3:2003). LST EN 25667 – 2:2001. Vandens kokybė. Mėginių ėmimas. 2 dalis. Nurodymai, kaip imti mėginius (ISO 5667 – 2:1991).

**1.12 Scientific literature:****1.13 Method developed by**

Jurate Kasperoviciene

jurate.kasperoviciene@gmail.com

Institute of Botany

**1.14 Method reported by**

Jelena Titova

j.titova@aaa.am.lt

Lithuanian Environmental Protection Agency

**1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

n.a.

**2.02 Short description**

Not stratified lakes: Euphotic zone integrated sample. If lakes deepest parts depth is <2 m, samples are sampled in depth 0,2 m and 1 m (it is necessary to shun bottom zone). If lakes parts depth is > or = 2 m, samples are sampled in depths 0.2 m, 2 m, 4 m, 6 m ... till 2S). Stratified lakes: 1. Euphotic zone integrated sample. If depth of 2S is < or = depth of underneath border of metalimnion, samples are sampled in depths 0.2 m, 2 m, 4 m, 6 m ... till 2S (till underneath border of metalimnion, include depth of underneath border of metalimnion). 2. Euphotic zone integrated sample. If depth of 2S is > depth of underneath border of metalimnion, samples are sampled in depths 0.2 m, 2 m, 4 m, 6 m ... till 2S (and till underneath border of metalimnion, not include depth of underneath border of metalimnion). 1 sample, fixed.

**2.03 Method to select the sampling/survey site or area:** n.a.**2.04 Sampling/survey device:** Water sampler**2.05 Specification:** Ruttner sample bottle**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)**2.07 Sampled/surveyed zones in areas with tidal influence:****2.08 Sampling/survey month(s):** August – September**2.09 Number of sampling/survey occasions (in time) to classify site or area**

It is 1 – 2 times per year.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1 replicate (1 in lake).

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Euphotic zone integrated sample, 1 replicate (1 l), 1 – 2 times per year.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 1µm (pikoplankton)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Different samples (1 l every) from different depths (0.2 m, 2 m ... ) are intermingled, and take 1 sample 1 l. Sample of phytoplankton is concentrated using method of sedimentation and filtration from 1 l -> 100 ml -> till 10 ml. Sample 10 ml are analyzed.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** n.a.  
Part of biomass of Cyanobacteria (%)  
in relation to n.a.  
Unit
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:**
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
biomass of Cyanobacteria/biomass of all phytoplankton\*100
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** Mean depth < 3 m - 5 lakes, mean depth 3 - 9 m - 11 lakes, mean depth > 9 m - 10 lakes.  
**Geographical coverage:** All areas of Lithuania  
**Location of sites:**  
**Data time period:** Historical data before 2004 (about 30 years period) and Lithuanian Environmental Protection Agency (EPA) data of monitoring of lakes since 2004 till 2006.  
**Criteria:**  
The absence of pressures had to be illustrated. The communities at the sites had correspond with the description of the reference community description. Spatio - temporal variability had to be taken into account of the community's composition and abundance affected, frequency of natural disturbances, e.g. hydrodynamism, grazing, by seasonal cycle of light period and intensity, and by limiting factors like nutrients.
- 3.08 Reference community description**  
At good status dominate Chrysophyceae and Bacillariophyceae, cyanobacteria subdominates in summer period. Chl a amounts increase almost 3 times at good boundary.
- 3.09 Results expressed as EQR?** No %

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** n.a.
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:**

### **Uncertainty**

- 3.13 Consideration of uncertainty:**

### 3.14 Comments:

Parameter - Part of biomass of Cyanobacteria (%) If type of lake is 1 (<3 m), reference value is 20, high class of ecologic state of lake is, then biomass (annual average) is  $\leq 40$ , good class of ecologic state of lake is, then biomass (annual average) is 41-60, moderate class of ecologic state of lake is, then biomass (annual average) is 61-80, poor class of ecologic state of lake is, then biomass (annual average) is 81-90, bad class of ecologic state of lake is, then biomass (annual average) is  $> 90$ .

If type of lake is 2 (3 - 9 m), reference value is 15, high class of ecologic state of lake is, then biomass (annual average) is  $\leq 30$ , good class of ecologic state of lake is, then biomass (annual average) is 31-50, moderate class of ecologic state of lake is, then biomass (annual average) is 51-70, poor class of ecologic state of lake is, then biomass (annual average) is 71-90,

bad class of ecologic state of lake is, then biomass (annual average) is  $> 90$ . If type of lake is 3 (> 9 m), reference value is 10,

high class of ecologic state of lake is, then biomass (annual average) is  $\leq 20$ , good class of ecologic state of lake is, then biomass (annual average) is 21-40, moderate class of ecologic state of lake is, then biomass (annual average) is 41-50, poor class of ecologic state of lake is, then biomass (annual average) is 51-70, bad class of ecologic state of lake is, then biomass (annual average) is  $> 70$ .

ID: 57

CHL-LT

## 1. General information

- 1.01 GIG:** Central-Baltic  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Lithuania
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment method of lakes using Chlorophyll-a*
- 1.07 Original name:** *Ežerų ekologinės būklės vertinimo metodas pagal chlorofilą "a"*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Ecological data from 30 lakes (< 3 m mean depth) were examined to establish pressure - impact relationship between phytoplankton (Chl "a") metrics and eutrophication gradient. The relationship for Chl "a" metrics and TP, TN (annual mean) showed correlation TN 0.2 - 0.3, TP 0.4 - 0.6 (p<0.05). Ecological data from 44 lakes (3 - 9 m mean depth) were examined to establish pressure - impact relationship between phytoplankton (Chl "a") metrics and eutrophication gradient. The relationship for Chl "a" metrics and TP, TN (annual mean) showed correlation TN 0.4 - 0.6 (p<0.05), TP 0.4 - 0.5 (p<0.05). Ecological data from 26 lakes (> 9 m mean depth) were examined to establish pressure - impact relationship between phytoplankton (Chl "a") metrics and eutrophication gradient. The relationship for Chl "a" metrics and TP, TN (annual mean) showed correlation TN 0.3 - 0.4 (p<0.05), TP 0.1 - 0.2 (p<0.05).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

LST EN ISO 56667-1-2007+AC2007, LAND 69-2005 "Vandens kokybė. Biocheminių parametų matavimas. Spektrometrinis chlorofilo "a" koncentracijos nustatymas."

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Jurate Kasperoviciene  
jurate.kasperoviciene@gmail.com  
Institute of Botany

**1.14 Method reported by**

Jelena Titova  
j.titova@aaa.am.lt  
Lithuanian Environmental Protection Agency

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Lorenzen, C.J., 1967. Determination of chlorophyll and phaeopigments; spectrophotometric equations, Limnol, Oceanogr. 12 p. 343-346.

**2.02 Short description**

Euphotic zone integrated sample (If lakes parts depth is <2 m, sample is sampled in depth 0,2 m. If lakes parts depth is > or = 2 m, samples are sampled in depths 0.2 m, 2 m, 4 m, 6 m ... till 2S). 3 l sample, not fixed, kept in refrigerator

**2.03 Method to select the sampling/survey site or area:** n.a.

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Ruttner sample bottle

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** March - November

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

2-9 times per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1 replicate (1 in lake).

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Euphotic zone integrated sample, 1 replicate (3 l), 2-9 times per year.

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 1 µm (pikoplankton)

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

Different samples from different depths (0.2 m, 2 m ... ) are intermingled, and take 1 sample 3 l. Replicates (3) up to 1 l per sample are analyzed.

**2.14 Level of taxonomical identification:** Other, Species/species groups

**2.15 Record of abundance:** Individual counts  
Chl "a" concentration, µg/L

**in relation to** Volume

**Unit** µg/L

**2.16 Quantification of biomass:** Chlorophyll-a concentration

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Annual mean concentration of Chl "a",  $\Sigma$ max concentration of Chl "a".

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** Mean depth < 3 m - 50 lakes, mean depth 3 - 9 m - 94 lakes, mean depth > 9 m - 46 lakes.

**Geographical coverage:** All area of Lithuania

**Location of sites:** n.a.

**Data time period:** Historical data before 2004 (about 30 years period) and Lithuanian Environmental Protection Agency (EPA) data of monitoring of lakes since 2004

**Criteria:**

The absence of pressures had to be illustrated. The communities at the sites had correspond with the description of the reference community description. Spatio - temporal variability had to be taken into account of the community's composition and abundance affected, frequency of natural disturbances, e.g. hydrodynamism, grazing, by seasonal cycle of light period and intensity, and by limiting factors like nutrients.

**3.08 Reference community description**

At good status dominate Chrysophyceae and Bacillariophyceae, cyanobacteria subdominates in summer period. Chl a amounts increase almost 3 times at good boundary.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** n.a.

Equidistant division of the EQR gradient (e.g. boundary setting at 0.07, 0.13, 0.32, 0.67).

**3.11 Boundary setting procedure**

Monitoring data of TP and TN for different quality classes were extracted based to boundaries values of Chl-a for different quality classes for all lake types. 25-th and 75-th percentiles of TP and TN were calculated for each lake quality class were calculated. Averages were calculated between 75 percentile (reference site) and 25 percentile (good status). These values were selected to represent threshold between high/good status. This procedure was applied for each subsequent class. Averages between 75 percentile (good) and 25 percentile (moderate status). These values were selected to represent threshold between good/moderate status.

**3.12 "Good status" community:** At good status annual mean Chl "a" amounts increase comparing to reference sites.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 213

Part of biomass of Bacillariophyta and Chrysophyta (%)

## 1. General information

**1.01 GIG:** Central-Baltic**1.02 Category:** Lakes**1.03 BQE:** Phytoplankton**1.04 Country:** Lithuania**1.05 Specification:****1.06 Method name:** *Assessment method of lakes using part of biomass of Bacillariophyta and Chrysophyta (%)***1.07 Original name:** *Ežerų ekologinės būklės vertinimo metodas pagal auksadumblių (Chrysophyta) ir titnagdumblių (Bacillariophyta)***1.08 Status:** Method is ~~not~~ used in ~~second round~~ (2015)**1.09 Detected pressure(s):** Eutrophication, General degradation**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Ecological data from 5 lakes (< 3 m mean depth) were examined to establish pressure - impact relationship between phytoplankton (part of biomass of Bacillariophyta and Chrysophyta (%)) metrics and eutrophication gradient. The relationship for phytoplankton metrics and TP, TN (annual mean) did not show correlation. Ecological data from 11 lakes (3 - 9 m mean depth) were examined to establish pressure - impact relationship between phytoplankton (part of biomass of Bacillariophyta and Chrysophyta (%)) metrics and eutrophication gradient. The relationship for phytoplankton metrics and TP, TN (annual mean) did not show correlation. Ecological data from 10 lakes (> 9 m mean depth) were examined to establish pressure - impact relationship between phytoplankton (part of biomass of Bacillariophyta and Chrysophyta (%)) metrics and eutrophication gradient. The relationship for phytoplankton metrics and TP, TN (annual mean) did not show correlation.

**1.10 Internet reference:****1.11 Pertinent literature of mandatory character:**

LAND 53-2003 "Fitoplanktono tyrimo metodika paviršinio vandens telkiniuose". LST EN 25667- 2, 2001. Vandens kokybė. Mėginių ėmimas. 2 dalis. Nurodymai, kaip imti mėginius (ISO 5667- 2, 1991). LST EN ISO 5667- 3, 2006. Vandens kokybė. Mėginių ėmimas. 3 dalis. Nurodymai, kaip konservuoti ir tvarkyti vandens mėginius (ISO 5667- 3, 2003).

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Jurate Kasperovicene  
jurate.kasperovicene@gmail.com  
Institute of Botany

**1.14 Method reported by**

Jelena Titova  
j.titova@aaa.am.lt  
Lithuanian Environmental Protection Agency

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

n.a.

**2.02 Short description**

Not stratified lakes: Euphotic zone integrated sample. If lakes deepest parts depth is <2 m, samples are sampled in depth 0,2 m and 1 m (it is necessary to shun bottom zone). If lakes parts depth is > or = 2 m, samples are sampled in depths 0.2 m, 2 m, 4 m, 6 m ... till 2S) Stratified lakes: 1. Euphotic zone integrated sample. If depth of 2S is < or = depth of underneath border of metalimnion, samples are sampled in depths 0.2 m, 2 m, 4 m, 6 m ... till 2S (till underneath border of metalimnion, include depth of underneath border of metalimnion). 2. Euphotic zone integrated sample. If depth of 2S is > depth of underneath border of metalimnion, samples are sampled in depths 0.2 m, 2 m, 4 m, 6 m ... till 2S (and till underneath border of metalimnion, not include depth of underneath border of metalimnion). 1 sample, fixed.

**2.03 Method to select the sampling/survey site or area:** n.a.**2.04 Sampling/survey device:** Water sampler**2.05 Specification:** Ruttner sample bottle**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant**2.08 Sampling/survey month(s):** March –May**2.09 Number of sampling/survey occasions (in time) to classify site or area**

It was 1 – 3 times per year. It is 1 time per year now.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1 replicate (1 in lake)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Euphotic zone integrated sample, 1 replicate (1 l), 1 time per year

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 1µm (pikoplankton)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Different samples (1 l every) from different depths (0.2 m, 2 m ... ) are intermingled, and take 1 sample 1 l. Sample of phytoplankton is concentrated using method of sedimentation and filtration from 1 l -> 100 ml -> till 10 ml. Sample 10 ml are analyzed.
- 2.14 Level of taxonomical identification:** Other, Species/species groups
- 2.15 Record of abundance:** n.a.  
Part of biomass of Bacillariophyta and Chrysophyta, %  
in relation to n.a.  
Unit
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
(biomass of Bacillariophyta + biomass of Chrysophyta)/biomass of all phytoplankton \* 100
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**
- Number of sites:** Mean depth < 3 m - 5 lakes, mean depth 3 - 9 m - 11 lakes, mean depth > 9 m - 10 lakes.
- Geographical coverage:** All area of Lithuania
- Location of sites:** n.a.
- Data time period:** Historical data before 2004 (about 30 years period) and Lithuanian Environmental Protection Agency (EPA) data of monitoring of lakes since 2004 till 2006.
- Criteria:**  
The absence of pressures had to be illustrated. The communities at the sites had correspond with the description of the reference community description. Spatio - temporal variability had to be taken into account of the community's composition and abundance affected, frequency of natural disturbances, e.g. hydrodynamism, grazing, by seasonal cycle of light period and intensity, and by limiting factors like nutrients.
- 3.08 Reference community description**  
At good status dominate Chrysophyceae and Bacillariophyceae, cyanobacteria subdominates in summer period. Chl a amounts increase almost 3 times at good boundary.
- 3.09 Results expressed as EQR?** No

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** n.a.
- 3.11 Boundary setting procedure**
- 3.12 "Good status" community:** n.a.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

Parameter - Part of biomass of Bacillariophyta and Chrysophyta (%)  
If type of lake is 1 (<3 m), reference value is 60, high class of ecologic state of lake is, then biomass (annual average) is  $\geq 50$ , good class of ecologic state of lake is, then biomass (annual average) is 49-30, moderate class of ecologic state of lake is, then biomass (annual average) is 29-20, poor class of ecologic state of lake is, then biomass (annual average) is 19-10, bad class of ecologic state of lake is, then biomass (annual average) is < 10.  
If type of lake is 2 (3 - 9 m), reference value is 70, high class of ecologic state of lake is, then biomass (annual average) is  $\geq 50$ , good class of ecologic state of lake is, then biomass (annual average) is 49-40, moderate class of ecologic state of lake is, then biomass (annual average) is 39-30, poor class of ecologic state of lake is, then biomass (annual average) is 29-20, bad class of ecologic state of lake is, then biomass (annual average) is < 20.  
If type of lake is 3 (> 9 m), reference value is 80, high class of ecologic state of lake is, then biomass (annual average) is  $\geq 70$ , good class of ecologic state of lake is, then biomass (annual average) is 69-60, moderate class of ecologic state of lake is, then biomass (annual average) is 59-50, poor class of ecologic state of lake is, then biomass (annual average) is 49-30, bad class of ecologic state of lake is, then biomass (annual average) is < 30.

ID: 144

KRW-maatlatten

## 1. General information

**1.01 GIG:** Central-Baltic  
L-CB1, L-CB2

**1.02 Category:** Lakes

**1.03 BQE:** Phytoplankton

**1.04 Country:** Netherlands

**1.05 Specification:** none

**1.06 Method name:** *WFD-metrics for natural watertypes*

**1.07 Original name:** *KRW-maatlatten voor natuurlijke watertypen*

**1.08 Status: Method is/will be used in** First RBMP (2009)

**1.09 Detected pressure(s):** Eutrophication, General degradation, Hydromorphological degradation

### *Has the pressure-impact-relationship been tested?*

Yes, with qualitative data (e.g. response at reference against impacted sites).

multi-metric for chlorophyll-a was extensively assessed during intercalibration. Correlation of median ratio for chlorophyll-a (mg/l) and tP (mg/l) was assessed for Dutch lakes (n = 2924) multi-metric for species composition was assessed for shallow (n = 307) and deep (n = 59) lakes. Species composition is strongly correlated to phosphorus and to lesser extend to nitrogen. [Further reading: Berg van den M.S., Pot R \[eds\] \(2008\): Background document on phytoplankton references and metrics for the Water Framework Directive \(in dutch\).](#)

**1.10 Internet reference:** [http://themas.stowa.nl/thema/ecologische\\_beoordeling/krw-maatlatten.aspx?mld=7213&rid=817](http://themas.stowa.nl/thema/ecologische_beoordeling/krw-maatlatten.aspx?mld=7213&rid=817)

### **1.11 Pertinent literature of mandatory character:**

Besluit Kwaliteitseisen en Monitoring Water, 2009. Ministry of Housing, Spatial Planning and the Environment (presently under public consultation).

### **1.12 Scientific literature:**

Vighi, M. & H. Ghiadani, 1985. A simple method to estimate lake phosphorus concentrations resulting from natural, background, loadings. *Wat. Res.* 19: 987-991.

### **1.13 Method developed by**

development by national expert group commissioned by STOWA, Bas van der Wal & RWS Waterdienst, Diederik van der Molen

b.van.der.wal@stowa.nl

STOWA Foundation for Applied Water Management Research & Rijkswaterstaat Waterdienst

### **1.14 Method reported by**

Roel Knobben

r.knobben@royalhaskoning.com

Rijkswaterstaat Waterdienst

### **1.15 Comments**

Description of KRWmaatlatten in Dutch.

## 2. Data acquisition

### ***Field sampling/surveying***

#### **2.01 Sampling/Survey guidelines**

Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) Quality Handbook Hydrobiology (in prep). 200 STOWA.

#### **2.02 Short description**

middle of the lake, sample the water column at intervals of 0.5 up to 1.0 m (depending on depth of the lake). Samples from all depth intervals are mixed.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** discrete-depth water-bottle sampler ; integrating sampler; Devices should be able to sample water at least 3 m from the shore; or up to a depth of 10 m.

**2.06 Sampled/surveyed habitat:** Single habitat(s)

middle of the lake, intervals of 0,5 up to 1 m; from water surface down to 0,5 - 1,0 m above

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** April- September

#### **2.09 Number of sampling/survey occasions (in time) to classify site or area**

minimum 6 occasions per year ( April- September), but classification preferably averaged over three years.

#### **2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

two replicates within a 20m radius.

#### **2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

depending on amount of intervals, volume of sampling device. species composition: Sub sample for lab analysis is 100-250 ml

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** Organisms of the complete sample are identified.  
mixed sample from several depth intervals is taken. From this mixed sample a sub sample is obtained for analysis.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Relative abundance  
abundance: chlorophyll a in mg/l  
**in relation to** Volume  
**Unit** species composition: cells/ml; abundance: chlorophyll a in mg/l
- 2.16 Quantification of biomass:** Chlorophyll-a concentration
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
Biological WFD monitoring is performed by 26 regional water boards (local/regional water systems) and 1 national water board (large rivers, large lakes, estuaries and coastal waters) . Small differences may occur in sampling strategies etc.

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
multimetric abundance (chlorophyll-a). This metric is based on relation between total P and chlorophyll-a. With a known chlorophyll a concentration the corresponding EQR-value can be looked up in a table given in the metrics (water type specific values) (more reading: KRWmaatlatten or Van den Berg M.s., Pot R. [eds] (2008): Background document references and metrics phytoplankton for the Water Framework Directive. ) multimetric species composition: metric based on algae blooms. Based on abundance of indicator species (cells/ml) a bloom is confirmed or not. If there is a bloom the corresponding EQR value is looked up in a table. When several blooms are occurring simultaneously the lowest EQR value is applied for the metric score. If there are no blooms occurring no score is awarded as the absence of blooms could be due to very good or a very poor water quality. In this case the EQR for phytoplankton is based on only the abundance multi metric.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions, Modelling (extrapolating model results)  
no actual existing natural sites in lakes;
- 3.07 Reference site characterisation**  
**Number of sites:** abundance: based on study/model by Vighi & Chiaudani (1985): USA, Canada, Germany, Italia. In addition data for Dutch lakes was used (n = 2924) and extensively assessed during intercalibration  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
All lakes in The Netherlands are (very) high hydromorphologically impacted, level fluctuation is completely controlled (less than 5 cm) and most of them are moderately to highly impacted by eutrophication. Too few lakes are assumed to meet the criteria of (almost) unimpacted
- 3.08 Reference community description**  
maximal phytoplankton biomass in spring. Seasonal succession of species. No blooms. Furthermore a general description is given (in Dutch) in: STOWA (2009) Referenties en maatlatten voor natuurlijke watertypen. report 2007-32
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise

Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient

### 3.11 Boundary setting procedure

Abundance: reference and boundary good-high derived from ratio chlorophyll and tP. For the tP-value the values from the P-metric were used, some the high-good boundary for chlorophyll-a is directly related to the high-good boundary for total-Phosphorus (Physico-chemical elements). Boundary for good-moderate is calculated : boundary high-good + 2\*(boundary high-good - median reference). The lower boundaries are calculated from the boundary one class higher (multiplying boundary by two) species composition. No actual boundary setting. Metric awards an EQR if blooms of indicator species are detected. EQR awarded is based on expert knowledge and historical data. An example: If abundance of *Stephanodiscus binderanus* is > 10000 cells/ml then a EQR of 0.3 is awarded.

- 3.12 "Good status" community:** Maximal phytoplankton biomass occurs in spring. There is a seasonal succession of species. Natural occurring blooms can be present for short periods, but there are no long lasting blooms of harmful phytoplankton.

## Uncertainty

- 3.13 Consideration of uncertainty:** Yes

Precision and uncertainty is regarded in Van Herpen, van Tongeren, Knoben, Baggelaar, van Loon (2009). Quick scan precision and confidence of KRW assessment (in Dutch). This study resulted in a statistical method to assess the level of precision and confidence monitoring results and status classifications (including identifying outliers and estimates for missing values). The confidence of a status classification is expressed as the probability of exceeding a chemical limit value or the biological status classification moderate/good. Recommendations from this study are incorporated in the Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) (see question B.0). In the multimetric for abundance (chlorophyll-a) the variance is incorporated as described in the REFCOND guidance.

### 3.14 Comments:

none

ID: 242

PMPL

## 1. General information

- 1.01 GIG:** Central-Baltic
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Poland
- 1.05 Specification:** Lowlands (all significant lake water bodies in Poland are lowland)
- 1.06 Method name:** *Phytoplankton Metric for Polish Lakes (PMPL)*
- 1.07 Original name:** *Metriks fitoplanktonowy dla jezior polskich*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Total P vs chl and biomass tested. About 500 records used; Three metrics: chl, total phytoplankton biomass and biomass of bluegreens combined into multimetric.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Hutorowicz A., Pasztaleniec A. 2009. Opracowanie metodyki oceny stanu ekologicznego jezior w oparciu o fitoplankton [Method for the estimation of ecological quality of lakes using the phytoplankton], Warszawa-Olsztyn, pp.22 (manuscript)

**1.12 Scientific literature:**

**1.13 Method developed by**

Andrzej Hutorowicz, Agnieszka Pasztaleniec  
ahut@infish.com.pl; paszta@ios.edu.pl  
Department of Freshwater Assessment Methods and  
Monitoring, Institute of Environmental Protection

**1.14 Method reported by**

Andrzej Hutorowicz, Agnieszka Pasztaleniec  
ahut@infish.com.pl, paszta@ios.edu.pl  
Inland Fisheries Institute, Institute of Environmental Protection

**1.15 Comments**

Article in preparation

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Hutorowicz A. (2004) Metody poboru prób i analiza ilościowo-jakościowa fitoplanktonu w jeziorach, Olsztyn, pp. 21 (manuscript) □ Hutorowicz A. (2005) Standardowe objętości komórek do szacowania biomasy wybranych taksonów □ glonów planktonowych wraz z określeniem sposobu pomiarów i szacowania. Olsztyn, pp. 40 (manuscript)

**2.02 Short description**

Integrated samples taken from euphotic zone (spring) or epilimnion (summer) - stratified lakes; whole water column - polymictic lakes.

**2.03 Method to select the sampling/survey site or area:** n.a.

**2.04 Sampling/survey device:** Plankton net, Water sampler

**2.05 Specification:** Ruttner-type water sampler

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
pelagial-epilimnion/euphotic zone

**2.07 Sampled/surveyed zones in areas with tidal influence:**

**2.08 Sampling/survey month(s):** March/April, June and August

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Three occasions per vegetation period

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

number of sites depends on the size and morphometry

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Sum of 1-4 spatial replicates à 0.12 l (taken from 5-9 liters of sampled water) = 0.12-0.50 liters water from epilimnion

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 2 micrometers

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

Sub-samples (5-15% of sample) are analyzed in two replicates. Algae are counted in a sedimentation chamber under an inverted microscope. The biomass of each species is estimated based on calculated cell volumes (Utermöhl technique).

**2.14 Level of taxonomical identification:** Family, Genus, Species/species groups

Most taxonomical group of phytoplankton to species level or genus level.

**2.15 Record of abundance:** Individual counts  
**in relation to** Volume  
**Unit** Biomass of individuals (mg) per liter (mg/l)

**2.16 Quantification of biomass:** Utermöhl technique

**2.17 Other biological data:** Length and width of organisms

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Biomass of phytoplankton [ $YBM = \text{coefficient} * LN(\text{biomass of phytoplankton})$ ], biomass and relative biomass of Cyanoprokaryota [ $YCY = \text{coefficient} * LN(\text{biomass of Cyanoprokaryota} + \text{biomass of Cyanoprokaryota} * \text{biomass of Cyanoprokaryota} / 2 * \text{biomass of phytoplankton})$ ], concentration of chlorophyll a [ $YChl = \text{coefficient} * LN(\text{chlorophyll})$ ]

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** 10

**Geographical coverage:** North-Eastern Poland

**Location of sites:** mainly Mazurian Lake District (Poland)

**Data time period:** 2005-2009

**Criteria:**

Intercalibration criteria adopted in lakes CB-GIG

**3.08 Reference community description**

At reference conditions the phytoplankton community was dominated by chrysophytes, cryptophytes and/or diatoms. Chlorophyta and Cyanoprokaryota constituted less important groups except of small species from Chroococaceae, Synechococcaceae and Merismopediaceae, which reached a great percentage share in total phytoplankton biomass in several lakes. Abundance of species was more or less equal.

**3.09 Results expressed as EQR?** No on standardised five-point scale

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites

**3.11 Boundary setting procedure**

Correlation between TP, chlorophyll a, SD and biomass following Carlson's procedure and German ecological status assessment system. Carlson's index value = 70 was treated as poor/bad boundary which responds to about 20 mg/L of total biomass. This value is regarded in Polish scientific literature as the boundary of bad status. The other boundaries were established based on the frequency of particular values within earlier adopted chlorophyll a classification system. Five grade scale was applied for total biomass, cyanoprokaryota biomass and chlorophyll for the purpose of data standardization.

**3.12 "Good status" community:** At good status, besides chrysophytes, cryptophytes and diatoms also Dinophyceae and chlorococcales Chlorophyta occurred. The filamentous Cyanoprokaryota and Microcystis genera were noted but do not reach a great abundance. Abundance of different species was more or less equal or 3-5 species are dominants.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

ID: 137

FR-BI-LA

## 1. General information

- 1.01 GIG:** Central-Baltic, Mediterranean  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** France
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment of benthic invertebrates in lakes*
- 1.07 Original name:** n.a.
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** n.a.
- Has the pressure-impact-relationship been tested?*  
0
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
0
- 1.14 Method reported by**  
Christine ARGILLIER  
christine.argillier@cemagref.fr  
Cemagref
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Method developed on the basis of the one published by Verneaux et al. in Ann. Limnol. - Int. J. Lim. 2004, 40 (1), 1-9. @The Lake Biotic Index (LBI): an applied method for assessing the biological quality of lakes using macrobenthos; the Lake Châlain (French Jura) as an example.
- 2.02 Short description**  
Two isobaths are defined : sublittoral (-3m) and central (0.75\*Zmax) where 7 and 5 replicates are sampled respectively. Each replicate is the sum of 2 or 3 grabs according to the type of grab. Sediment of these 2 or 3 grabs are filtered (250µm) and stored in 1l bottles. Formaldehyde is used to conserve the samples. All the individuals are counted (sometimes after dilution). The level of determination depends of the taxa (see original publication Verneaux et al., 2004).
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** Grab
- 2.05 Specification:** Grab (Ekman or Friedinger)
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
sediment - sublittoral and central
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Intertidal zone
- 2.08 Sampling/survey month(s):** early spring
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one campaign per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
7 replicates on the sub-littoral isobath, 5 on the central isobath
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
between 675\*12cm<sup>2</sup> (Ekman Grab) and 700\*12 cm<sup>2</sup> (Friedinger GRAB)

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 250 µm (mesh-size of the sieve)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.  
dilution following AFNOR. 2005. Détermination de l'indice oligochètes de bioindication lacustre (IOBL).@Norme française NF T 90-391
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
In general, the level of taxonomical identification is genus level except for@- Nematodes : phylum level@- Diptera other

Chironomidae : family level- Oligochaeta : species level- Moreover, immature forms must be identified for Lumbricidae, Dorydrillidae and Tubificidae. Tubificidae is a subfamily of Naididae and corresponds to the old family Tubificidae (Erseus et al., 2008). For immature Tubificidae, specify with or without setae.

**2.15 Record of abundance:** Abundance classes, Individual counts, Relative abundance  
**in relation to** Area  
**Unit** number of individuals /m2

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

This protocol is applied only in deep lakes (mean depth over 3m and maximal depth over 8m). For the shallow lakes and for reservoirs, others protocols are currently checked.

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Under development

**3.02 Does the metric selection differ between types of water bodies?** n.a.

**3.03 Combination rule for multi-metrics:** n.a.

**3.04 From which biological data are the metrics calculated?**

n.a.

#### Reference conditions

**3.05 Scope of reference conditions:** n.a.

**3.06 Key source(s) to derive reference conditions:**

n.a.

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** n.a.

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** n.a.

#### Boundary setting

**3.10 Setting of ecological status boundaries:** n.a.

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** n.a.

**3.14 Comments:**

none

ID: 139

FR-FI-LA

## 1. General information

**1.01 GIG:** Central-Baltic, Mediterranean  
n.a.

**1.02 Category:** Lakes

**1.03 BQE:** Fish Fauna

**1.04 Country:** France

**1.05 Specification:** none

**1.06 Method name:** *Assessment of fish fauna in lakes*

**1.07 Original name:** n.a.

**1.08 Status: Method is/will be used in** n.a.

**1.09 Detected pressure(s):** n.a.

*Has the pressure-impact-relationship been tested?*

0

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

0

**1.14 Method reported by**

Christine ARGILLIER

christine.argillier@cemagref.fr

Cemagref

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

CEN standard - sampling of fish with multi-mesh gillnets.

**2.02 Short description**

Stratified random sampling during summer period (June-October, temperature over 15°C). The sampled lakes were divided in depth strata and random samplings were performed within each depth stratum. Fish sampling was performed with benthic multi-mesh gillnets which are 30m long and 1.5m deep, made out of homogenous, uncoloured nylon. The gillnets are composed of 12 different mesh-sizes ranging between 5mm to 55mm knot to knot following geometry series, with a ratio between mesh-sizes of about 1.25. The number of nets increases with lakes' depth and area.

**2.03 Method to select the sampling/survey site or area:** Random sampling/surveying, Stratified sampling/surveying

**2.04 Sampling/survey device:** Gill net

**2.05 Specification:** nordic gillnets - 12 mesh sizes

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** summer month, water temperature > 15°C

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

one campaign per year - length depending on area and depth of the lake

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

effort standardised by the protocol - depending on size and depth of the lake

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Different strata covering the whole lake area

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** about 6 mm

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts, Relative abundance

**in relation to** n.a.

the lake

**Unit** number of individuals or biomass/unit effort (for exemple : m2 of gillnet \* unit of time)

**2.16 Quantification of biomass:** n.a.

determination of fresh weight during field campain

**2.17 Other biological data:** lenght and sometimes scales and otholith collected to assess age

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Under development

**3.02 Does the metric selection differ between types of water bodies?** n.a.

**3.03 Combination rule for multi-metrics:** n.a.

**3.04 From which biological data are the metrics calculated?**

n.a.

#### Reference conditions

**3.05 Scope of reference conditions:** n.a.

**3.06 Key source(s) to derive reference conditions:**

n.a.

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** n.a.

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** n.a.

#### Boundary setting

**3.10 Setting of ecological status boundaries:** n.a.

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** n.a.

**3.14 Comments:**

none

ID: 141

FR-MA-LA

## 1. General information

- 1.01 GIG:** Central-Baltic, Mediterranean  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes
- 1.04 Country:** France
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment of macrophytes in lakes*
- 1.07 Original name:** n.a.
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** n.a.

*Has the pressure-impact-relationship been tested?*

0

- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Vincent BERTRIN  
vincent.bertrin@cemagref.fr  
Cemagref

- 1.14 Method reported by**  
Vincent BERTRIN  
vincent.bertrin@cemagref.fr  
Cemagref

**1.15 Comments**

The French Lake Macrophyte Index is currently under development. A first version will be available in 2010.

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

n.a.

**2.02 Short description**

Frequency of surveys : one campaign in the summer period. Regarding the WFD implementation in FR, one macrophyte campaign per management plan (each management plan is about 6 years long). Macrophytes are sampled on observation units (1 section of shore and 3 perpendicular profiles). These observation units are located by applying the Jensen's method (geometric positioning) and selected according to the description of the shore such that the main types of riparian zone around the lake are represented (description of the vegetation structures and/or anthropic alterations of the shore). The number of observation units can never be less than 3 for a lake of 50 to 250 ha, 6 for lakes of 250 to 10 km<sup>2</sup> and reach 8 for a lake of over 10 km<sup>2</sup>, the aim being to locate at least one observation unit on each major category of shore in order to provide the most representative image possible of the macrophyte population of the whole water body. Survey of the littoral zone: the width of the area explored depends on the slope of the bottom, finishing when the depth reaches 1m. In the event of a shallow slope the width explored will reach at least 10 metres. The record will also include the occurrence of helophytes and wetland plants up to 1 metre above the high water line. Profiles perpendicular to the shore: For each of the profiles (3 profiles, at least 50 m long), thirty or so samples will be taken by point contact in a random manner using a rake or a grapnel depending on the depth. Data to be collected : list of taxa and relative abundances (1 to 5 scale) for each taxa (littoral zone and each profile); substrate and depth (recorded on each contact point for each profile), maximum colonization depth. We also take into account helophytes, amphiphytes, macroalgae, bryophytes...

**2.03 Method to select the sampling/survey site or area:** n.a.

**2.04 Sampling/survey device:** Grapnel, Rake  
Grapnel used for samples > 4

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** July to September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

one campaign per year (vegetation period)

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

A sampling site = Observation Unit = 1 survey of littoral zone (100 m long) + 3 perpendicular profiles (50 m long)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Number of Observation Units/lake : at least 3 for lakes from 50 to 250 ha, 6 for lakes from 250 ha to 10 km<sup>2</sup>, 8 for a lakes > 10

km<sup>2</sup>.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Phanerogams, pteridophytes, bryophytes and Characea algae : species level  
Macroscopic algae (filamentous, gelatinous, thallose) : genus level
- 2.15 Record of abundance:** Abundance classes  
in relation to Volume  
Unit 1 to 5 scale
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** Some lake types are not surveyed because of the absence of macrophytes : e.g. high altitude lakes and reservoirs (water level fluctuations > 2 m).
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Under development
- 3.02 Does the metric selection differ between types of water bodies?** n.a.
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
n.a.

### **Reference conditions**

- 3.05 Scope of reference conditions:** n.a.
- 3.06 Key source(s) to derive reference conditions:**  
n.a.
- 3.07 Reference site characterisation**  
Number of sites: n.a.  
Geographical coverage: n.a.  
Location of sites: n.a.  
Data time period: n.a.  
Criteria:  
n.a.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** n.a.

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** n.a.
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** n.a.
- 3.14 Comments:**  
none

ID: 143

IPLAC

## 1. General information

**1.01 GIG:** Central-Baltic, Mediterranean  
n.a.

**1.02 Category:** Lakes

**1.03 BQE:** Phytoplankton

**1.04 Country:** France

**1.05 Specification:** none

**1.06 Method name:** *Lake phytoplankton index*

**1.07 Original name:** *Indice Planctonique LACustre*

**1.08 Status: Method is/will be used in** n.a.

**1.09 Detected pressure(s):** Eutrophication

*Has the pressure-impact-relationship been tested?*

0

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

0

**1.14 Method reported by**

Christophe Laplace-Treuture

christophe.laplace-treuture@cemagref.fr

Cemagref

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

#### 2.01 Sampling/Survey guidelines

Laplace- Treuture, C., J.Barbe, A. Dutartre, J.C. Druart, F. Rimet & O. Anneville, 2009. Standard protocol for Sampling, Conservation, Observation and Counting of Lake Phytoplankton for application of the WFD - version 3.3.1. Cemagref, Bordeaux, Research Unit Networks, water treatment and water quality. Report, 42 p.

#### 2.02 Short description

4 sampling campaigns is the number recommended during the year with 3 during the "summer" period i.e. from May to October. The timing should be as follows: 1- the first between mid-February and the end of March; 2- the second from mid-May to the end of June; 3- the third is in July or August; 4- the fourth is between September and mid-October. A single measurement site is sufficient, located vertically above the deepest point of the lake. One sample is taken from the euphotic layer (2.5 x Secchi disk). One part is fixed with Lugol for phytoplankton analysis, another one for chlorophyll and chemical analysis. At the sampling site, various physico-chemical measurements essential for the study of phytoplankton must be made: transparency of the water and vertical profiles, from the surface to 1 meter above the bottom, of the temperature, the pH, the conductivity and the dissolved oxygen.

**2.03 Method to select the sampling/survey site or area:** n.a.

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Hydrobios water sampler

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
euphotic layer at the deepest point of the lake

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** February-March, May-June, July-August and September-October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

4 per year (with 3 during the growing period)

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

n.a.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

euphotic layer

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** all

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

One sub-sample is sedimented and analyzed by inverted microscope

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts  
in relation to Volume

**Unit** number of cells per milliliter and cubic-millimeter per litre

**2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**  
n.a.

**3.02 Does the metric selection differ between types of water bodies?** n.a.

**3.03 Combination rule for multi-metrics:** n.a.

**3.04 From which biological data are the metrics calculated?**  
n.a.

#### Reference conditions

**3.05 Scope of reference conditions:** n.a.

**3.06 Key source(s) to derive reference conditions:**  
n.a.

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** n.a.

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** n.a.

#### Boundary setting

**3.10 Setting of ecological status boundaries:** n.a.

**3.11 Boundary setting procedure**  
n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** n.a.

**3.14 Comments:**  
none

ID: 55

LTDI

## 1. General information

**1.01 GIG:** Central-Baltic, Northern  
Not intercalibrated to date. Environment Agency, England & Wales is planning a package of work, for  
**1.02 Category:** UK experts to lead on IC of Diatoms in Lakes, across GIG's. Martyn Kelly to lead. From December 2009 to review what diatom methods types of lakes data are

**1.03 BQE:** Benthic Diatoms

**1.04 Country:** United Kingdom

**1.05 Specification:** none

**1.06 Method name:** *Lake Trophic Diatom Index Method*

**1.07 Original name:** *Lake Trophic Diatom Index Method*

**1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)

**1.09 Detected pressure(s):** Eutrophication, General degradation

### *Has the pressure-impact-relationship been tested?*

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient). Relationships between LTDI score and the pressure metrics Chlorophyll a, SRP, TP, Total Oxidised Nitrogen and Total Nitrogen were tested from c200 lakes. Significant relationships were found for TP and TN, type specific R2 values ranged from 0.48-0.69, no significance was found in Low alkalinity lakes. Full data have been presented in: Use of diatoms for evaluating ecological status in UK freshwaters. SCO301030/SR1 Environment Agency, 2007. and Kelly et al, 2008, Assessment of ecological status in U.K. rivers using diatoms, Freshwater Biology (2008) 53, 403-422

**1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/river%20phytobenthos%20method%20statement](http://www.wfduk.org/bio_assessment/bio_assessment/river%20phytobenthos%20method%20statement)

### **1.11 Pertinent literature of mandatory character:**

Water Framework Directive - United Kingdom Advisory Group (WFD-UKTAG) (2008 UKTAG lake assessment methods. macrophytes and phytobenthos - diatom assessment of lake ecological quality (DARLEQ1).

[http://www.wfduk.org/bio\\_assessment/bio\\_assessment/lakes\\_phytobenthos\\_darleq](http://www.wfduk.org/bio_assessment/bio_assessment/lakes_phytobenthos_darleq)

Use of diatoms for evaluating ecological status in UK freshwaters. Environment Agency report, 2007. SCO301030/SR1.

Environment Agency England & Wales use these operational instructions and National Standards (regularly reviewed):

EA Ref. No. 027\_07 Sampling diatoms from rivers and lakes

EA Ref. No. 087\_07 Fixing phytoplankton and diatom samples with Lugol's iodine

EA Ref. No. 028\_07 Diatom sample digestion and slide preparation

EA Ref. No. 029\_07 Diatom slide analysis, recording and archiving

EA Ref. No. 198\_07 Quality Assurance Scheme for diatom samples

EA Ref. No. 387\_09 Interpreting and reporting freshwater ecology data

### **1.12 Scientific literature:**

Kelly, M.G., L. King, G. Clarke, H. Bennion & M. Yallop, 2006. Recommendations for sampling littoral diatoms in lakes for ecological status assessments. *Journal of Applied Phycology* 18: 15-25. Kelly, M.G., S. Juggins, H. Bennion, A. Burgess, M. Yallop, H. Hirst, L. King, B.J. Jamieson, R. Guthrie & B. Rippey, 2007. Use of diatoms for evaluating ecological status in UK freshwaters. Environment Agency Report SCO30103/SR. Kelly et al., 2008. Assessment of ecological status in U.K. rivers using diatoms. *Freshwater Biology* 53: 403-422. Kelly, M.G., L. King, R. Jones, P. Barker & B.J. Jamieson, 2008. Validation of diatoms as proxies for phytobenthos when assessing ecological status in lakes. *Hydrobiologia* 610: 125-129. Kelly, M.G., H. Bennion, A. Burgess, J. Ellis, S. Juggins, R. Guthrie, B.J. Jamieson, V. Adriaenssens & M. Yallop, 2009. Uncertainty in ecological status assessments of lakes and rivers using diatoms. *Hydrobiologia* 633: 5-15. Yallop, M., H. Hirst, M. Kelly, S. Juggins, B.J. Jamieson & R. Guthrie, 2009. Validation of ecological status concepts in UK rivers using historic diatom samples. *Aquatic Botany* 90: 289-295. Kelly et al., 2008. Assessment of ecological status in U.K. rivers using diatoms. *Freshwater Biology* 53: 403-422. Kelly, M.G., H. Bennion, A. Burgess, J. Ellis, S. Juggins, R. Guthrie, B.J. Jamieson, V. Adriaenssens & M. Yallop, 2009. Uncertainty in ecological status assessments of lakes and rivers using diatoms. *Hydrobiologia* 633: 5-15. Kelly, M.G., L. King, G. Clarke, H. Bennion & M. Yallop, 2006. Recommendations for sampling littoral diatoms in lakes for ecological status assessments. *Journal of Applied Phycology* 18: 15-25. Kelly, M.G., L. King, R. Jones, P. Barker & B.J. Jamieson, 2008. Validation of diatoms as proxies for phytobenthos when assessing ecological status in lakes. *Hydrobiologia* 610: 125-129. Kelly, M.G., S. Juggins, H. Bennion, A. Burgess, M. Yallop, H. Hirst, L. King, B.J. Jamieson, R. Guthrie & B. Rippey, 2007. Use of diatoms for evaluating ecological status in UK freshwaters. Environment Agency Report SCO30103/SR. Yallop, M., H. Hirst, M. Kelly, S. Juggins, B.J. Jamieson & R. Guthrie, 2009. Validation of ecological status concepts in UK rivers using historic diatom samples. *Aquatic Botany* 90: 289-295.

### **1.13 Method developed by**

first point of contact, Dr Martyn Kelly  
MGKelly@bowburn-consultancy.co.uk  
Bowburn Consultanc

### **1.14 Method reported by**

Jane Jamieson  
jane.jamieson@environment-agency.gov.uk  
Environment Agency (EA)

### **1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

#### **2.01 Sampling/Survey guidelines**

EN 13946, 2003. Water Quality – Guidance Standard for the Routine Sampling and Pretreatment of Benthic Diatoms from

Rivers.

Kelly, M.G., A. Cazaubon, E. Coring, A. Dell'Uomo, L. Ector, B. Goldsmith, H. Guasch, J. Hürlimann, A. Jarlman, B. Kawecka, J. Kwandrans, R. Laugaste, E.-A. Lindstrøm, M. Leitao, P. Marvan, J. Padisák, E. Pipp, J. Prygiel, E. Rott, S. Sabater, H. van Dam & J. Vizinet, 1998. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *Journal of Applied Phycology* 10: 215-224.

King, L., G. Clarke, H. Bennion M.G. Kelly & M.L. Yallop, 2006. Recommendations for sampling littoral diatoms in lakes for ecological status assessments. *Journal of Applied Phycology* 18: 15-25.

Validation of diatoms as proxies for phytobenthos when assessing ecological status in lakes' and 'Sampling littoral diatoms in lakes for ecological status assessments: a literature review'. EA Science reports:

Environment Agency England & Wales also uses these operational instructions and National Standards (regularly reviewed):

EA Ref. No. 027\_07 Sampling diatoms from rivers and lakes

EA Ref. No. 087\_07 Fixing phytoplankton and diatom samples with Lugol's iodine

EA Ref. No. 028\_07 Diatom sample digestion and slide preparation

EA Ref. No. 029\_07 Diatom slide analysis, recording and archiving

EA Ref. No. 198\_07 Quality Assurance Scheme for diatom samples

EA Ref. No. 387\_09 Interpreting and reporting freshwater ecology data

## 2.02 Short description

In standing waters, if cobbles are the dominant littoral substratum, collect samples from cobbles. If cobbles or small boulders are absent or the bed is dominated by fine sediments with only a few large stones, collect samples from submerged stems of emergent plants such as *Phragmites australis*, *Sparganium erectum*, *Glyceria maxima* or *Typha* spp. *Phragmites australis* is widespread in UK standing waters and should be used wherever possible. To apply the method, samples of benthic diatom species should be collected by brushing or scraping the upper surface of cobbles or small boulders obtained from the littoral zones of lakes in order to remove the biofilm with a clean toothbrush, This passed to a tray with a little stream water. The resulting suspension collected in a plastic bottle, fixed with Lugol's iodine and stored prior to analysis. Where the bed of the lake is dominated by fine sediments, samples should be collected from submerged stems of emergent macrophytes such as *Phragmites australis*, *Sparganium erectum*, *Glyceria maxima* or *Typha* species. The national standard method should be followed – Environment Agency National Standard Ref. No. 027\_07 Sampling diatoms from rivers and lakes. EN 13946 : 2003 Water quality – Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying, Stratified sampling/s

**2.04 Sampling/survey device:** Airlift sampler, Artificial substrate, Brush, Corer, Dredge, Dredge, Grab, Grapnel, Hand net, Multipl  
Toothbrush

**2.05 Specification:** toothbrush; strong scissors; white plastic tray; wide-mouthed plastic sample bottles with watertight lids; waterproof permanent marker pen or another means of labelling samples; (house bricks with holes in, and polypropylene rope – only if using introduced substrata in absence of cobbles)

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

Generally cobbles but other habitats when cobbles are not present. Sample habitat is

**2.07 Sampled/surveyed zones in areas with tidal influence:** which is appropriate for optimising the presence of diatoms at a site.

**2.08 Sampling/survey month(s):** Great Britain (England, Wales and Scotland): Spring (May) and Autumn (September to end November)/ year Northern Ireland: Spring & Summer

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

At least six replicates are required within a three-year classification period to ensure 95% confidence of class at the middle of

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

one

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

If cobbles or small boulders are absent or the bed is dominated by fine sediments with only a few large stones, collect samples from submerged stems of emergent plants such as *Phragmites australis*, *Sparganium erectum*, *Glyceria maxima* or *Typha* spp. *Phragmi*

## Sample processing

**2.12 Minimum size of organisms sampled and processed:** n.a.

**2.13 Sample treatment:** n.a.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts, Relative abundance

**in relation to** Area, Time, Volume

sampled 5 cobbles/small boulders free from algae

**Unit**

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** Other photosynthetic organisms e.g. filamentous algae Cover of sewage fungus above and below stones, presence and density

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

The LTDI is based on the weighted average equation of Zelinka and Marvan (1961).  $TDI = \frac{\sum a_j \cdot s_j}{\sum a_j}$  where  $a_j$  the abundance or proportion of valves of species  $j$  in sample;  $s_j$ , the revised nutrient sensitivity class (1–5) of species  $j$ ; WMS, the weighted mean score. The second step was performed to present the TDI on a score ranging from 0 (very low nutrients) to 100 (very high nutrients).

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

##### 3.04 From which biological data are the metrics calculated?

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Expert knowledge, Historical data hindcasting methods, e.g. paleolimnology

##### 3.07 Reference site characterisation

**Number of sites:** 45 sites across Scotland, England and Wales and Northern Ireland were used to derive reference conditions for the method.

**Geographical coverage:** Scotland, England and Wales and Northern Ireland

**Location of sites:** Scotland, England, Wales

**Data time period:** Reference sites were identified from Spr/Aut 2004–2006 Scottish sites also included 2003.

##### Criteria:

Derived using a number of methods including spatial state schemes, expert judgement and modelling. For the latter, hindcasting methods such as palaeolimnology (the study of the lake sediment record) are given as one such technique (Pollard and Huxham, 1998; European Union, 2000). In order to identify a set of reference sites to assist in tool development, a combination of the above methods were employed. One data source was the set of reference lakes identified in June 2005 by the phytoplankton classification project, following discussion with both SEPA and the Environment Agency, to support the development of a GB-calibrated morphoedaphic index model (MEI). The lakes are assumed to have no significant anthropogenic sources of phosphorus (P) and thus represent high status lakes in the context of their total P (TP) concentration. A second set of reference lakes was identified for the EU Rebecca project by the Centre for Ecology and Hydrology (CEH) based on an analysis of reference conditions for TP and chlorophyll a. This list is being used as a basis for the identification of intercalibration reference lakes for the Northern Geographical Intercalibration Group (GIG). A further set of high alkalinity reference lakes has been identified by the Central GIG on the basis that they have no point sources of P, < 10% non-natural land use and < 10 inhabitants km<sup>2</sup>. A list of the lakes used for each of the above purposes is documented in an Excel spreadsheet (LTT\_106a\_GP\_QRY\_RefList\_Mar06) and further details are given in TAG/LTT 106 (Phillips, 2006). A further body of data for identifying potential reference lakes is the palaeoecological database held by the Environmental Change Research Centre (ECRC). Data were collated from all UK lakes where palaeoecological diatom studies have been undertaken.

##### 3.08 Reference community description

High relative abundance of Achnanthydium spp. (many sites also contained A. biasolettiana and/or A. microcephalum), attached taxa Gomphonema spp, and loosely-attached Fragilariophyceae (Fragilaria capucina was the most abundant, but Meridion circulare, Hannae arcus and Tabellaria flocculosa were all common at lower alkalinities), but few motile taxa. A few lower alkalinity sites were dominated by Achnanthes oblongella, and Cocconeis placentula was also abundant on some occasions.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites Using paired metrics that respond in different ways to the influence of the pressure

### 3.11 Boundary setting procedure

The procedure for defining the class boundaries follows that of the DARES approach for rivers, which has been successfully intercalibrated. The method is based on the approach for many UK classification methods set out in Phillips et al (2003). The assessment of ecological quality of lakes in the Great Britain Ecoregion: an update on thinking and a possible approach for phytoplankton (TeemaNord 2003, 547,p35-39). The Good/Moderate boundary was determined as the point at which the proportion of nutrient sensitive and nutrient tolerant taxa intersect when plotted on an axis of decreasing EQR (increased impact). At this point both the reference and impact communities are represented and thus the impact community, considered to be undesirable, is not yet dominant. There were insufficient reference sites to determine the High/Good boundary from the distribution of EQR from type specific reference sites. Thus a point approximately mid-way (position depended on lake type) between the GM boundary and an EQR of 1 was selected. The remaining gradient Moderate – Bad was divided into 3 equal classes, although only a Moderate/Poor boundary is used in the final method as it was considered that the diatom method in isolation could not identify Bad status as defined in the Normative Definitions.

- 3.12 "Good status" community:** A. minutissimum, F. capucina, F. vaucheriae and N. dissipata were present in a majority of sites at 99.5%, 69.2%, 70.6% and 70.6% respectively, but the maximum relative abundance recorded was lower than in samples at high status (62.5%, 25.7%, 26.0% and 41.6% relative abundance respectively). Other species including G. parvulum, A. pediculus, Planothidium lanceolatum, Reimeria sinuata and motile species including N. gregaria, N. lanceolata, Navicula minima and N. dissipata were present in over 70% of all samples in this status class.
- Concerning these species the highest maximum of relative abundance was recorded for G. parvulum (61.4%).

## Uncertainty

### 3.13 Consideration of uncertainty: Yes

The approach assumes that the estimated diatom EQR is normally distributed with a standard deviation that is a modelled function of EQR. Using the estimated standard deviation and number of samples collected we determine the confidence that the observed mean EQR lies within particular class boundaries. The approach follows that of Ellis (1990) (available at <http://publications.environmentagency.gov.uk/epages/eapublications.storefront/4b100774024a67a6273fc0a802960648/Product/View/GEHO1006BLOR&2DE&2DE>). The approach is described in chapter 6 of the report: Use of diatoms for evaluating ecological status in UK freshwaters (Environment Agency report SCO301030/SR1, 2007). In the chapter we address two questions: What is the uncertainty associated with a single sample as an estimate of ecological status on the day that the sample was collected? How well does this sample reflect the long-term average condition of the biology? These questions are addressed separately. The former uses a nested analysis of variance that examines variation in metrics associated with variability on a slide nested within variability at a site. No attempt has been made to separate (natural) spatial variability from variability introduced by the operator but the latter sources of error were minimised by use of standard methods. Errors associated with making slides are relatively small and differences between lakes and rivers are minor. If analysts adhere to protocols, one slide per sample is sufficient to estimate the taxonomic composition and derived indices from a sample. The variance between replicate samples taken at one time from one location in lakes was much smaller than in rivers. There is a large amount of temporal variation at single sampling locations in rivers and reliable indications of status class will need to be based on repeated sampling from the same location. Results suggest that at least six replicates (i.e. two per year for three years or three per year for two years) will be required in order to provide a firm basis for regulation. A sampling intensity greater than this might be at risk of 'pseudo-replication'. The risk of misclassification depends on the proximity of the mean EQR for a site to the status class boundary. When the EQR value is very close to the boundary, the risk of misclassification will be approximately 50%, regardless of the number of samples available.

### 3.14 Comments:

none

ID: 99

MILA

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Multimetric Index for Lake Acidity*
- 1.07 Original name:** *Multimetric Index for Lake Acidity*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Acidification

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

**1.10 Internet reference:****1.11 Pertinent literature of mandatory character:**

Johnson, R.K. & W. Goedkoop, 2007. Bedömningsgrunder för bottenfauna i sjöar och vattendrag – Användarmanual och bakgrundsdokument, Swedish University of Agricultural Sciences, Report 2007: 4, 84 p. [Background report for benthic fauna in lakes and watercourses - User manual and background document]. Report 2007: 4. Department of Environmental Analysis Swedish University of Agricultural Sciences (SLU).

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

R.K. Johnson and W. Goedkoop  
richard.johnson@vatten.slu.se  
Dept. of Aquatic Sciences and Assessment, SLU

**1.14 Method reported by**

Richard K. Johnson  
richard.johnson@vatten.slu.se  
Dept. Aquatic Sciences and Assessment, SLU, Uppsala, Sweden

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Standardized Kick-sampling, SSEN-27828 (20 s x 1 m; 0,5 mesh; 5 replicates taken in autumn).

**2.02 Short description**

Substratum is disturbed by kicking for 20 s and moving a distance upstream of 1 m

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying**2.04 Sampling/survey device:** Hand net**2.05 Specification:** Kick net**2.06 Sampled/surveyed habitat:** Single habitat(s)  
hard bottom**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant**2.08 Sampling/survey month(s):** September to November**2.09 Number of sampling/survey occasions (in time) to classify site or area**

one occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

5 replicates per site

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

0.25 (width of kick net) x 1 m

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 0.5 mm mesh**2.13 Sample treatment:** Organisms of the complete sample are identified.**2.14 Level of taxonomical identification:** Species/species groups**2.15 Record of abundance:** Individual counts

in relation to Time

Unit Catch per unit effort

**2.16 Quantification of biomass:** n.a.

- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

MILA is constructed from six different simple indices and responds to acidity. The indices are (1) relative abundance (%) of mayflies (Ephemeroptera), (2) relative abundance (%) of true flies (Diptera), (3) the number of mollusc taxa (Gastropoda) (4) the number of mayfly taxa (5) the value for the British AWIC index, and (6) the relative abundance (%) of predators in the sample. Values for these simple indices must be normalised so that each has a value (indexnorm) between 0 and 10 according to Table 6.6. The normalised values are then added together and re-scaled by dividing the sum of the normalised index values by the number of simple indices included (a mean value) and multiplying this mean value by 10 according to the following:  $MILA = 10 * \text{sum indexnorm} / 6$ . MILA thus acquires a value that can vary between 0 and 100. Table 6.6. Normalisation of index values (Indexnorm) for the six simple indices to values between 0 and 10. In the next step MILA is calculated as a mean value for these normalised indices. "ASTERICS nomenclature" relates to the software program at <http://www.aqem.de>. Index ASTERICS- nomenclature Indexnorm=10 if the index Indexnorm=0 if the index Otherwise Indexnorm=% mayflies (of total abundance) -Ephemeroptera |%| >27 <0.05 % true flies (of total abundance) - Diptera |%| <26 >86 % Molluscs (number of taxa) -Gastropoda >8 <0 % Mayflies (number of taxa) -Ephemeroptera >6 <1 % AWICfamily index AWIC Index >5.4 <4.8 % predators (of total abundance) -|%| Predators <8.7 >19 MILA shows the benthic fauna's response to acidity. It cannot be determined from the MILA classification whether the acidity is natural or of anthropogenic origin. The ecological quality ratio (EQR) is calculated as follows:  $EQR = \text{calculated MILA} / \text{reference value}$ . Reference values and class boundaries are given in Table 6.7.

- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Worst quality class
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**
- Number of sites:** ca 300
- Geographical coverage:** whole of Sweden
- Location of sites:** whole of Sweden
- Data time period:** 2000 national survey and Trend Streams (national monitoring programme)
- Criteria:**  
Use of pressure filter to identify reference conditions.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** Yes  
Determination of type 2 error frequency using independent data.
- 3.14 Comments:**  
none



ID: 95

ASPT

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Average Score per Taxon*
- 1.07 Original name:** *Average Score per Taxon*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Johnson, R.K. & W. Goedkoop, 2007. Bedömningsgrunder för bottenfauna i sjöar och vattendrag – Användarmanual och bakgrundsdokument, Swedish University of Agricultural Sciences, Report 2007: 4, 84 p. [Background report for benthic fauna in lakes and watercourses - User manual and background document]. Report 2007: 4. Department of Environmental Analysis Swedish University of Agricultural Sciences (SLU).

**1.12 Scientific literature:**

Armitage, P.D., D. Moss, J.F. Wright & M.T. Furse, 1983. The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-waters. *Water Research* 17: 333-347.

**1.13 Method developed by**

Armitage et al.

**1.14 Method reported by**

Richard K. Johnson

richard.johnson@vatten.slu.se

Dept. Aquatic Sciences and Assessment, SLU, Uppsala, Sweden

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Standardized Kick-sampling, SSEN-27828 (20 s x 1 m; 0,5 mesh; 5 replicates taken in autumn).

**2.02 Short description**

Substratum is disturbed by kicking for 20 s and moving a distance upstream of 1 m

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying

**2.04 Sampling/survey device:** Hand net

**2.05 Specification:** Kick net

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
hard bottom

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** September to November

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

one occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

5 replicates per site

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

0.25 (width of kick net) x 1 m

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 0.5 mm mesh

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

**in relation to** Time

**Unit** Catch per unit effort

**2.16 Quantification of biomass:** n.a.

- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

ASPT exploits the differences in tolerance among different families of benthic macroinvertebrates and the order Oligochaeta (earthworms). Very sensitive families give high indicator values, while those with high tolerance give low indicator values. The index value for ASPT is a mean value for included taxa and is calculated by adding indicator values and dividing them by the number of included taxa (families). Table 7.3. Indicator values for ASPT for different families. Indicator value Family

10 Aphelocheiridae, Beraeidae, Brachycentridae, Capniidae, Chloroperlidae, Ephemeridae, Ephemerellidae, Goeridae, Heptageniidae, Lepidostomatidae, Leptoceridae, Leptophlebiidae, Leuctridae, Molannidae, Odontoceridae, Perlidae, Perlodidae, Phryganeidae, Potamanthidae, Sericostomatidae, Siphonuridae, Taeniopterygidae

8 Aeshnidae, Astacidae, Agriidae, Cordulegasteridae, Corduliidae, Gomphidae, Lestidae, Libellulidae, Philopotamidae, Psychomyiidae

7 Caenidae, Limnephilidae, Nouridae, Polycentropodidae, Rhyacophilidae (incl. Glossosomatidae)

6 Ancylidae, Coenagriidae, Corophiidae, Gammaridae, Hydroptilidae, Neritidae, Platycnemididae, Unionidae, Viviparidae

5 Chrysomelidae, Clambidae, Corixidae, Curculionidae, Dendrocoelidae, Dryopidae, Dytiscidae, Elminthidae, Gerridae, Gyrinidae, Haliplidae, Heledidae, Hydrophilidae (incl. Hydraenidae), Hydropsychidae, Hygrobiidae, Hydrometridae, Mesoveliidae, Naucoridae, Nepidae, Notonectidae, Planariidae, Pleidae, Simuliidae, Tipulidae (inkl. Pediciidae)

4 Baetidae, Piscicolidae, Sialidae

3 Asellidae, Erpobdellidae, Glossiphoniidae, Hirudidae, Hydrobiidae, Lymnaeidae, Planorbidae, Physidae, Sphaeriidae, Valvatidae

2 Chironomidae

1 Oligochaeta

The ecological quality ratio (EQR) is calculated as follows:  $EQR = \text{calculated ASPT} / \text{reference value}$

Reference values and class boundaries are given in Table 7.4.

- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Worst quality class
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**
- Number of sites:** ca 300
- Geographical coverage:** whole of Sweden
- Location of sites:** whole of Sweden
- Data time period:** 2000 national survey and Trend Streams (national monitoring programme)
- Criteria:**  
Use of pressure filter to identify reference conditions.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** Yes  
Determination of type 2 error frequency using independent data.
- 3.14 Comments:**  
none

ID: 97

BQI

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Benthic Quality Index*
- 1.07 Original name:** *Benthic Quality Index*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Johnson, R.K. & W. Goedkoop, 2007. Bedömningsgrunder för bottenfauna i sjöar och vattendrag- Användarmanual och bakgrundsdokument, Swedish University of Agricultural Sciences, Report 2007: 4, 84 p. [Background report for benthic fauna in lakes and watercourses - User manual and background document]. Report 2.

**1.12 Scientific literature:**

Wiederholm, T., 1980. Use of zoobenthos in lake monitoring. Journal of the Water Pollution Control Federation 52: 537-547.

**1.13 Method developed by**

Torgny Wiederholm

**1.14 Method reported by**

Richard K. Johnson

richard.johnson@vatten.slu.se

Dept. Aquatic Sciences and Assessment, SLU, Uppsala, Sweden

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Use of Ekman sampler, SS 028190 (0.5 mesh; 5 replicates taken in autumn).

**2.02 Short description**

Five Ekman samples are collected from a 100 m<sup>2</sup> area in the middle of the lake (or over the deepest region).

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying

**2.04 Sampling/survey device:** Grab

**2.05 Specification:** Ekman sampler

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
Soft bottom (profundal)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** September to November

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

one occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

5 replicates per site

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

n.a.

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 0.5 mm mesh

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

in relation to Time

**Unit** Number of individuals per square meter

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** wet weight (blot dried) biomass

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

BQI exploits knowledge of the varying tolerance of different species of midges to low oxygen levels at lake bottoms. BQI is calculated on the basis of the presence and population density of different indicator taxa of midge larvae in the samples. BQI is calculated as follows:  $BQI = \frac{\sum (W_i \cdot n_i)}{N}$  Where:  $W_i$  = 5 for *Heterotrissocladius subpilosus* (Kieff.),  $W_i$  = 4 for *Paracladopelma* sp., *Micropsectra* sp., *Heterotanytarsus apicalis* (Kieff.), *Heterotrissocladius grimshawi* (Edw.), *Heterotrissocladius marcidus* (Walker) and *Heterotrissocladius maeaeeri* (Brundin)  $W_i$  = 3 for *Sergentia coracina* (Zett.), *Tanytarsus* sp. and *Stictochironomus* sp.,  $W_i$  = 2 for *Chironomus anthracinus* (Zett.),  $W_i$  = 1 for *Chironomus plumosus* L.,  $W_i$  = 0 if these indicator taxa are not present in the sample  $n_i$  = the number of individuals within the indicator group  $n$  = the total number of individuals in all indicator groups  $N$  = the total number of individuals in all indicator groups The ecological quality ratio (EQR) is calculated as follows:  $EQR = \frac{BQI}{Reference\ value}$  Reference values and class boundaries are given in Table 6.5.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Worst quality class

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites

**3.07 Reference site characterisation**

**Number of sites:** ca 110

**Geographical coverage:** whole of Sweden

**Location of sites:** whole of Sweden

**Data time period:** Trend lakes (national monitoring programme of ca 110 lakes)

**Criteria:**

Use of pressure filter to identify reference conditions.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

Determination of type 2 error frequency using independent data.

**3.14 Comments:**

none

ID: 75

CPET

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** United Kingdom
- 1.05 Specification:** Areas at No – only used in Scotland, England, Wales. Not currently used in Northern Ireland
- 1.06 Method name:** **Chironomid pupal exuvial technique**
- 1.07 Original name:** *Chironomid pupal exuvial technique*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Acidification, Eutrophication

NOTE: CPET currently only being used to classify for eutrophication pressure

### **Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Yes, with quantitative data. Ecological data from 203 lakes (representing all WFD types available in UK and 0.2 – 315 mg l<sup>-1</sup> CaCO<sub>3</sub>). Explored using Canonical Correspondence Analysis to produce optima scores and niche breadth from species abundance-weighted data. Monte Carlo randomisation tested (Bonferroni-adjusted) and validated for significant taxa response to nitrogen and phosphorus impact (eutrophication) Nutrient impact scores were significantly related to a compound pressure metric (Total Nitrogen x Total Phosphorus/mean depth) R<sup>2</sup> 0.78 n = 166

- 1.10 Internet reference:** Method Statement: [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/lakes\\_cpnet](http://www.wfduk.org/bio_assessment/bio_assessment/lakes_cpnet) Further details at <http://publications.environment-agency.gov.uk/epages/eapublications.storefront/4b13bb3403a11362273fc0a80296065b/Product/View/SCH00609BQFJ&2DE&2DE>

- 1.11 Pertinent literature of mandatory character:**  
[http://www.wfduk.org/bio\\_assessment/bio\\_assessment/lakes\\_cpnet](http://www.wfduk.org/bio_assessment/bio_assessment/lakes_cpnet)

- 1.12 Scientific literature:**  
Ruse, L.P., 2002. Lake reference state deduced from chironomid pupal skin data. International Symposium of Chironomidae, University of Minnesota, USA. Chironomid pupal exuviae as indicators of lake status. Arch. Hydrobiol. 3: 367-390. [Ruse, L.P., 2009. Classification of nutrient impact using the chironomid pupal exuvial technique. Ecological Indicators \(in press\).](#) [Ruse, L.P. & S. Brooks, 2005. A guide to the identification of chironomid pupal exuviae occurring in Britain and Ireland. Freshwater Biological Association, UK.](#) [Ruse, L.P. & S. Brooks, 2009. Lake reference state deduced from chironomid pupal skin data. International Symposium of Chironomidae, University of Minnesota, USA.](#)

- 1.13 Method developed by**  
Les Ruse  
[les.ruse@environment-agency.gov.uk](mailto:les.ruse@environment-agency.gov.uk)  
Environment Agency

- 1.14 Method reported by**  
Les Ruse  
[les.ruse@environment-agency.gov.uk](mailto:les.ruse@environment-agency.gov.uk)  
Environment Agency, England & Wales

- 1.15 Comments**  
none

## 2. Data acquisition

### **Field sampling/surveying**

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
Collect floating debris at leeward shore (to which wind is blowing), if no discernable wind you will have to walk shore to find where debris has been deposited. Best to compose sample from several points along the leeward shore.
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** 250 micron mesh net attached to extendable lightweight pole
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** During April to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
4 recommended, 2 minimum
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
A single sample is spatially and temporally integrated
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Single sample will at least be representative of the wind transect across the lake from the collection point.

## **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** All pupae of chironomid species are retained by a 250 micron mesh net, smallest have a length of 1.5 mm
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Refloat sample in a bowl, stir and use small sieve to remove aliquots, ideally of about 30 pupal exuviae each time until 200 pupal exuviae are removed in the case of 4 samples being collected from the lake in different months. If only 2 sampling visits po
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Assessment is valid both at full species level but also at the genus/species-group level possible using Wilson & Ruse (2005) key (op. cit. A-22).. Data at genus/species group is used for classifications used for WFD
- 2.15 Record of abundance:** Individual counts  
Individual counts but >50 individuals of a taxa, then this is by estimation.  
**in relation to** n.a.  
Although individual counts the data are relative because of the 200 exuviae subsample.  
**Unit** Percentage of whole sample
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
Abundance data were used to develop the assessment tool and determine each species impact score but for lake assessment it has been demonstrated that qualitative data are as efficient as quantitative data in measuring impact of anthropogenic nutrient enrichment

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Average impact score of all taxa collected from lake survey of 4 samples
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**  
**Number of sites:** 20  
**Geographical coverage:** England, Wales and Scotland  
**Location of sites:** Hampshire, Cumbria, mid- and north- Wales, Islay, West and North Scotland  
**Data time period:** Earliest reference survey 1999, latest 2007.  
**Criteria:**  
Chosen from their relative proportions of impact-sensitive and tolerant species across all WFD lake types available in UK where possible. Reservoirs were not used because their anthropogenic physical characteristics would distort models of reference condition. Lakes were also not considered as reference if urban land-use was greater than 10 per cent or if they had a catchment population density greater than 10/km<sup>2</sup>. Acidified lakes were not used as reference lakes for nutrient tool.
- 3.08 Reference community description**  
Reference community not described. Relationship between lake nutrient impact score and pressure metric developed. Reference nutrient impact scores determined by regression model using best sub-set regression Log lake area, log mean lake depth, log retention time, log catchment area R<sup>2</sup> = 0.79
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Using paired metrics that respond in different ways to the influence of the pressure

Other: Approach to boundary setting set out in Phillips et al 2003. The assessment of ecological quality of lakes in the Great Britain Ecoregion: an update on thinking and a possible approach for phytoplankton. TeemaNord 2003, 547, 35-39.

### 3.11 Boundary setting procedure

Boundaries derived from a plot of the relative frequency of sensitive species and tolerant species for all surveys except. The best fit describing each data set was a quadratic equation. For species-level nutrient assessment the relative frequency of sensitive species exceeded tolerant species at an EQR of 0.64 at 16.24 per cent with a SD 7.07 for the fit of tolerant species. In terms of frequency of tolerant species the High/Good boundary was placed at the crossover point minus 7.07, 9.2 which has an EQR of 0.725. The Good/Moderate boundary occurred at a tolerant species frequency of  $16.24 + 7.07$ , 23.3 which has a EQR of 0.56. The Moderate/Poor boundary was taken as the fitted 0 per cent sensitive species at EQR 0.37. Below an EQR of 0.21, where no sensitive species occurred and observed scores were well below reference scores, was taken as the Poor/Bad boundary. Generic level boundaries were derived from the species boundaries as In a linear regression genus-level EQR equalled  $0.1854 + 0.8105 * \text{species EQR}$  with an  $r^2$  of 93.6 per cent ( $p < 0.001$ ).

**3.12 "Good status" community:** No prescriptive taxa description, CPET provides a surrogate assessment for all benthic macroinvertebrates. Good status species EQR  $0.560 > < 0.725$ , generic EQR  $0.639 > < 0.773$ .

## Uncertainty

**3.13 Consideration of uncertainty:** Yes

CPET overcomes spatial and operator sampling error due to the passive collection of material from a large area of the lake. The largest source of variation unrelated to ecological status was the contingency of which months samples were collected in. Full details of methods are provided by Ruse, L. (2006). Sampling efficiency using the chironomid pupal exuvial technique in a survey of Cotswold Water Park Lake 12 [online]. Available from: <http://www.freshwaterlife.org/>. CPET data for the UK study were collected over four visits among the seven months from April to October. There are 35 possible combinations of 4 months from April to October. EQR<sub>nutr</sub> were calculated for all 35 combinations to measure the variation due to the contingency of which four months were sampled. This would include spatial variation as samples were taken at the leeward shore on each visit and not necessarily at the same point on the lake. The frequency distribution of all possible EQR<sub>nutr</sub> was normal about the mean with a Ryan and Joiner correlation of 0.996, normal probability  $> 10$  per cent. The seasonal sampling exercise with Cotswold Lake data provided an EQR Standard Deviation which was used to plot per cent confidence of class points based on a symmetrical SD v mean EQR curve (Ellis J. and Adriaenssens V). Uncertainty estimation for monitoring results by the WFD biological classifications tools [online]. Environment Agency, Science Report, February 2006. Available from: [enquiries@environment-agency.gov.uk](mailto:enquiries@environment-agency.gov.uk)

**3.14 Comments:**

none

ID: 33

LEAFPACS(Lakes)

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
LCB1 LCB2 101 102 201 202 301 302
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes
- 1.04 Country:** United Kingdom
- 1.05 Specification:** only applied in England, Wales and Scotland. "Free Index" developed by Ireland is used in Northern Ireland
- 1.06 Method name:** *Ecological Classification of Lakes using Macrophytes*
- 1.07 Original name:** *Ecological Classification of Lakes using Macrophytes*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

If yes, please specify pressure and impact metrics, the amount of used, statistical significance of pressure. The relationship of all metrics with pressure (Total P, total oxidised nitrogen [TON], Chlorophyll a) were investigated using 2700 macrophyte surveys of UK lakes. The nutrient metric (LMNI) was the most significantly related to nutrient pressure (Correlation of LMNI to TP r-squared = 0.5). The relationship was dependent on alkalinity and tended to plateau at TP values >100ug/l, particularly in very high alkalinity lakes. Richness metrics showed unimodal response to nutrients with a reduction in diversity in higher alkalinity lake types.

- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/lakes%20leafpacs%20method%20statement](http://www.wfduk.org/bio_assessment/bio_assessment/lakes%20leafpacs%20method%20statement)
- 1.11 Pertinent literature of mandatory character:**  
Water Framework Directive - United Kingdom Technical, 2009. UKTAG river assessment methods macrophyte and phytobenthos macrophytes (Lake leafpacs). Version 2. [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/lakes%20leafpacs%20method%20statement](http://www.wfduk.org/bio_assessment/bio_assessment/lakes%20leafpacs%20method%20statement) Willby, N.J., J. Pitt & G.L. Phillips, 2010. The ecological classification of UK lakes using aquatic macrophytes. Environment Agency Science Report.

- 1.12 Scientific literature:**  
n.a.

- 1.13 Method developed by**  
Nigel Willby  
n.j.willby@stir.ac.uk  
University of Stirling

- 1.14 Method reported by**  
Geoff Phillips  
Geoff.phillips@environment-agency.gov.uk  
Environment Agency(England & Wales)

- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Willby, N.J., J. Pitt & G.L. Phillips, 2010. The ecological classification of UK lakes using aquatic macrophytes. Environment Agency Science Report - Derived from JNCC (2005). Common standards monitoring guidance for freshwater habitats and species. First version – March 2005, ISSN 1743-8160.
- 2.02 Short description**  
Each sector consists of 100 m parallel to shore perimeter survey, 5 wader transects perpendicular to 100 m shore survey and 1 boat survey perpendicular to 100m shore survey extending to maximum depth of colonisation. Surveyor record frequency of occurrence per species per transect at up to 20 points per transect.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Grapnel, Rake  
bathyscope
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** June – September inclusive
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Dependent on confidence required: recommended 2 years of the 6-year RBMP reporting period – achieves 97% confidence
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Dependent on confidence required: recommended 4 replicates to achieve the mid point of moderate.
- Spatial replicates are not used. Survey is of "whole lake" by combining data from structured sampling of 4-8 sectors of lake depending on lake area
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
4 – 8 sectors each covering 100m parallel to shore, plus a transect in each sector extending out to max depth of colonisation

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Macroalgal filaments
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Most macroalgae to genus level only
- 2.15 Record of abundance:** Abundance classes  
**in relation to** n.a.  
frequency  
**Unit** % frequency over colonised area Abundance of macrophytic vegetation at each recording point scored from 1-3. Macroalgae scored separately on same scale.
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** Maximum depth of colonisation (for use in future development)
- 2.18 Special cases, exceptions, additions:** In some cases boat surveys are not possible and survey is based only on perimeter and wader surveys.
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Lake Macrophyte Nutrient Index(LMNI),Number of Functional Groups, Number of Taxa, Macrophyte Cover, Filamentous Algal Cover. Formulae too complex to enter, detailed in report
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores, Worst metric score  
Worst diversity metric, worst cover metrics for macrophytes and filamentous algae are combined with each other and LMNI using weighted average depending on position of water body along a natural fertility
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Historical data, Modelling (extrapolating model results)  
Cover of highly sensitive and sensitive taxa. Taxa defined using modelled (CCA) relationships with pressure, subsequently verified and adjusted by comparing regression of metric scores of these indicative taxa groups against a morpho edaphic index to ensure that UK data used to select groups were drawn from a full gradient of pressure. Resulting taxa compared with historic records
- 3.07 Reference site characterisation**  
**Number of sites:** c600 surveys (mixture of historic and contemporary surveys)  
**Geographical coverage:** Surveys from whole of UK (England, Wales, Scotland & Northern Ireland)  
**Location of sites:** Available on request  
**Data time period:** Surveys selected from data set covering 1830-2005  
**Criteria:**  
Sites selected by iterative application of biological and physicochemical criteria.  $\leq$ 15% total cover of pressure tolerant taxa, highly pressure sensitive species present, cover of highly tolerant species <10% total cover, number of aquatic taxa and functional groups > 25th percentile of type specific richness after allowance for lake area, mean cover score per species within global mean interquartile range. no established invasive alien or translocated species, dominant acid tolerant taxa <30% relative cover (the 75th percentile cover of lakes where acid deposition below critical load), filamentous green algae < 10% relative cover. Mean annual concentration of TP < Good/Moderate boundary value determined by UK MEI approach (site specific, details in  $\mu$ , No evidence of hydromorphological modification, impacted land cover <20% of catchment area.
- 3.08 Reference community description**  
Macrophyte community dominated by highly sensitive taxa, tolerant taxa are strongly subordinate and highly tolerant taxa occur only as transients and are never established. Typical macrophyte mediated functions (habitat support, bed and bank stabilisation, biogeochemical cycling, aesthetics) are intact.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites Using paired metrics that respond in different ways to the influence of the pressure Approach to boundary setting set out in Phillips et al 2003. The assessment of ecological quality of lakes in the Great Britain Ecoregion: an update on thinking and a possible approach for phytoplankton. TeemaNord 2003, 547, 35-39.

#### **3.11 Boundary setting procedure**

The relative positions of High-Good and Poor-Bad boundaries are effectively symmetrical with sensitive species overwhelmingly dominant at one and solerant species overwhelmingly dominant at the other. Using logistic regressions for each lake type the LMNI scores at which tolerant taxa composed 15, 35, 65 and 90% of the community were identified and converted to EQR values using a type specific reference LMNI taken as the mid point of LMNI values in reference surveys. The mean of these type specific EQRs (weighted by the abundance of lakes of different types in the UK) were used to set the HG, GM, MP and PB boundaries. EQR boundaries are subsequently adjusted to equidistant divisions (by a piecewise linear transformation) to enable combination by weighted averaging.

**3.12 "Good status" community:** Sensitive taxa dominate, highly sensitive taxa are scarcer and account for about half the contribution of sensitive taxa. Tolerant taxa present but remain subordinate. Highly tolerant taxa if present are rare. Macrophyte functions at high status all remain intact, undesirable disturbances are rare and macrophyte cover is stable over time.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

If the modelled relationship between observed mean EQR and EQR SD, taking into account sampling, temporal and spatial sources of variation accepted as the best available estimate of the error associated with a given EQR we can combine this with information on class boundaries and therefore predict the confidence with which a site can be assigned to a given class. This approach effectively assumes that the errors associated with a given EQR are normally distributed about that mean with a distribution equivalent to the modelled EQR SD. Given this information one can assess the impact of different survey frequencies on confidence of class. The procedure for calculating confidence of class is outlined by Ellis (2006) available at <http://publications.environment-agency.gov.uk/epages/eapublications.storefront/4b100774024a67a6273fc0a802960648/Product/View/GEHO1006BLOR&2DE&2DE> . The risk of face-value misclassification (i.e. of assigning a site to the wrong class) is then computed as the sum of confidences of membership of all classes except for the observed class. The risk of misclassification will always be at least 50% for an EQR that lies exactly on a class boundary but will fall to a minimum moving towards the middle of that class.

#### **3.14 Comments:**

none

ID: 211

IE-PP-LA

## 1. General information

**1.01 GIG:** Central-Baltic, Northern  
Inter-calibration was initially in the Atlantic GIG and Northern GIG but now currently in CBGIG and

**1.02 Category:** NGIG.

**1.03 BQE:** Phytoplankton

**1.04 Country:** Ireland

**1.05 Specification:**

**1.06 Method name:** *Chlorophyll-a metric - Phytoplankton Biomass*

**1.07 Original name:** *Chlorophyll a metric - Phytoplankton Biomass*

**1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)

**1.09 Detected pressure(s):** Eutrophication

*Has the pressure-impact-relationship been tested?*

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Pressure: Eutrophication Impact metrics: Chlorophyll a Amount of data used: 61 lakes n=2: 44.3%

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Internal standard.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Environmental Protection Agency, Ireland

**1.14 Method reported by**

Gary Free, Wayne Trodd

g.free@epa.ie; w.trodd@epa.ie

Environmental Protection Agency - Ireland

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Internal EPA guidelines.

**2.02 Short description**

Sub-surface samples are taken from mid-lake stations. Samples are taken in 2.5 L bottles. Chlorophyll a is determined using spectrophotometric analysis

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Sub-surface dip-sample using bottle

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
Mid-lake

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** January - December

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Maximum of 12 times per year, minimum of 4 times per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Depends on lake size. Minimum of one.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

2.5 L of water per sampling site.

### Sample processing

**2.12 Minimum size of organisms sampled and processed:**

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** n.a.

Chlorophyll a value is determined. No taxa are identified.

**2.15 Record of abundance:** n.a.

Chlorophyll a used as proxy for phytoplankton biomass.

**in relation to** n.a.

Chlorophyll a used as proxy for phytoplankton biomass.

**Unit** Chlorophyll a ug/l

**2.16 Quantification of biomass:** Chlorophyll-a concentration

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** In bad weather some shore sampling takes place.

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time  
Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites

**3.07 Reference site characterisation**

**Number of sites:** 62

**Geographical coverage:** National

**Location of sites:** National

**Data time period:** Current monitoring and data from 2002

**Criteria:**

Reference sites were selected by expert opinion and also through palaeolimnological validation: Leira M, Jordan P, Taylor D, Dalton C, Bennion H, Rose N, Irvine K. 2006. Assessing the ecological status of candidate reference lakes in Ireland using palaeolimnology. *Journal of Applied Ecology* 43: 816–827.

**3.08 Reference community description**

Decided at GIG level. Chlorophyll a reference values set out in 'Water Framework Directive Intercalibration Technical Report - Part 2: Lakes'. Edited by Sandra Poikane, JRC.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.

**3.11 Boundary setting procedure**

Reference and High/Good boundary values of AGIG and CBGIG. Reference and HG boundary values were determined from the distribution of chlorophyll a concentrations from reference lakes confirmed by palaeolimnology. The median value was taken as an estimate of the reference value and the 75th percentile as an estimate of the HG boundary. The 75th percentile was selected because lake years were used with some lakes possibly deviating from reference. The results were compared with similar analysis carried out for CGIG lakes for a similar type (L-CB1). The results - reference and HG boundary values - were similar and thus validated or supported each other. The Type Reference value was taken as the median value 3.2 ug/L as a growing season mean. The Type H/G boundary value was taken as 6 ug/L as a growing season mean with an EQR of 0.53. The GIG notes that the proposed CGIG values for the same type are 3.2 ug/L and 5.8 ug/L which gives an EQR of 0.55. The chlorophyll values are not significantly different. The AGIG subsequently agreed to adopt the CGIG HG boundary and EQR. (a) Good Moderate Boundary Ireland had preliminary views of a lower GM boundary derived by using total phosphorus to determine points of ecological changes for macrophytes among others- compatible with the normative definitions- along the pressure gradient eutrophication. The total phosphorus value at the GM boundary was subsequently used to determine the corresponding chlorophyll a by a regression equation from the N American literature. This was further supported by a chlorophyll a vs TP relationship from the MS dataset. The reference and HG boundaries values for L-A1/2 lakes are similar to L-CB1 lakes, the analysis of these lakes (which included lakes from AGIG) and the resulting boundaries can be applied to L-A1/2 lakes (CGIG Milestone 6 Report Annex C). The GM boundary value was taken as 10 ug/L as a growing season mean. The NGIG has made general descriptions of degradation of the lake ecosystem from high to bad status for

clearwater lakes and for humic lakes separately. (a) The H/G boundaries were set primarily from the 90th percentile or the 75th percentile of the reference lake distribution. These were compared with response curves of taxonomic indicators in conjunction with MS statistical analysis of reference populations. The final boundaries were based on harmonized EQRs supported by expert judgement. (b) The G/M boundaries were primarily set from the breakpoints in the response curves for indicator groups from REBECCA data plots. Below the breakpoint there are only slight changes from reference conditions and above there is a more rapid increase in impact taxa. In good status, there is a rapid increase in early warning taxa and a clear decrease in reference taxa, distinguishing this class from the high status class in which the reference taxa are more common. The reference taxa are however still present in relatively high abundance in lakes at good status. The G/M boundary was set at the maximum for the early warning taxa for some lake types, beyond which they are decreasing in relative abundance.

(c)

These values were compared with statistical distributions of chlorophyll from all NGIG lakes using the equal log class distribution approach, based on the worst value in the whole type-specific population of REBECCA lakes (both ref. and impacted lakes). The difference between H/G boundary and the worst value was equally distributed for the other class boundaries using log scale intervals. (c) The final boundary values were derived by slightly adjusting the values derived from point a and b above, obtaining chlorophyll values which were consistent with the GIG's expert judgement of the ecological expectations of the differences between the lake types. These expectations were that chlorophyll should increase with alkalinity and humic content and decrease with depth, altitude and latitude.

- 3.12 "Good status" community:** Good status description outlined in section C-15 under boundary setting procedure. See 'Water Framework Directive Intercalibration Technical Report - Part 2: Lakes'. Edited by Sandra Poikane, JRC. and Milestone reports for AGIG/CBGIG and NGIG.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 212

IPI

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
Intercalibration of phytoplankton composition in the CBGIG and NGIG is ongoing.
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Ireland
- 1.05 Specification:**
- 1.06 Method name:** *Irish Phytoplankton composition and abundance Index*
- 1.07 Original name:** *Irish Phytoplankton composition and abundance Index*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Yes, with eutrophication pressure using the IPI. The  $r^2$  between the IPI and log transformed TP was 0.67 ( $p < 0.0001$ ,  $n = 129$ ). The metric was applied across diverse lake types.
- 1.10 Internet reference:** [https://www.epa.ie/downloads/pubs/research/water/Final%20Report%20\(2000-FS1-M1\).pdf](https://www.epa.ie/downloads/pubs/research/water/Final%20Report%20(2000-FS1-M1).pdf) PAGES 51-60
- 1.11 Pertinent literature of mandatory character:**  
Internal standard.
- 1.12 Scientific literature:**  
Free, G., R. Little, D. Tierney, K. Donnelly & R. Caroni, 2006. A reference based typology and ecological assessment system for Irish lakes. Preliminary investigations. 266pp. EPA, Wexford, Ireland.
- |  |  |
|--|--|
| <b>1.13 Method developed by</b><br>Gary Free, Rossana Caroni, Karol Donnelly with further development by Wayne Trodd.<br>g.free@epa.ie, w.trodd@epa.ie<br>Environmental Protection Agency, Ireland | <b>1.14 Method reported by</b><br>Gary Free, Wayne Trodd<br><br>g.free@epa.ie; w.trodd@epa.ie<br>Irish Environmental Protection Agency |
|--|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Internal EPA guidelines.
- 2.02 Short description**  
Sub-surface samples are taken from mid-lake stations. Samples are taken in 2.5 L bottles from which a 100 ml sub-sample is preserved with Lugol's iodine.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** Sub-surface dip-sample using bottle
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Mid-lake
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** 1st of June to 7th of September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Ideally twice in summer
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
One per site
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
2.5 L of water.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** A quantitative water sample is taken and taxa are processed under light microscopy
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Following mixing a sub-sample is placed into a settling chambers of 10ml volume and counted using the Utermöhl technique.
- 2.14 Level of taxonomical identification:** Genus

- 2.15 Record of abundance:** Individual counts  
**in relation to** Volume  
**Unit** number of cells or colonies per ml
- 2.16 Quantification of biomass:** Chlorophyll-a concentration
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** In bad weather some shore sampling takes place.
- 2.19 Comments**  
Some lakes have shore sites only.

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

1) Nine 'eutrophic' taxa or groups of taxa that showed a positive growth response to TP are used: *Scenedesmus* spp., *Pediastrum* spp., *Anabaena* spp., *Cryptomonas* spp., *Rhodomonas* spp., *Aphanizomenon* spp., *Oocystis* spp., *Sphaerocystis* spp. and *Melosira* + *Aulacoseira* spp. 2) The response of each of the above groups is awarded a score ranging from 1 to 0.1, descending with increasing TP concentration (See Table 4.10 Free et al 2006 for scores). 3) Sample chlorophyll a is awarded a score ranging from 1 to 0.1, descending with increasing TP. 4) The scores are averaged to produce an index value. 5) Index values from two sampling occasions are averaged for each year of sampling.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

##### 3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

Metric is calculated for each single sampling occasion, but metric score is averaged for dates.

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites

##### 3.07 Reference site characterisation

**Number of sites:** 62

**Geographical coverage:** National

**Location of sites:** National

**Data time period:** Current monitoring and data from 2002

##### Criteria:

Reference sites were selected by expert opinion and also through palaeolimnological validation: Leira M, Jordan P, Taylor D, Dalton C, Bennion H, Rose N, Irvine K. 2006. Assessing the ecological status of candidate reference lakes in Ireland using palaeolimnology. *Journal of Applied Ecology* 43: 816–827.

##### 3.08 Reference community description

A description of reference conditions is provided on pages 49-50 and Table 4.9 of Free et al (2006). Essentially indicator values were calculated using indicator species analysis (Dufrene and Legendre, 1997) for the most common taxa occurring in a set of reference lakes for 9 lake types. This provides a succinct statistical description of the affinity of taxa for a type in reference condition. Reference values were also calculated for the phytoplankton index which ranged from 1 to 0.84 (Standard deviation ranged from 0 to 0.14) depending on type.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.  
Status boundaries are currently under development as intercalibration is ongoing for phytoplankton composition and abundance. National approach will look at discontinuities / breakpoints and secondary effects.

##### 3.11 Boundary setting procedure

Status boundaries are currently under development as intercalibration is ongoing for phytoplankton composition and abundance. National approach will look at discontinuities / breakpoints and secondary effects with macrophytes. See Free et al (2006) p216-227 for initial work on this issue.

**3.12 "Good status" community:** This is under development and will be finalized by the work of the NGIG and CBGIG. Initial

work at national level indicated that good status is characterised by an initial increase of 'eutrophic' taxa but not an accelerated growth resulting in an undesirable disturbance. See figures 9.2 and 9.3 of Free et al (2006).

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

The IPI is being currently calculated but as no composition and abundance metrics have yet been intercalibrated it is not foreseen that it will be used for the First RBMP2 (2009). However, it is likely following intercalibration that it will be used in the Second RBMP (2015).

ID: 110

Lake Phytoplankton

## 1. General information

- 1.01 GIG:** Central-Baltic, Northern  
LCB-1, LCB-2, LN-1, LN-2a, LN-2b, LN-5
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** United Kingdom
- 1.05 Specification:** none
- 1.06 Method name:** *Lake Phytoplankton – Chlorophyll-a and Percentage nuisance cyanobacteria*
- 1.07 Original name:** *Lake Phytoplankton – Chlorophyll-a and Percentage nuisance cyanobacteria*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

Chlorophyll a v Total P & Total N relationship tested within REBECCA project, 1000 lakes(global data R2 0.80 for TP, 0.58 for TN, R2 0.52 – 0.81 for lakes grouped by type) Ref Phillips et al 2008 Chlorophyll nutrient relationships of different lake types using a large European dataset, Aquat Ecol 42 213-226. Relationship between cyanobacteria and TP investigated. No significant linear relationship with TP, but clear increasing upper quantile boundary (detected but not tested for significance) demonstrated increasing probability of increased cyanobacteria. For this reason cyanobacteria and chlorophyll metric combined using worst of either, absence of cyanobacteria is not considered an indicator of good status, but presence above a threshold is. Overall metric compared with TP as part of IC in CBGIG technical report.

- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/lake\\_phytoplankton](http://www.wfduk.org/bio_assessment/bio_assessment/lake_phytoplankton)
- 1.11 Pertinent literature of mandatory character:**  
[http://www.wfduk.org/bio\\_assessment/bio\\_assessment/lake\\_phytoplankton](http://www.wfduk.org/bio_assessment/bio_assessment/lake_phytoplankton)
- 1.12 Scientific literature:**  
n.a.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Geoff Phillips<br>geoff.phillips@environment-agency.gov.uk<br>Environment Agency | <b>1.14 Method reported by</b><br>Sian Davies<br>p.sian.davies@environment-agency.gov.uk<br>Environment Agency |
|---|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Sample processing is carried out according to CEN guidance on the enumeration of phytoplankton (CEN TC 230/WG 2/TG 3/N83 Water quality – Guidance standard for the routine analysis of phytoplankton abundance and composition using inverted microscopy (Utermöhl technique)) and biovolume assessments are carried out in a way which complies with early proposals in CEN for a biovolume assessment method.
- 2.02 Short description**  
Integrated sample taken from the mid point or deepest point of a lake using and integrated sampling tube or sub surface sample taken from the outflow or other suitable location near the outflow of the lake.
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** Water sampler  
Subsurface dip bottle or long t
- 2.05 Specification:** Wide-mouthed bottle for subsurface dip, 2cm diameter flexible tube, length dependant on the lake in question, for the integrated sample collection
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Euphotic zone of lake
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** January – December for chlorophyll, July – September for taxonomic composition
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Minimum of 12 samples per year for chlorophyll but recommendation to use two years or more of data. Minimum of three
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
No spatial replication is used
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Samples are presumed to be representative of the whole lake phytoplankton

## **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Pico plankton
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Whole lake water samples are analysed for chlorophyll. Whole lake water samples are preserved using Lugol's iodine and subsamples of these are used for enumeration, biovolume assessment and identification. For taxonomic composition, a subsample of known volume
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
Taxa are identified to species where ever possible, and if this is not possible, usually genus level is used, occasionally family or class level. There are always some phytoplankton taxa which cannot be identified and these are recorded under dump codes according to size and presence of flagella.
- 2.15 Record of abundance:** Individual counts  
Biovolume assessment is made of individual samples in addition to a chlorophyll analysis to provide an estimation of total biomass.  
**in relation to** Volume  
**Unit**
- 2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Annual geometric mean chlorophyll concentration. % by biovolume of nuisance cyanobacteria taxa, average across all samples collected.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Worst metric score
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**
- Number of sites:** The reference conditions for chlorophyll are derived on a site specific basis using a predicted reference Total Phosphorus concentration from alkalinity and lake depth (Morpho-edaphic index). A type-specific relationship between chlorophyll and total phosphorus
- Geographical coverage:** Sites from the REBECCA project were used to derive reference conditions as described above. The majority of lakes were from England, Scotland, the NGIG countries, particularly Norway and Finland and CBGIG, mainly Germany. Reference conditions for % nuisance cyanobacteria
- Location of sites:** For chlorophyll, there are too many lakes to list, refer to Cavalho et al (2006)\_WFD Project 38\_Chlorophyll and Phosphorus Classification for UK lakes for details. For % nuisance cyanobacteria, the fact that the conditions were derived from expert judgement means it is not possible to provide a list of lakes used.
- Data time period:** See comments in C-09
- Criteria:**  
Land-use pressures based on >90% natural or semi natural land-use, <5% artificial land-use and <10 pop/km<sup>2</sup>. Also, absence of other non-land-use factors like discharges of sewage effluent to the lake and fish-farming/rearing activities within the lake.
- 3.08 Reference community description**  
There will be no more on average, than 5-7% by biovolume (depending on lake type), of nuisance cyanobacteria in the samples taken over the summer period. Other taxa present in the phytoplankton community are not considered in their own right in this method, they purely contribute to the total biovolume of the phytoplankton.
- 3.09 Results expressed as EQR?** Yes

## **Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise

### **3.11 Boundary setting procedure**

Boundary EQR values for the H/G and G/M boundaries are those agreed during the first phase of intercalibration in the CBGIG and NGIG and were set in terms of relationship to pressure, secondary effects and expert judgement. For low and moderate alkalinity lakes boundaries were set by determining break points in percentage of Chrysophytes, Pennate Diatoms and Cyanobacteria (NGIG). For high alkalinity lakes boundaries were set in relation to the 2nd impacts on macrophytes using models of maximum depth of colonisation, macrophyte cover and proportion of cyanobacteria. Full documentation of the approaches for lake phytoplankton can be found in the GIG milestone reports and technical annexes.

**3.12 "Good status" community:** There will be, on average, no more than 20-25% (depending on lake type) by biovolume of nuisance cyanobacterial taxa present in the samples taken from the summer period. Composition of other taxa within the phytoplankton community are not considered in this method.

## **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Uncertainty for chlorophyll a is determined using a modelled estimate of variance which allows for seasonal variation, determined by subsampling of data from lakes with a long period of frequent sampling. This allows an estimate of variation on the annual geometric mean which is independent of variation caused by seasonality (Cavalho et al (2006)\_WFD Project 38\_Chlorophyll and Phosphorus Classification for UK lakes). For cyanobacteria a modelled estimate of variance as a function of EQR was determined from available replicated data using the method outlined in Ellis 2006 (available from <http://publications.environment-agency.gov.uk/epages/eapublications.storefront/4b100774024a67a6273fc0a802960648/Product/View/GEHO1006BLOR&2DE&2DE>). For both chlorophyll and cyanobacteria the confidence of class is determined by assuming the estimated mean Chlorophyll a value and Cyanobacteria EQRs are normally distributed about the mean with standard deviations determined from the above models.

**3.14 Comments:**

none

ID: 204

HUNGPHYTOBENTLAKE

## 1. General information

- 1.01 GIG:** Eastern Continental
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Diatoms
- 1.04 Country:** Hungary
- 1.05 Specification:**
- 1.06 Method name:** *Improvement of the Hungarian ecological water qualification system - Phytobenthos in Lakes*
- 1.07 Original name:** *A magyarországi ökológiai minősítési rendszer továbbfejlesztése, fitobentosz*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Ács, É., 2007. A Velencei-tó bevonatlakó algáinak tér- és időbeli változása, kapcsolata a tó ökológiai állapotával. (Spatial and temporal change of epiphytic algae and their connection with the ecological condition of shallow lake Velencei-tó (Hungary). Acta Biologica Debrecina Oecologica Hungarica 17, Hydrobiological Monographs 1: 9-111. @Ács, É., G. Borics, G. Fehér, K.T. Kiss, M.N. Reskóné, M. Nagy, C. Stenger-Kovács, A. Tóth & G. Várbiro, 2009. A fitobentosz élőlénycsoport zárójelentése az ökológiai minősítési rendszer továbbfejlesztéséről @ 10.
- 1.12 Scientific literature:**  
Ács, É., B. Bolla, G. Borics, K.T. Kiss, M.N. Reskóné, M. Nagy, C. Stenger-Kovács & G. Várbiro, NN. Recommendations for ecological status assessment of lake balaton (largest shallow lake of central europe), based on benthic diatom communities. – submitted paper (Vie Milieau).
- 1.13 Method developed by** **1.14 Method reported by**  
Eva Acs and Gabor Borics **statt 161 Dr. Eva Acs**  
evaacs@freemail.hu, boricsg@gmail.com **evaacs@freemail.hu**  
Hungarian Danube Research Station; Environmental Protection, **Hungarian Danube Research Station of Hung. Acad. Sci., Göd**  
Nature Conservation and Water Authority of Transiszanian Region
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Ács, É. & K. Szabó, 2004. Bentikus algák gyűjtése és feldolgozása (Collection and investigation methods for benthic algae). In Ács, É. & K.T. Kiss (eds), Algológiai praktikum. (Algological practice). ELTE Eötvös Kiadó, Budapest: 35-46.
- 2.02 Short description**  
Cut across the reed-stem with a strong clipper at the water surface, then cutting across the underwater-section as well, remove the substrate, and cut a 10-20 cm long underwater section of the stem into a labelled bottle. After collecting 5 stems, put a little bit of tap water into the bottle, so that the biofilm could rest in a moist place, and close the bottle. Samples should be kept in a cool, dry place until transporting to laboratory. It is suggested to wash out the biofilm in the laboratory. To wash out the biofilm, put the stems into a Petri-dish filled with few tap water, and rub the coating from the surface (one by one) with the help of a brush. Wash out the substrate and pour the suspension into a labelled bottle.
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying
- 2.04 Sampling/survey device:** Brush
- 2.05 Specification:** clipper
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
reed stems
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** June to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
5
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
5x30=150 cm<sup>2</sup>

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** every diatoms
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Relative abundance  
in relation to Area  
Unit number of valves per 400 valves
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
relative abundance of taxa with indicator and sensitivity values for organic material and nutrients (diatom indices calculated by OMNIDIA and self-developed program)
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** -  
**Geographical coverage:** -  
**Location of sites:** n.a.  
**Data time period:** -  
**Criteria:**  
It was practically impossible to find reference conditions, so we used the so called "Least Disturbed Sites" for boundary setting.
- 3.08 Reference community description**  
-
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
We calculated the median of the used diatom index in every type. This median was the H/G boundary. If a type there was any pressure, the H/G boundary was the median-1, if a type there was multiply pressures, the H/G boundary was the median-2. The rest was divided into 4 equal parts.
- 3.12 "Good status" community:** At good status stands of the sensitive taxa are well developed. They are dominant, but significantly decreasing at good-moderate boundary and replaced by tolerant taxa. The median of the index values of the selected LDS sites were considered as high/good (H/G) class boundaries.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none



ID: 194

QBAP-L

## 1. General information

- 1.01 GIG:** Eastern Continental
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Hungary
- 1.05 Specification:**
- 1.06 Method name:** *Macroinvertebrate based assessment system - Lakes*
- 1.07 Original name:** *Makrogerincteleneken alapuló minősítési rendszer - Tavak*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation, Habitat destruction, Hydromorphological degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Zoltán Müller et al.  
mullez@bioaquapro.hu  
BioAquaPro Plc.
- 1.14 Method reported by**  
Béla Csányi  
bela.csanyi@gmail.com  
Inst. of Env. Protect. & Water Management Plc.
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
AQEM Protocol.
- 2.02 Short description**  
See: AQEM Protocol
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** FBA Pondnet
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** April-October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Once a year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
10 replicates re taken (25x25 cm per each)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
0.25 cm<sup>2</sup>

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 950 micron(mesh size of handnet)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Other, Species/species groups  
Oligochaeta, Chironomidae and other Diptera: max. until Genus
- 2.15 Record of abundance:** Individual counts, Relative abundance  
**in relation to** Area  
**Unit** ind/m<sup>2</sup>
- 2.16 Quantification of biomass:** n.a.

- 2.17 Other biological data: none
- 2.18 Special cases, exceptions, additions: none
- 2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics  
Only data collection takes place, the development of lake assessment system is still going on. Further development is needed
- 3.02 Does the metric selection differ between types of water bodies?
- 3.03 Combination rule for multi-metrics: n.a.
- 3.04 From which biological data are the metrics calculated?  
n.a.

#### Reference conditions

- 3.05 Scope of reference conditions: Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:  
Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation  
Number of sites:  
Geographical coverage:  
Location of sites: n.a.  
Data time period:  
Criteria:  
It was practically impossible to find reference conditions, so we will try to use the so called "Least Disturbed Sites" for boundary setting.
- 3.08 Reference community description  
n.a.
- 3.09 Results expressed as EQR? n.a.

#### Boundary setting

- 3.10 Setting of ecological status boundaries: n.a.
- 3.11 Boundary setting procedure
- 3.12 "Good status" community: n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty: No (to be done)
- 3.14 Comments:  
none

ID: 198

HU-FI-LA

## 1. General information

1.01 GIG: Eastern Continental

1.02 Category: Lakes

1.03 BQE: Fish Fauna

1.04 Country: Hungary

1.05 Specification:

1.06 Method name: *Assessment of fish fauna in lakes*

1.07 Original name: *n.a.*

1.08 Status: Method is/will be used in *n.a.*

1.09 Detected pressure(s): *n.a.*

*Has the pressure-impact-relationship been tested?*

1.10 Internet reference:

1.11 Pertinent literature of mandatory character:

*n.a.*

1.12 Scientific literature:

*n.a.*

1.13 Method developed by

1.14 Method reported by

Zoltan Szaloky

szaloky@vituki.hu

VITUKI Environmental and Water Management Research Institute  
Non-profit Ltd.

1.15 Comments

*none*

## 2. Data acquisition

### Field sampling/surveying

2.01 Sampling/Survey guidelines

EN 14011 Water quality. Sampling of fish with electricity. EN 14757 Water quality. Sampling of fish with multi-mesh gillnets. ECOSURV Protocol.

2.02 Short description

See: EN 14011 Water quality - Sampling of fish with electricity EN 14757 Water quality - Sampling of fish with multi-mesh gillnets and ECOSURV Protocol

2.03 Method to select the sampling/survey site or area: Expert knowledge

2.04 Sampling/survey device: Electrofishing gear, Gill net

We plan to use the echo sound

2.05 Specification: Battery powered backpackers and electrofishers with engine and multi mesh gill net.

2.06 Sampled/surveyed habitat: All available habitats per site (Multi-habitat)

2.07 Sampled/surveyed zones in areas with tidal influence: not relevant

2.08 Sampling/survey month(s): May to October

2.09 Number of sampling/survey occasions (in time) to classify site or area

One occasion per sampling season

2.10 Number of spatial replicates per sampling/survey occasion to classify site or area

*n.a.*

2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area

150-1500 m (based on lake type)

### Sample processing

2.12 Minimum size of organisms sampled and processed: 5 mm (mesh-size of catcher bow net)

2.13 Sample treatment: Organisms of the complete sample are identified.

2.14 Level of taxonomical identification: Species/species groups

2.15 Record of abundance: Relative abundance

in relation to Area, Time

Unit Number of individuals per one square-metre

2.16 Quantification of biomass: *n.a.*

2.17 Other biological data: Length and agegroup (0+, adult) of individual specimens.

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### **Evaluation**

3.01 List of biological metrics

3.02 Does the metric selection differ between types of water bodies?

3.03 Combination rule for multi-metrics: n.a.

3.04 From which biological data are the metrics calculated?

n.a.

#### **Reference conditions**

3.05 Scope of reference conditions:

3.06 Key source(s) to derive reference conditions:

n.a.

3.07 Reference site characterisation

Number of sites:

Geographical coverage:

Location of sites: n.a.

Data time period:

Criteria:

n.a.

3.08 Reference community description

n.a.

3.09 Results expressed as EQR? n.a.

#### **Boundary setting**

3.10 Setting of ecological status boundaries: n.a.

3.11 Boundary setting procedure

3.12 "Good status" community: n.a.

#### **Uncertainty**

3.13 Consideration of uncertainty:

3.14 Comments:

none

ID: 210

HLPI

## 1. General information

**1.01 GIG:** Eastern Continental

**1.02 Category:** Lakes

**1.03 BQE:** Phytoplankton

**1.04 Country:** Hungary

**1.05 Specification:**

**1.06 Method name:** *Hungarian Lake Phytoplankton Index*

**1.07 Original name:** *Magyar Tavi Fitoplankton Index*

**1.08 Status: Method is/will be used in** First RBMP (2009)

**1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic matter  
in case of alkaline saline lakes decrease of conductivity has been tested.

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

In case of the naturally eutrophic, alkaline lakes the dilution is the most frightening impact. In this lake type the chlorophyll-a is not involved in the index, exclusively the composition metric (Q) is used for assessment. Relationship between the Q index and the conductivity has been tested on 10 data from a saline lake. The relationship was significant ( $R=0,68$ ). TP Chl-a relationship was tested on 673 lake-year data. There was no significant relationship! Lowess technic indicated that extreme eutrophication may appear above 500ug/l TP. HLPI was tested against several measures of the organic pollution. Data of 20 lakes (~196 data) has been used. R-square values were the followings: HLPI-BOD  $R^2=0,37$ ; HLPI-CODcr  $R^2=0,66$ ; HLPI-COD Mn  $R^2=0,72$ ; HLPI-NH4  $R^2=0,28$ ; HLPI-Inorg.N  $R^2=0,12$

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

Padisák, J., G. Borics & I. Grigorszky et al., 2006. Use of phytoplankton assemblages for monitoring ecological status of lakes within the Water Framework Directive: the assemblage index *Hydrobiologia* 553: 1-14.

**1.13 Method developed by**

Judit Padisák, Gábor Borics

boricsg@gmail.com

Environmental Protection Nature Conservation and Water Authority Trans-Tisa Region Debrecen

**1.14 Method reported by**

Gábor Borics

boricsg@gmail.com

Environmental Protection Nature Conservation and Water Authority Trans-Tisa Region Debrecen

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

MSZ EN 15204, 2006. Vízminőség. Útmutató szabvány a fitoplanktonok inverz mikroszkópiás számlálására (Ütermöhl - technika). MSZ ISO 10260. Water quality. Measurement of biochemical parameters. Spectrometric determination of the chlorophyll-a concentration.

**2.02 Short description**

Water samples are taken from the representative site(s) on the lake. In case of the deeper lakes the euphotic layer is sampled (~2.5x Secchi transparency). In case of the very shallow lakes the whole water column is sampled. Three samples are taken by tube sampler from a boat. The samples are mixed in a dish of larger volume. 0.3 litres sample is taken from the dish and preserved with Lugol's solution on the spot.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Hard-wall plastic tube sampler.

**2.06 Sampled/surveyed habitat:** Single habitat(s)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** May to October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

3 years duration, 4samples/year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Number of samples depends on lake size and morphometry. Three samples are taken at a single sample site, and mixed on the spot.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

3x2 litres of samples are taken.

### **Sample processing**

**2.12 Minimum size of organisms sampled and processed:**

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

in relation to Volume

Unit: phytoplankton biomass (mg/l)

**2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

## **3. Data evaluation**

### **Evaluation**

**3.01 List of biological metrics**

$HPLI = NChl-a/2 + NQ/2$   
HPLI: Hungarian Lake Phytoplankton Index. NChl-a: Normalised value of the Chl-a  
NQ: Normalised value of the Chl-a

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:**

**Geographical coverage:**

**Location of sites:** n.a.

**Data time period:** Lake Balaton: Historical data before 1950s, covering ~2 decades

**Criteria:**

Hasn't established yet!

**3.08 Reference community description**

Dominance (80% of total biomass) of those functional groups of algae that (on basis of the historical data, modelling or expert opinion) can be considered as reference for the given lake type.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** n.a.

given percentiles of the data derived from the whole lake population.

**3.11 Boundary setting procedure**

Boundaries for chl-a were set by the 25 (H/G), 75(G/M), 90(M/P), 95(P/B) percentiles of the dataset. The boundaries of the composition metric were set by proposed ratio of the functional groups of algae.

**3.12 "Good status" community:** In lakes of good status the value of the Q metric is >0.46. Practically it means that relative abundance of the most impacted functional groups is <15%, the ratio of the functional groups with 3,4,5 factor numbers (considered moderate, good and excellent for the lake) is >70%.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Three categories were established for uncertainty estimation. Low uncertainty (category 1) means that there are at least 4 samples/year, and both the chl-a and the phytoplankton composition were measured. If the number of samples are <4, the

uncertainty is "medium" (category 2). If one of the metric (Chl-a or composition metric) is missing the uncertainty shifts to a lower category (from 1 to 2, or from 2 to 3).

**3.14 Comments:**

none

ID: 235

LN-FITO

## 1. General information

- 1.01 GIG:** Eastern Continental
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Romania
- 1.05 Specification:**
- 1.06 Method name:** *Assessment system for natural lakes status based on Phytoplankton according to the WFD*
- 1.07 Original name:** *Sistem de evaluare a starii lacurilor naturale (metodologie) pe baza fitoplanctonului, conform cerintelor Directivei*
- 1.08 Status: Method is/will be used in:** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, General degradation, Pollution by organic matter  
*Has the pressure-impact-relationship been tested?*
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
gabriel.chiriac@rowater.ro
- 1.14 Method reported by**  
Serban Iliescu, Gabriel Chiriac  
serban.iliescu@rowater.ro, gabriel.chiriac@rowater.ro  
Romanian Water Authority - Department of Water Resource Monitoring

### 1.15 Comments

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Guidance on quantitative and qualitative sampling of phytoplankton from inland waters. (Draft N109: 2008, experimentally).
- 2.02 Short description**  
500 ml from representative sampling sites ( e.g. middle of the lake), based on euphotic zone (transparency Secchi' Disk), integrated sample. Alkaline Lugol's solution for preservation.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
water column
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** April to June / July / August to September / October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
3 - 4 times / year based on algae growth
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
One integrated sample
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
500 ml

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 10 - 25 µm
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Standard SR EN ISO CEI 15204:2007
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Cyanobacteria: genus/species level; Bacillariophyta - species level; Chryptophyta, Dinophyta, Euglenopyta, Chlorophyta - genus/species level
- 2.15 Record of abundance:** Individual counts  
in relation to Volume  
Unit Number of algal objects dm<sup>3</sup> / liter

- 2.16 Quantification of biomass:** Utermöhl technique
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments:** none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics:** Biomass
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Mean quality class
- 3.04 From which biological data are the metrics calculated?** Data from single spatial replicate

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:** Existing near-natural reference sites, Expert knowledge, Historical data
- 3.07 Reference site characterisation**
- Number of sites:** 8
- Geographical coverage:** Carpathians, Sub-Carpathian Hills
- Location of sites:** Retezat Park; Calimani National Park; Maramures Zone; Transylvanian Plateau; Fagaras Mountains; Romanian Plain
- Data time period:** 1960s; 1970s; 2004 - 2007
- Criteria:** n.a.
- 3.08 Reference community description** Presence of sensitive taxa, high diversity, absence of algal bloom, historical data.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure** biomass (mg/L); organic pollution matter and eutrophication process (phosphorus) ; ecological status boundaries RO LN01 type H/G = 5; G/M = 7
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:** none

ID: 5

BQIL Hw

## 1. General information

- 1.01 GIG:** Mediterranean  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Italy
- 1.05 Specification:** none
- 1.06 Method name:** *Benthic Quality Index for Italian Lakes*
- 1.07 Original name:** *Indice di qualità bentonico per i laghi italiani*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Ecological data including 1659 samples of macroinvertebrates from 37 lakes belonging to different lake types were examined to establish pressure-impact relationship between macroinvertebrates metrics and eutrophication gradient. The relationship between two macroinvertebrate metrics and eutrophication measures showed highly significant correlation. Product moment Correlation Coefficient between BQI and TSI = 0.63  $p < 0.01$ , between BQI and TP = -0.46,  $p < 0.01$ , between BQI and transparency = 0.48,  $p < 0.01$ , between BQI and % sat oxygen = 0.28,  $p < 0.01$ , between weighted diversity index and TP = -0.14  $p < 0.01$ .

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

AA.VV. 2009. Indici per la valutazione della qualità ecologica dei laghi. CNR-ISE 2/09.

**1.12 Scientific literature:**

Rossaro, B., A. Boggero, V. Lencioni, L. Marziali & A. Solimini, 2006. Tools for the development of a benthic quality index for Italian lakes. *Journal of Limnology* 65 (1): 41-51. Rossaro, R., L. Marziali, A.C. Cardoso, A. Solimini, G. Free & R. Giacchini, 2007. A biotic index using benthic macroinvertebrates for Italian lakes. *Ecological Indicators* 7: 412-429.

**1.13 Method developed by**

Bruno Rossaro  
bruno.rossaro@unimi.it  
Dipartimento di Protezione dei Sistemi Agroalimentare ed  
Urbano e Valorizzazione delle Biodiversità - Univ. Milano

**1.14 Method reported by**

Angela Boggero  
a.boggero@ise.cnr.it  
CNR-ISE Institute of Ecosystem Study

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Bazzanti, M., A. Boggero, V. Lencioni, L. Mastrantuono, B. Rossaro & A. Solimini, 2007. Protocollo di campionamento e analisi dei macroinvertebrati negli ambienti lacustri. MATTM-APAT Roma: 16 pp.

**2.02 Short description**

The method follows US-EPA methodologies (<http://www.epa.gov/owow/monitoring>). 3 sampled areas (littoral, sublittoral and profundal) along transects are considered with 3-5 replicates per site (minimum effort to obtain good data set). The number of transects depends on lake area and for major lakes (like L. Maggiore, Garda and Como the highest lake areas and maximum depths in Italy, i.e. > 300 m) the number of transects refers to the number of sub-basins.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge, Stratified sampling/surveying

**2.04 Sampling/survey device:** Grab  
scuba divers

**2.05 Specification:** Ponar, Ekman

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** Lakes: February-April, September-October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Biannual: turn-over and stratification periods

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

3 sampled areas (littoral, sublittoral and profundal) along transects are considered with 3-5 replicates per site depending on grab area

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

one square-metres

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 250-300 µm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups  
Chironomids, Oligochaetes: species level; Others: genus level
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per one square-metre
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Sum of (relative abundance of taxa \* log relative abundance of taxa \* indicator weight)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates

### **Reference conditions**

- 3.05 Scope of reference conditions:** n.a.
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**  
**Number of sites:** Not yet defined  
**Geographical coverage:** Not yet defined  
**Location of sites:** Not yet defined  
**Data time period:** Not yet defined  
**Criteria:**  
Not yet defined.
- 3.08 Reference community description**  
Not yet defined.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** n.a.  
Not yet defined
- 3.11 Boundary setting procedure**  
Not yet defined
- 3.12 "Good status" community:** Not yet defined.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** n.a.  
Not yet defined
- 3.14 Comments:**  
none

ID: 234

MacrolMMI

## 1. General information

- 1.01 GIG:** Mediterranean
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Italy
- 1.05 Specification:** only north Italy, Alpine ecoregion
- 1.06 Method name:** **Macrophytes Italian MultiMetrics Index (MacrolMMI)**
- 1.07 Original name:** *Indice multimetrico italiano per le macrofite*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Biological data from 19 lakes, between 0.64 and 3.86 meq l-1 TALK and with mean depth < 125 m, were examined to establish relationship between macrophyte abundance and eutrophication (TP) pressure. The relationship between the five metrics and TP (measured in winter stratification) showed significant correlation.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Italian law and "CNR ISE Report 2/09. Indici per la valutazione della qualità ecologica dei laghi"

**1.12 Scientific literature:**

Oggioni, A., 2010. Proposal of trophic value for the aquatic plants in lake: first step for a italian macrophytes index. Atti Società Italiana di Ecologia. In press.

**1.13 Method developed by**

a.oggioni@ise.cnr.it

**1.14 Method reported by**

Alessandro Oggioni  
a.oggioni@ise.cnr.it  
CNR - Institute of Ecosystem Study

**1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Buraschi, E., F. Buzzi, L. Garibaldi, G. Morabito, A. Oggioni, G. Tartari & N. Salmaso et al., 2008. Protocollo di campionamento di macrofite acquatiche in ambiente lacustre. Metodi biologici per le acque. Parte I. APAT: 16 pp.

**2.02 Short description**

The sampling is to go from 1 to 4 transects - perpendicular to the coastline - the number of which depends on the extent of the site along shore. The site is represented by a homogeneous area in terms of macrophytes cenosis. For each depth interval along the transect, you made an assessment of the abundance of each species found on a scale of 1 to 4 caps for 4 positions around the boat.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Grapnel, Rake  
GPS e underwater camera

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
All littoral habitat within the maximum growth of macrophytes

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** July to September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
From 1 to 4 replicated depending to extension of shore with an homogenous species composition

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Number of transects per site is 1 up to an extent of 50 m, 2 between 50 and 200 m, 3 between 200 and 1000 m, or 4

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** In the survey method we don't consider a macroalgae organism

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Abundance classes

**in relation to** Area

**Unit** along a transect: number of presence of specie at any sampling point; in the lakes: weight-mean abundance of each transect for lake perimeter

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** only hydrophytes, pleustophytes and carophytes are sampling

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Maximum depth colonization (Z-cmax); percent frequencies of submerged species (sub) and of exotic species (exot) where the frequency is the  $f_k = \text{number of point with submerged or exotic species} / \text{total point with vegetation}$ ; Trophic score (sk) = sum of (abundance of specie \* trophic score) / sum of all species abundance.

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Site-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data

**3.07 Reference site characterisation**

**Number of sites:** In this moment we don't have a reference condition site in Italy

**Geographical coverage:**

**Location of sites:** n.a.

**Data time period:**

**Criteria:**

**3.08 Reference community description**

**3.09 Results expressed as EQR?** n.a.

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Using discontinuities in the relationship of anthropogenic pressure and the biological response.

**3.11 Boundary setting procedure**

**3.12 "Good status" community:**

#### Uncertainty

**3.13 Consideration of uncertainty:**

**3.14 Comments:**  
none

ID: 94

n.a.

## 1. General information

- 1.01 GIG:** Mediterranean  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Cyprus
- 1.05 Specification:** none
- 1.06 Method name:** *Mediterranean Assessment System for Reservoirs Phytoplankton*
- 1.07 Original name:** *Μεσογειακό Σύστημα Αξιολόγησης του φυτοπλαγκτού σε ταμειτήρες νερού*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

### *Has the pressure-impact-relationship been tested?*

Yes, with qualitative data (e.g. response at reference against impacted sites).

Chlorophyll-a concentration & total biovolume (as indicators of phytoplankton biomass) and % cyanobacteria biovolume & Catalan index (as indicators of phytoplankton composition) data were applied to a set of previous data on Spanish reservoirs in order to know their suitability for the Mediterranean reservoirs (C. de Hoyos, 2005). The metrics showed a significant relationship with Total Phosphorus (TP) as indicator of the eutrophication pressure ( $r = 0.858$  for Chlorophyll-a,  $0.881$  for total biovolume,  $0.747$  for % cyanobacteria biovolume &  $0.91$  for Catalan index). When the dataset from sampled reservoirs during summer 2005 was included in the analysis, correlation significance decreased. This should not be surprising, bearing in mind that the agreed data sampling programme did not intend to cover the whole gradient of impact.

### 1.10 Internet reference:

### 1.11 Pertinent literature of mandatory character:

Commission Decision of 30 October 2008 (2008/915/EC). Establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the intercalibration exercise.

### 1.12 Scientific literature:

For chlorophyll concentration: [APHA, AWWA, WPCF 21st Edition, 2005](#). Edited by Eaton, A.D., L.S. Clesceri, E.W. Rice, A.E. Greenberg. Method 10200 H. Chlorophyll. [Standard Methods for the examination of water & wastewater](#). For the assessment of phytoplankton abundance: [CEN 1520, 2006](#). Water quality. Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique). For the assessment of phytoplankton biovolume: [CEN TC 230/WG 2/TG 3](#). (Draft version), 2006. Phytoplankton biovolume determination using inverted microscopy – Utermöhl technique. For description of the GAI index: [Catalan, J., M. Ventura, A. Munné & L. Godé, 2003](#). Desenvolupament d'un index integral de qualitat ecològica i regionalització ambiental dels sistemes lacustres de Catalunya. Agència Catalana del Aigua. Generalitat de Catalunya. <http://mediambient.gencat.net/aca/ca//planificacio/directiva/treballs.jsp>

### 1.13 Method developed by

Members of L-M GIG. The taxonomic composition metric I.Catalan (IGA) was designed by Dr. Jordi Catalan.  
cdhoyos@cedex.es  
CEDEX

### 1.14 Method reported by

Polina Polykarpou  
ppolycarpou@wdd.moa.gov.cy  
Water Development Department, Cyprus

### 1.15 Comments

none

## 2. Data acquisition

### Field sampling/surveying

#### 2.01 Sampling/Survey guidelines

Lake Mediterranean GIG, 2007. Milestone 6 Report – Lake Mediterranean, updated 26 November 2007. European Commission, Directorate General Joint Research Centre, Institute of Environment and Sustainability.

#### 2.02 Short description

Deepest lake point is detected. At this point depth of the euphotic layer is determined by using Secchi disk (euphotic layer = 2.5 \* Secchi disk depth). This layer is then sampled, using an integrating water sampler

#### 2.03 Method to select the sampling/survey site or area:

n.a.

#### 2.04 Sampling/survey device:

Water sampler

#### 2.05 Specification:

Integrating water sampler

#### 2.06 Sampled/surveyed habitat:

All available habitats per site (Multi-habitat)

#### 2.07 Sampled/surveyed zones in areas with tidal influence:

not relevant

#### 2.08 Sampling/survey month(s):

June & September

#### 2.09 Number of sampling/survey occasions (in time) to classify site or area

2 samplings in the summer

#### 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area

One integrated water sample from the euphotic layer

#### 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area

For chlorophyll concentration: 12 L of water & For phytoplankton analyses (Utermöhl technique): 0.4 L of water

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 1-2  $\mu\text{m}$
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
For chlorophyll concentration: Sub-sampling depends from phytoplankton concentration in the original sample. For phytoplankton analyses (Utermöhl technique): Sub-sampling depends from phytoplankton concentration in the sample. The most appropriate volume
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
If identification to the species level is not possible or doubtful, identification remains at the genus level.
- 2.15 Record of abundance:** Individual counts  
in relation to Volume  
Unit Number of individuals per mL
- 2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
- Indicators of phytoplankton biomass: (1) chlorophyll-a concentration ( $\text{mg}/\text{m}^3$ ) =  $[26.7 * (664b - 665a) * V1] / (V2 * L)$  & (2) total biovolume ( $\text{mm}^3/\text{L}$ ) = (average cell biovolume of taxon \* number of individuals) / 10-9  
- Indicators of phytoplankton composition: (1) % cyanobacteria biovolume = (cyanobacteria biovolume \* 100) / total biovolume & (2) Catalan index =  $[1 + 0.1 * \text{Cryptophyceae} + \text{colonial Chrysophyceae} + 2 * (\text{colonial Diatoms} + \text{colonial Chlorococcales}) + 3 * \text{colonial Volvocales} + 4 * \text{Cyanobacteria}] / [1 + 2 * (\text{Dinoflagellates} + \text{Chrysophyceae not colonial}) + \text{Chlorococcales not colonial} + \text{Diatoms not colonial}]$
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores  
Average of (average of biomass metrics & average of composition metrics)
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
n.a.  
Existing reference reservoirs
- 3.07 Reference site characterisation**  
**Number of sites:** 10 (1 from Cyprus)  
**Geographical coverage:** Mediterranean region  
**Location of sites:** Cyprus, France, Greece, Portugal, Romania & Spain  
**Data time period:** Summer of 2005 (4 months: from June to September)  
**Criteria:**  
Cyprus criteria: CORINE land cover, 90% of land in the catchment area is covered by semi-natural coniferous forest; 8% is agricultural land. No industry, nor significant human settlements.
- 3.08 Reference community description**  
It corresponds totally, or nearly totally, to undisturbed conditions, aside from the hydromorphological alterations calling for HMWB designation. For Phytoplankton composition, the maximum ecological potential corresponds to a composition of algae groups coherent with undisturbed conditions. Very minor % of bloom-forming Cyanobacteria biovolume is expected. Phytoplankton biomass (chlorophyll concentration and total biovolume) show low values.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise

### 3.11 Boundary setting procedure

L-M GIG did not consider the H/G or Max/G boundary in the IC exercise, bearing in mind the WFD only requires for AWB and HMWB to be reported the ecological potential "good and above" as a whole, with no distinction between Good and Maximum Ecological Potential. G/M boundary value was set as a percentile of the distribution of the data collected for each index, namely 95th percentile for the biomass metrics and 90th percentile for the composition metrics. The G/M boundary values of the four biological indices were calculated for each type: Siliceous Arid, Siliceous Wet and Calcareous.

**3.12 "Good status" community:** It corresponds to a slightly deviation from reference conditions. The composition of algae groups does not become affected by longer changes although some taxa begin to change. The values of both % of bloom-forming Cyanobacteria biovolume and composition indices might be higher than at maximum ecological potential.

## Uncertainty

**3.13 Consideration of uncertainty:** Yes

Partly: Successful participation in Proficiency test phytoplankton 2008 (State Reservoirs Administration of Saxony) & participation in Mediterranean WISER phytoplankton counter workshop (22-23 Oct. 2009)

**3.14 Comments:**

none

ID: 2

MedPTI

## 1. General information

- 1.01 GIG:** Mediterranean  
LM-5,7,8
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Italy
- 1.05 Specification:** deep (average>15m) reservoirs of the Mediterranean ecoregion
- 1.06 Method name:** **Mediterranean Phytoplankton Trophic Index**
- 1.07 Original name:** *Mediterranean Phytoplankton Trophic Index*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

The index was calibrated using data from 30 reservoirs in Sardinia, and calibrated using further 48 annual data from 10 reservoirs (data sets not overlapping). The index is significantly related with the logarithm of total P concentration  $r=0.93$  in the calibration data set,  $r=0.62$  in the validation data set

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

CNR ISE Report 2/09. Indici per la valutazione della qualità ecologica dei laghi.

**1.12 Scientific literature:**

Marchetto, A., B.M. Padedda, M.A. Mariani, A. Lugliè & N. Sechi, 2009. A numerical index for evaluating phytoplankton response to changes in nutrient levels in deep mediterranean reservoirs. *Journal of Limnology* 68: 106-121.

**1.13 Method developed by**

Aldo Marchetto  
a.marchetto@ise.cnr.it  
CNR ISE and University of Sassari, Dept. of Botany

**1.14 Method reported by**

Aldo Marchetto  
a.marchetto@ise.cnr.it  
CNR National Research Council, ISE Institute of Ecosystem Study

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Buraschi, E., F. Buzzi, L. Garibaldi, A. Lugliè, E. Legnani, G. Morabito, A. Oggioni, S. Pozzi, N. Salmaso & G. Tartari, 2008. Protocollo per il campionamento di Fitoplancton in ambiente lacustre. APAT. [http://www.apat.gov.it/site/\\_files/Pubblicazioni/Metodi\\_bio\\_acque/laghi\\_fito.pdf](http://www.apat.gov.it/site/_files/Pubblicazioni/Metodi_bio_acque/laghi_fito.pdf)

**2.02 Short description**

Standard integrated sample for phytoplankton analysis, obtained by mixing discrete samples taken every meter from lake surface to 2.5 times Secchi depth.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
Water column

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** ice free season

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Six per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

1 water column, sampled every meter up to 2.5 times the Secchi disk depth

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** n.a.

**2.13 Sample treatment:** n.a.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** n.a.

biovolume  
**in relation to** Volume  
**Unit** cubic micrometer per cubic milimeter or per liter

**2.16 Quantification of biomass:** Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

MedPTI=sum of (Taxa biovolume \* trophic weight \* indicator value) / sum of (Taxa biovolume \* indicator value)  
 % biovolume of (eutrophic) cyanobacteria ÷ total biovolume ÷ chlorophyll a concentration

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

average normalized EQR

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites

**3.07 Reference site characterisation**

**Number of sites:** 3 reservoirs in the Mediterranean ecoregion

**Geographical coverage:** Rumania, Cyprus, Spain

**Location of sites:** see Mediterranean GIG reports

**Data time period:** One year of data

**Criteria:**

Upstream water demand < 3% per domestic use, < 1.5% per industrial use, <10% per agriculture ÷ Catchment area classified as natural by CORINE.

**3.08 Reference community description**

Phytoplankton mainly composed of diatoms and chlorophytes.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Using discontinuities in the relationship of anthropogenic pressure and the biological response.

**3.11 Boundary setting procedure**

A discontinuity was found at MedPTI=2.45 separating reservoirs with Total P concentration lower or higher than 40 micrograms per litre. This was used as G/M. ÷ The difference between the median reference MedPTI and the G/M boundary was assumed equal to two class widths, and one class width was used to identify class boundaries. ÷ G/M boundary was confirmed during the intercalibration calculating MedPTI for 14 reservoirs in Cyprus, Spain, Rumania, Portugal and Sardinia.

**3.12 "Good status" community:** Eutrophic cyanobacteria develop but does not dominate the assemblages.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

Interannual variability of MedPTI was evaluated in 3 reservoirs.

**3.14 Comments:**

In spite of the high variability in hydraulic conditions of the Mediterranean reservoirs, standard deviation of MedPTI was only .06-.16 ÷ The main problem in the Lake Mediterranean GIG is the very small number of natural lakes, and of reservoirs in good ecological conditions, as most reservoirs have been built to cope with strong water needs in these arid countries.

ID: 208

ES-PP-LA

## 1. General information

- 1.01 GIG:** Mediterranean  
Mediterranean reservoirs (L-M GIG). Types L-M 5/7 and L-M 8
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Spain
- 1.05 Specification:** Reservoirs
- 1.06 Method name:** *Mediterranean Assessment System for Reservoirs Phytoplankton*
- 1.07 Original name:** *Mediterranean Assessment System for Reservoirs Phytoplankton*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Relations between the phytoplankton metrics selected and the Total Phosphorus were made with data from 33 Spanish reservoirs sampled from 1999 to 2001. Each reservoir was sampled only during one year, 1-6 times during the period Jun-Oct at 1-3 different depths in the euphotic zone. Results: Chlorophyll a/TP. R2=0.74 p=0 Biovolume/TP. R2=0.78 p=0 Cyanobacteria. R2=0.56 p=0.00001. Catalan (IGA). R2=0.83 p=0

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

ORDEN ARM/2656/2008 de 10 de septiembre por la que se aprueba la Instrucción de Planificación Hidrológica (BOE 22-11-08).

**1.12 Scientific literature:**

IGA, 2003. Desenvolupament d'un índex integral de qualitat ecològica i regionalització ambiental dels sistemes lacustres de Catalunya. Agència Catalana de l'Aigua.

**1.13 Method developed by**

Members of L-M GIG. (The taxonomic composition metric I. Catalan (IGA) was designed by Dr. Jordi Catalan)  
cdhoyos@cedex.es  
CEDEX

**1.14 Method reported by**

Carmen Coletó Fiaño  
ccolet@mma.es  
Subdirección General de Gestión Integrada del Dominio Público  
Hidráulico - Ministerio de Medio Ambiente, Medio Rural y Marino

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Phytoplankton sampling protocol for Lakes and Reservoirs. Protocolo de muestreo de fitoplancton en lagos y embalses.

**2.02 Short description**

Sampling stations: In general, one sampling station at the deepest site. Could be more in accordance with the morphometric characteristics. Sampling frequency: twice in summer (at the beginning of July and in September) Vertical samples: Integrated sample of the euphotic zone (2.5\* Secchi depth). Either by 1-m steps or by integrating sampler. In operational and investigative monitoring as well as in the reference network also samples at the chlorophyll a peaks, as detected with a fluorometric probe. In situ Filtration of chlorophyll a samples, and subsequent Preservation with acid or alkaline Lugol iodine solution. Others parameters to be measured: In situ: Temperature, pH, conductivity, dissolved oxygen, fluorimetric profile of chlorophyll a In laboratory: TP, TN, PO43-, NH4+, NO3-, Total alkalinity

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Hydrographic bottle, integral sampler or Hose-integrated sample

**2.06 Sampled/surveyed habitat:** Single habitat(s)

The euphotic zone in the deepest site at aprox. 200 m from the dam.

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** about July and about September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Twice during the summer estratification period

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

none

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

n.a.

### **Sample processing**

**2.12 Minimum size of organisms sampled and processed:**

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

Species whenever is possible

**2.15 Record of abundance:** Individual counts

**in relation to** Volume

**Unit** mm<sup>3</sup>/m<sup>3</sup>

**2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

## **3. Data evaluation**

### **Evaluation**

**3.01 List of biological metrics**

Biomass: Chlorophyll a, Biovolume  
Composition: % Cyanobacteria (Chroococcales species should be excluded except Microcystis and Woronichinia) I. Catalan (IGA)  $IGA = [1 + 0.1Cr + Cc + 2(Dc + Chc) + 3Vc + 4Cia] / [1 + 2(D + Cnc) + Chnc + Dnc]$   
IGA - Group of algae composition index  
Cr - Cryptomonads  
Cc - Colonial Chrysophyte  
Dc - Colonial Diatoms  
Chc - Colonial Chlorococcales  
Vc - Colonial Volvocales  
Cia - Cyanobacteria  
D - Dinoflagellates  
Cnc - Chrysophyte not colonial  
Chnc - Chlorococcales not colonial  
Dnc - Diatoms not colonial

**3.02 Does the metric selection differ between types of water bodies?**

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

**3.05 Scope of reference conditions:**

**3.06 Key source(s) to derive reference conditions:**

n.a.

**3.07 Reference site characterisation**

**Number of sites:**

**Geographical coverage:**

**Location of sites:** n.a.

**Data time period:**

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise

**3.11 Boundary setting procedure**

The approach adopted by the L-M GIG was to set the G/M boundary value as a percentile of the distribution of the data collected for each index, namely 95th percentile for the biomass metrics and 90th percentile for the composition metrics. This assumption is based on the fact that nearly all the reservoirs sampled for the IC exercise were those firstly proposed for the IC site register, as a preliminary required step in the IC process. It is to be remembered that according to WFD Annex V.1.4.1, this site register is intended to form the IC network, representing the high/good and good/moderate class boundaries. The G/M boundary values of the four biological indices were calculated for each type: Siliceous Wet and Calcareous. A validation of these results was made with data of Spanish reservoirs along the whole gradient of pressures and to identify the behaviour of some groups of algae in relation to eutrophication process. This approach allows analyzing whether the narrow range of G/M boundary values according to the IC sites corresponds with the changes in taxonomic composition as described in the conceptual model of the WFD normative definitions.

**3.12 "Good status" community:** To be reported at a later date.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 136

BQI

## 1. General information

- 1.01 GIG:** Northern  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Finland
- 1.05 Specification:** none
- 1.06 Method name:** *Benthic Quality Index*
- 1.07 Original name:** *Pohjanlaatuindeksi*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication

***Has the pressure-impact-relationship been tested?***

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

The index is tested against total P with many different data sets. In general, there has been statistically significant ( $p < 0.05$ ) relationship, but the amount of explained variation has been rather low (25 -35 %).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Pintavesien ekologisen luokittelun vertailuolot ja luokan määrittäminen. Finnish Environment Institute, Finnish Game and Fisheries Research Institute 2009.

**1.12 Scientific literature:**

Jyväsjärvi, J., J. Nyblom & H. Hämäläinen, 2009. Palaeolimnological validation of estimated reference values for a lake profundal macroinvertebrate metric (Benthic Quality Index). *Journal of Paleolimnology* (in press).  
Wiederholm, T., 1980. Use of benthos in lake monitoring. *J. Wat. Pollut. Cont. Fed.* 52: 537–547.

**1.13 Method developed by**

Jussi Jyväsjärvi  
jussi.jyvasjarvi@jyu.fi  
University of Jyväskylä

**1.14 Method reported by**

Heikki Mykrä  
heikki.mykra@ymparisto.fi  
Finnish Environment Institute (SYKE)

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Hellsten, H., M. Järvinen, S.M. Karjalainen, K. Meissner, H. Mykrä & K.-M. Vuori, 2009. Jokien ja järvien biologinen seuranta: näytteenotosta tiedon tallentamiseen. Finnish Environment Institute.

**2.02 Short description**

Six replicate samples are taken from the deepest point of lake.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge, Stratified sampling/surveying

**2.04 Sampling/survey device:** Grab

**2.05 Specification:** Ekman

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
Profundal

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** September to October

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Six replicates

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

surface area = 250–300 cm<sup>2</sup> per Ekman-grab sample

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 500 µm

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts  
in relation to Area

Unit Density / square-meter

- 2.16 Quantification of biomass:** n.a.  
**2.17 Other biological data:** none  
**2.18 Special cases, exceptions, additions:** none  
**2.19 Comments:** none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Site-specific prediction of expected value of Benthic Quality Index with linear regression using lake mean depth or log(sampling depth) as predictor variable.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**  
**Number of sites:** 80  
**Geographical coverage:** Whole Finland  
**Location of sites:** n.a.  
**Data time period:** Data has been collected between 1992 and 2006  
**Criteria:**  
No point source pollution, percentage of agriculture within catchment less than 15 %.
- 3.08 Reference community description**  
No description of reference communities, only abiotic issues are considered in reference lake selection.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
Boundaries are derived as follows: H/G = 0.75, G/M = 0.60, M/P = 0.30, and P/B = 0.10
- 3.11 Boundary setting procedure**  
Pressure relationships has not been used in setting the class boundaries.
- 3.12 "Good status" community:** Only EQRs are used to define ecological quality classes.

#### Uncertainty

- 3.13 Consideration of uncertainty:** Yes  
Different type-specific and site-specific approaches has been tested for prediction of expected values for BQI. Best performing approach (currently used) was selected based on its precision and performance in detection of impairment.
- 3.14 Comments:** none

ID: 105

IE-BI-LA

## 1. General information

- 1.01 GIG:** Northern  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Ireland
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment of littoral macroinvertebrates in lakes*
- 1.07 Original name:** *Assessment of littoral macroinvertebrates in lakes*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Acidification, Eutrophication
- Has the pressure-impact-relationship been tested?**  
0  
method currently under development
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
method currently under development  
  
method currently under development
- 1.14 Method reported by**  
Ruth Little  
r.little@epa.ie  
Environmental Protection Agency, Ireland
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
CEN (European Committee for Standardization), 1994. Water –quality. Methods of biological sampling – Guidance on hand net sampling of aquatic benthic macroinvertebrates. EN 27828: 1994 E.
- 2.02 Short description**  
A single exposed stony shoreline is selected in each lake from which samples are collected in water no deeper than 0.5m using a 25 cm square pond net with a 670 mm mesh net.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** 25 cm square pond net with a 670 micron mesh net
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
exposed stony lake shorelines
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** April
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
One
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
2 minute kick sample

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 670 microns
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
Most insecta, Crustacea, hirudinea, Mollusca to species level. ☐Oligochaetes, Hydracarina, Chironomids, Ceratopogonidae, other Diptera. Gammarus sp. identified as such.
- 2.15 Record of abundance:** Individual counts  
**in relation to** Time

Unit number of individuals per sample

2.16 Quantification of biomass: n.a.

2.17 Other biological data: none

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

3.01 List of biological metrics  
method currently under development

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: Not relevant

3.04 From which biological data are the metrics calculated?  
Data from single sampling/survey occasion in time

#### Reference conditions

3.05 Scope of reference conditions: Surface water type-specific

3.06 Key source(s) to derive reference conditions:  
Existing near-natural reference sites, Expert knowledge  
paleolimnology confirmed reference sites

3.07 Reference site characterisation  
Number of sites: currently under development  
Geographical coverage: Ecoregion 17  
Location of sites: Ecoregion 17  
Data time period: currently under development  
Criteria:  
currently under development

3.08 Reference community description  
currently under development

3.09 Results expressed as EQR? n.a.

#### Boundary setting

3.10 Setting of ecological status boundaries: n.a.  
currently under development

3.11 Boundary setting procedure  
currently under development

3.12 "Good status" community: Currently under development.

#### Uncertainty

3.13 Consideration of uncertainty: n.a.

3.14 Comments:  
none

ID: 7

LAMM

## 1. General information

- 1.01 GIG:** Northern  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** United Kingdom
- 1.05 Specification:** Areas at risk from acidification
- 1.06 Method name:** *Lake Acidification Macroinvertebrate Metric*
- 1.07 Original name:** *Lake Acidification Macroinvertebrate Metric*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Acidification

***Has the pressure-impact-relationship been tested?***

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Macroinvertebrate kick samples taken in spring from 49 clear-water lakes (< 5mg/l DOC) and 35 humic-water lakes (=> 5 mg/l DOC) and matched to chemistry from the preceding year. A minimum of two chemical samples had to be available. Only sites with mean ANC <150 ueq/l and mean Ca < 5mg/l, were included. Linear regression of LAMM v Cantrell ANC at clear-water lakes resulted in R-square =0.64, P = <0.001. Linear regression of LAMM v Cantrell ANC at humic-water lakes resulted in R-square =0.82, P = <0.001.

- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/lakes\\_invertebrates](http://www.wfduk.org/bio_assessment/bio_assessment/lakes_invertebrates)
- 1.11 Pertinent literature of mandatory character:**  
UKTAG Lake Assessment Methods. Benthic Invertebrate Fauna. Lake Acidification Macroinvertebrate Metric (LAMM). Water Framework Directive. [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/lakes\\_invertebrates](http://www.wfduk.org/bio_assessment/bio_assessment/lakes_invertebrates)
- 1.12 Scientific literature:**  
McFarland, B.P., F. Carse & L. Sandin, 2009. Littoral macroinvertebrates as indicators of lake acidification within the UK. Aquatic Conservation. Pre-print.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Ben McFarland<br>ben.mcfarland@environment-agency.gov.uk<br>Environment Agency | <b>1.14 Method reported by</b><br>Ben McFarland<br>ben.mcfarland@environment-agency.gov.uk<br>Environment Agency, England & Wales |
|---|---|
- 1.15 Comments**  
McFarland et al (2009) currently on online as Early View only. Due to go into next volume.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Sampling guidelines are outlined in UKTAG Lake Assessment Methods. Benthic Invertebrate Fauna. Lake Acidification Macroinvertebrate Metric (LAMM). Water Framework Directive - UK Advisory Group.
- 2.02 Short description**  
To apply the method, invertebrates should be collected from a stony-bottomed section of the littoral zone of the lake with a depth of ≤ 75 cm. Two samples should be collected from each location sampled. Sampling should normally be undertaken between March and May. The invertebrates should be collected by disturbing the substratum with the feet ("kick sampling") and passing a hand net (nominal mesh size: 1 mm) through the water above the disturbed area. All habitats in the chosen sampling site should be sampled within a 3-minute period. In addition, a pre-sample sweep to collect surface dwelling invertebrates and a post sample manual search, lasting one minute, should be undertaken during which any invertebrates attached to submerged plant stems, stones, logs or other solid surfaces should be collected by hand and placed in the net. The sampling method used should comply with BS EN 27828:1994, ISO 7828-1985 Water quality. Methods for biological testing. Methods of biological sampling: guidance on handnet sampling of aquatic benthic macro-invertebrates.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Hand net
- 2.05 Specification:** pond net
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Stony littoral zone
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** Spring (March-June)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
2 samples are taken in each spring survey. One spring survey is enough for classification. However, 3 years worth of data will
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

from 2 \* 3 minute kick samples in one year to 2 \* 3 minute kick samples from 3 years (subject to resources)

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:** 1mm

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Family, Genus, Species/species groups

Ephemeroptera - species  
Plecoptera - species  
Trichoptera - species  
Gastropoda - species  
Leeches - species  
Bivalvia - genus  
Diptera - family  
Coleoptera - species

**2.15 Record of abundance:** Individual counts

Individual counts but >50 individuals of a taxa, then this is by estimation.

**in relation to** Time

**Unit** Percentage contribution towards all scoring taxa (<5%, 5-20%, >20%)

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

**3. Data evaluation**

**Evaluation**

**3.01 List of biological metrics**

The observed value of the parameter, LAMM, should be calculated using the equation:  $\frac{\sum \text{Shk} * \text{Whk} * \text{Hhk}}{\sum \text{Whk} * \text{Hhk}}$  where: "Shk" is the acid sensitivity score for taxon "k" "Whk" is the corresponding indicator weighting score for taxon "k" "Hhk" is the relative abundance score.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

**Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge, Historical data, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** 8 sites for clear-water lakes, 6 for humic-water lakes  
**Geographical coverage:** Representative lakes throughout UK at risk from acidification  
**Location of sites:** Representative lakes throughout UK at risk from acidification  
**Data time period:** Historical data from 2005-2008

**Criteria:**

Reference sites screened using the Damage matrix. See table 6.1 in 'Macroinvertebrate Classification Diagnostic Tool Development' SNIFFER Report WFD60. This matrix assesses sites based on their Acid Neutralising Capacity (ANC) in relation to Ca content.

**3.08 Reference community description**

Reference community characterised by high abundances of highly sensitive Ephemeroptera. Presence of sensitive species of Trichoptera and Plecoptera. Often species of gastropod, leeches and sensitive Coleoptera present.

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** Using discontinuities in the relationship of anthropogenic pressure and the biological response.  
Where discontinuities could not be found then partitioning based on the Damage Matrix was used.

### 3.11 Boundary setting procedure

Distinct discontinuities along the ANC pressure gradient were only found at humic sites at ANC 23 ueq/l, to derive a good-moderate boundary. These were consistent using pressure metrics (e.g. LAMM), diversity measures (e.g. Shannon) and functional groups (e.g. grazers). Where there were no consistent breakpoints/step-changes were found, sites were grouped by the damage matrix according to class. The mean LAMM scores of the two adjacent classes were then added together and divided by two to form the boundary.

**3.12 "Good status" community:** Expected to be lower abundances of some HS taxa. Lower end of good status classes tend to have higher number of tolerant species as a percentage contribution to the metric.

### Uncertainty

**3.13 Consideration of uncertainty:** Yes

The approach to uncertainty assessment assumes that the estimated mean LAMM EQR is normally distributed with a standard deviation that is a modelled function of EQR. Using the estimated standard deviation and number of samples collected we determine the confidence that the observed mean EQR lies within particular class boundaries. The approach follows that of Ellis (1990) (available at <http://publications.environment-agency.gov.uk/epages/eapublications.storefront/4b100774024a67a6273fc0a802960648/Product/View/GEHO1006BLOR&2DE&2DE>) and has been used for the majority of the UK classification methods.

**3.14 Comments:**

none

ID: 80

EQR4

## 1. General information

- 1.01 GIG:** Northern  
Not yet defined; pilot study for IC in phase two is going on in Northern-GIG (L-N-F)
- 1.02 Category:** Lakes
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Finland
- 1.05 Specification:** applied in Ecoregion 22 (Fennoscandian Shield) covering > 95% of the area
- 1.06 Method name:** *Finnish Lake Fish Classification Index*
- 1.07 Original name:** *Suomen järvien kalastoindeksi*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, General degradation
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
The response of the Finnish Lake Fish Classification Index (EQR4) along the eutrophication gradient was examined by relating the EQR4 values from 248 lakes to the total phosphorus concentrations of the lakes. The correlation between the EQR4 and TP was 0.56 ( $p < 0.001$ )
- 1.10 Internet reference:** [http://www.rktl.fi/kala/vesipuitedirektiivin\\_kalastotutkimukset/vesien\\_ekologisen\\_tilan\\_luokittelu](http://www.rktl.fi/kala/vesipuitedirektiivin_kalastotutkimukset/vesien_ekologisen_tilan_luokittelu) (includes only the list of metrics)
- 1.11 Pertinent literature of mandatory character:**  
Sutela, T., M. Olin, T. Vehanen & M. Rask, 2007. Hajakuormituksen vaikutukset järvien ja jokien kalastoon ja ekologiseen tilaan. Riista- ja kalatalouden tutkimuslaitos. Kala- ja riistaraportteja 411: 35 p. Tammi, J., M. Rask & M. Olin, 2006. Kalayhteisöt järvien ekologisen tilan arvioinnissa ja seurannassa- Alustavan luokittelujärjestelmän perusteet. Riista- ja kalatalouden tutkimuslaitos. Kala- ja riistaraportteja 383: 51 p.
- 1.12 Scientific literature:**  
Rask, M., M. Olin & J. Ruuhijärvi, 2009. Fish based assessment of ecological status of Finnish lakes loaded by diffuse nutrient pollution from agriculture. Fisheries Management and Ecology (pre-print). Sairanen, S., M. Rask, S. Stridsman & K. Holmgren, 2008. Fish communities of 15 lakes in River Torne basin: aspects of lake typology and ecological status. In Luokkanen, E., P. Olofsson, V. Hokka & B. Sundström (eds), TRIWA II – Management of an international river basin district – Torne River. The Finnish Environment 10/2008: 64-88.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Mikko Olin<br>mikko.olin@helsinki.fi<br>Department of Biological and Environmental Sciences, University of Helsinki | <b>1.14 Method reported by</b><br>Martti Rask<br>martti.rask@rktl.fi<br>Finnish Game and Fisheries Research Institute |
|--|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Olin, M., M. Rask, J. Ruuhijärvi, M. Kurkilahti, P. Ala-Opas & O. Ylönen, 2002. Fish community structure in meso- and eutrophic lakes of southern Finland: the relative abundances of percids and cyprinids along a trophic gradient. J. Fish Biol. 60: 593-612. Water quality. Sampling fish with multi-mesh gillnets. CEN standard 14757: 2005. 27 p.
- 2.02 Short description**  
Fish community data from the lakes is collected during mid June - early September using NORDIC multimesh survey nets (1.5 × 30 m; 12 panels with mesh size 5 to 55 mm from knot to knot; European Standard EN 14757:2005). Stratified random sampling with respect to lake area and depth relations is applied. The number of unit efforts per lake is 6 to 68 net nights from ca. 8 pm to 8 am, according to size and depth relations of the lakes. Depth zones are < 3m, 3-10 m, 10-20 m, > 20 m.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying, Stratified sampling/s
- 2.04 Sampling/survey device:** Gill net
- 2.05 Specification:** Nordic multimesh surveynets (CEN 14757:2005)
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** Mid July to early September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
1-8 sampling nights per sampling season (depending on the lake size and depth)
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
5 to 68 gillnet nights, depending on lake size and depth
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Lake area 0.01-10 km<sup>2</sup>, in larger lakes a representative sub area up to 10 km<sup>2</sup> is selected by expert judgement. Duration 1-8

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** The smallest mesh in the Nordic survey net is 5 mm - smallest fish are 40 mm, 0+ fish
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
     **in relation to** n.a.  
     Relative abundance: n / gillnet night and g / gillnet night  
     **Unit** Number or weight of fish species per gillnet night (or n or g per 100 m<sup>2</sup> of gillnet per night), each mesh size can be expressed separately
- 2.16 Quantification of biomass:** n.a.  
     Total biomass as g of fish per gill net, each species separately, each mesh size separately
- 2.17 Other biological data:** Length of individual fish at 1 cm interval for length distribution
- 2.18 Special cases, exceptions, additions:** In the case of high catches, a subsample of 30 individuals of a fish species is measured from a net panel for length distribution
- 2.19 Comments**  
     none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
     The metrics of the Finnish Lake Fish Classification index EQR4 are 1) total biomass of fish per gillnet night (BPUE), 2) total number of fish individuals per gillnet night (NPUE), 3) biomass proportion of cyprinid fish and 4) occurrence of indicator species.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
     Median metric scores
- 3.04 From which biological data are the metrics calculated?**  
     Aggregated data from multiple sampling/survey occasions in time  
     Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
     Existing near-natural reference sites, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
     **Number of sites:** 125  
     **Geographical coverage:** Fairly evenly throughout the country  
     **Location of sites:** Reference sites found in Finnish River Basin Districts 1-7  
     **Data time period:** 1995-2007  
     **Criteria:**  
     The main criteria of reference sites is the lack or minor presence of human induced environmental pressures. This was ascertained by using lake type specific threshold values of main nutrients (P<sub>tot</sub>, N<sub>tot</sub>) for reference conditions obtained from a water quality database covering a period of over 30 years (Finnish Environment Institute 2009), land use information, including Corine land cover, and nutrient load model calculations obtained from the Finnish Environment Institute.
- 3.08 Reference community description**  
     Natural fish communities from reference lakes, usually 1 to 10 species per lake. The most common species of Finnish lakes, perch (*Perca fluviatilis*), pike (*Esox lucius*), roach (*Rutilus rutilus*), ruffe (*Gymnocephalus cernuus*), almost always present and, additionally, some of the sensitive indicator species with higher environmental quality demands, for example vendace (*Coregonus albula*) and burbot (*Lota lota*)
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
     High-good boundary derived from metric variability at near-natural reference sites

### 3.11 Boundary setting procedure

Median values of the fish parameters BPUE, NPUE and cyprinid proportion in type specific reference lakes were used as reference values for the scores of ecological quality ratio (EQR = reference / observed value). High/Good (H/G) class boundary was set to the 25th percentile of the EQR-distribution of fish parameters in reference lakes of each lake type. The range of EQR values of a lake type from the H/G class boundary to the extreme EQR recorded, for example the one found in a eutrophicated lake with highest BPUE value, was then divided into even distances for determination of the other class boundaries. The fourth metric, indicator species, was not based on reference / observed relation but on the occurrence of certain sensitive species with high environmental quality demands or on the normality of the population structure of perch, pike and roach.

**3.12 "Good status" community:** Good status fish communities in Finnish lakes are close to those in reference conditions including the possible occurrence of sensitive indicator species. The main deviation from high status may be slight increases in the biomass and abundance of fish and in the biomass proportion of cyprinid fishes as early symptoms of eutrophication/general degradation towards the good-moderate boundary.

### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 71

EQR8

## 1. General information

- 1.01 GIG:** Northern  
Not decided yet
- 1.02 Category:** Lakes
- 1.03 BQE:** Fish Fauna
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment criteria for ecological status of fish in Swedish lakes*
- 1.07 Original name:** *Bedömningsgrunder för fiskfaunans status i sjöar*
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** Acidification, Eutrophication, General degradation

**Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

Pressure and impact metrics used as reference criteria for high plus good status lakes: acidity (pH > 6), nutrients (total phosphorous < 20 µg/l) and land use (agriculture < 25 % and built-up area < 1 % of the catchment). Reference group: 116 high plus good status lakes. Impacted group: 168 lakes not passing the reference filter plus 224 limed (previously acidified) lakes. Metrics selected for the multimetric index responded significantly (t-tests) to either acidity or nutrients/landuse. The performance of the multimetric index was tested using two-ways ANOVA, showing significant effect of impact as well as the interaction between impact and liming. The index value was significantly lower in acidic lakes than in reference lakes, and in lakes impacted by nutrients/landuse compared to reference lakes.

- 1.10 Internet reference:** [https://www.fiskeriverket.se/download/18.88bd54c111926b5289800545/Finfo+2007\\_3.pdf](https://www.fiskeriverket.se/download/18.88bd54c111926b5289800545/Finfo+2007_3.pdf)
- 1.11 Pertinent literature of mandatory character:**  
Naturvårdsverket, 2007. Status, potential och kvalitetskrav för sjöar, vattendrag, kustvatten och vatten i övergångszon. En handbok om hur kvalitetskrav i ytvattenförekomster kan bestämmas och följas upp. Naturvårdsverket Handbok 2007: 4, Utgåva 1. ISSN 1404-8590. Naturvårdsverket, 2008. Naturvårdsverkets föreskrifter och allmänna råd om klassificering och miljökvalitetsnormer avseende ytvatten. NFS 2008:1. ISSN-1403-8234.
- 1.12 Scientific literature:**  
Holmgren, K., A. Kinnerbäck, S. Pakkasmaa, B. Bergquist & U. Beier, 2007. Assessment criteria for ecological status of fish in Swedish lakes – development and application of EQR8 (in Swedish with English Summary). Fiskeriverket Informerar 2007: 3-54 pp.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Kerstin Holmgren, Anders Kinnerbäck, Susanna Pakkasmaa,<br>Björn Bergquist & Ulrika Beier<br>kerstin.holmgren@fiskeriverket.se<br>Swedish Board of Fisheries, Institute of Freshwater Research | <b>1.14 Method reported by</b><br>Kerstin Holmgren<br>kerstin.holmgren@fiskeriverket.se<br>Swedish Board of Fisheries, Institute of Freshwater Research |
|---|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
CEN, 2005. Water quality - Sampling of fish with multi-mesh gillnets. EN 14757:2005:E, 27 p.
- 2.02 Short description**  
The standard effort of gillnets is set during one to several consecutive nights. The site of each net is recorded, along with minimum and maximum depth at the site, on a map of the lake. The positions are sometimes recorded by GPS. Weather conditions are briefly described, and Secchi disc depth and a water temperature profile is recorded at least once during the field sampling campaign. The catch within each gillnet is registered as number of individuals and total weight of each species. The total length of each individual is additionally registered (tracked to individual gillnets).
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** Gill net
- 2.05 Specification:** Benthic and pelagic gillnets as specified in EN 14757
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** Late summer (usually between July 15 and August 31), when deep lakes are thermally stratified
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
8-68 benthic gillnets, depending on lake area and maximum depth
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Standard effort of benthic gillnets (see B-11) set for 12 hours (+/- 1 hour) including dusk and dawn.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Fish caught by the smallest mesh size (5 mm, knot to knot), down to about 3 cm total length for some fish species
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Family, Species/species groups  
An exception from species/species groups levels is if possible hybrids of cyprinids are identified at the family level.
- 2.15 Record of abundance:** Individual counts  
**in relation to** n.a.  
Number or area of gillnets and 12 hours fishing  
**Unit** Number per gillnet (or gillnet area) and 12 hours fishing
- 2.16 Quantification of biomass:** n.a.  
Biomass of all fish caught by the standard effort
- 2.17 Other biological data:** Length of individual specimens
- 2.18 Special cases, exceptions, additions:** Delimited subareas have sometimes been used for fish sampling in very large lakes. Then the stratified random sampling is applied within the subarea instead of the whole lake. So far the Swedish assessment criteria for ecological status have, however, not been adapted for use in subareas of large lakes.
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

#### **3.01 List of biological metrics**

1) Number of native fish species. 2) Simpson's Dn (diversity index based on number of individuals): calculated as  $1 / (\sum Pi^2)$ , where Pi = numerical proportion of species i, and the sum is taken for all species in the catch. 3) Simpson's Dw (diversity index based on biomass): calculated as  $1 / (\sum Pi^2)$ , where Pi = biomass proportion of species i, and the sum is taken for all species in the catch. 4) Relative biomass of native fish species: total biomass (g) of all native species, divided by number of nets. 5) Relative abundance of native fish species: total number of individuals of all native species, divided by number of nets. 6) Mean mass: biomass of all species (g) divided by the number of individuals. 7) Proportion of piscivorous percids (based on biomass in the total catch): The proportion of potentially piscivorous perch is 0 at fish length less than 120 mm and 1 at length above 180 mm. At intermediate length the proportion is calculated as  $1 - ((180 - \text{length}) / 60)$ . Individual mass of perch (g) is estimated as  $a \cdot \text{length (mm)}^b$ , where  $a = 3.377 \cdot 10^{-6}$ , and  $b = 3.205$ . Each individual mass is multiplied with the length-specific proportion piscivorous perch. The sum of the products is the biomass of piscivorous perch, which is then added to any biomass of pikeperch. Finally, the total sum of piscivorous percids is divided by the total biomass of all species in the catch. 8) Ratio perch / cyprinids (based on biomass): total biomass of perch divided with total biomass of all native cyprinids.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

### **Reference conditions**

**3.05 Scope of reference conditions:** Site-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Least Disturbed Conditions, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** 116 high plus good status lakes (see A-13)

**Geographical coverage:** All parts of Sweden

**Location of sites:** Covering the following range of environmental factors: altitude 10 – 894 m above sea level, lake area 2 – 4236 ha, maximum depth 1 – 65 m, annual mean in air temperature -2 – 8 °C.

**Data time period:** Fish data were extracted from the National Register of Survey Test-fishing in 2005, and the latest date of standardised sampling was used for each lake.

**Criteria:**

Criteria for high plus good status sites as in A-13. Fish metrics at reference sites are expected to have low deviation from site-specific reference values, rendering a high value of the multimetric index EQR8. Procedure: Step 1) Transformation of some environmental factors: The altitude is transformed as  $\log_{10}(x+1)$ , and  $\log_{10}(x)$  is used for lake area and maximum depth. Step 2) Estimation of reference values: Use linear regression models,  $Y = a + b_1 \cdot X_1 + \dots + b_n \cdot X_n$ , where  $a$  is intercept and  $b_1 - b_n$  are specified coefficients of regression for environmental factors ( $X_1 - X_n$ ). Step 3) Transformation of some observed metric values: Metrics 4-5 are transformed as  $\log_{10}(x+1)$  and  $\log_{10}(x)$  is used for metrics 6 and 8. Step 4) Calculation of deviations from reference values (residuals): The residual of each metric is calculated as observed (or transformed) value minus reference value. Step 5) Calculation of standardised residuals (Z-values): Transformation to Z-values is achieved by division with the metric-specific standard deviation (SD) of residuals (SDresid) in the reference data set. Step 6) Transformation to probabilities (P-values) in the distribution of reference values: Get a two-tailed P-value for each Z-value, by using any statistical software (e.g., SPSS where  $P = 2 \cdot \text{CDF.NORMAL}(-\text{ABS}(Z\text{-value}), 0, 1)$ ). Step 7) Calculation of the combined fish index: Calculate EQR8 as mean value of P-values for the 3-8 metrics that can be calculated from a given catch.

### 3.08 Reference community description

Not explicitly described, but dependent on environmental factors used for modelling reference values of eight fish metrics (see C-01), i.e., altitude, lake area, maximum depth, annual mean in air temperature, and location below (0) or above (1) the highest coast line after deglaciation (HC).

### 3.09 Results expressed as EQR? Yes

## **Boundary setting**

### 3.10 Setting of ecological status boundaries: n.a.

According to procedures used in the FAME project, for development of a European fish index for rivers

### 3.11 Boundary setting procedure

The good-moderate boundary was set at the EQR8 value which minimised the risk for type I and type II errors, i.e. at minimised probability of misclassification of 116 reference (high plus good status) as well as 113 impacted lakes (moderate - bad status). The high-good boundary was conservatively set at the 95th percentile of EQR values in reference lakes, and the poor-bad boundary at the 10th percentile of EQR values in impacted lakes. The moderate-poor boundary was more arbitrarily set at the mean of EQR values at good-moderate and moderate-poor boundaries.

### 3.12 "Good status" community: Not explicitly described, but dependent on environmental factors used for modelling reference values of eight fish metrics (see C-01), i.e. altitude, lake area, maximum depth, annual mean in air temperature, and location below (0) or above (1) the highest coast line after deglaciation (HC).

## **Uncertainty**

### 3.13 Consideration of uncertainty: Yes

A general measure of uncertainty is recommended when assessment is based on only one sampling occasion. The general uncertainty measure was set as the median standard deviation of the EQR8 value in a dataset of 113 lakes with at least 3 years of data.

### 3.14 Comments:

none

ID: 200

FINLAKMAC

## 1. General information

- 1.01 GIG:** Northern
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Finland
- 1.05 Specification:**
- 1.06 Method name:** *Lake ecological status assessment method by aquatic macrophytes*
- 1.07 Original name:** *Järvien ekologinen luokittelu vesikasvien avulla*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** General degradation

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Finnish dataset consists of 117 reference and 131 impacted lakes. In impacted clear water lakes relationship between totP and EQR is relatively good ( $R^2=0,518$ ), but so far current method is not working very well in small humic lakes which are isolated. However, method describes also relatively well hydromorphological pressure expressed by winter drawdown ( $R^2=0,576$ ).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Vuori, K.-M., S. Mitikka & H. Vuoristo (eds), 2009. Pintavesien ekologisen tilan luokittelu Osa I: Vertailuolot ja luokan määrittäminen Osa II: Ihmistoiminnan ympäristövaikutusten arvio. Suomen ympäristökeskuksen ohjeita. 88 p.

**1.12 Scientific literature:**

Alahuhta, J., K.-M. Vuori, S. Hellsten, M. Järvinen, M. Olin, M. Rask, A. Palomäki & K. Pekka Korhonen, 2009. Defining the ecological status of small forest lakes using multiple biological quality elements and paleolimnological analysis. *Fundamental and Applied Limnology* 175 (3): 203–216.

**1.13 Method developed by**

Seppo Hellsten  
Seppo.Hellsten@ymparisto.fi  
Finnish Environment Institute, Freshwater Centre, Monitoring and Assessment Unit

**1.14 Method reported by**

Seppo Hellsten  
Seppo.Hellsten@ymparisto.fi  
Finnish Environment Institute (SYKE) Freshwater Centre, Monitoring and Assessment Unit

**1.15 Comments**

Preliminary version of method was included in 1. intercalibration round. Correlation with common ICCM was weak although comparison by contingency tables gave very good results. It was decided to exclude from intercalibration.

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

At present Finnish method is following CEN-lake standard, it is described in detail in: Kuoppala, M., S. Hellsten & A. Kanninen, 2008. Sisävesien vesikasviseurantojen laadunvarmennus. Suomen ympäristö 36/ 2008. Note: classification system is largely based on older data with more diverse methodology.

**2.02 Short description**

The main belt-transect method is also recommended for lake monitoring consistent with the WFD [Leka et al. 2002, Leka and Kanninen 2003, Leka 2005]. Method is based on 5 m wide transect, where frequency and coverage (percent scale: 0,5, 1, 2, 3, 5, 7, 10, 15, 20, 30...90, 100) of all detected species estimated. Currently only one value per transect is applied and only depth limits between different belts are estimated. Method is currently applied very widely and also annual training courses are organised since 2006 (Kuoppala et al. 2008).

**2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying

**2.04 Sampling/survey device:** Rake  
Main belt transect method is used

**2.05 Specification:** Main belt transect method is used, there are several sampling devices depending on depth zone

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** According to QA guidance from July to mid September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Only once

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Number of transects are depending on lake size starting from 8 transects (0.5 km<sup>2</sup>) to 25 transects (large lakes or water body unit). 2/3 of transects are situated at representative sites (not too open nor sheltered), 1/3 of transects are on sheltered shallow sites. Restored sites are not monitored.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Main belt transect is 5 meters wide and run from uppermost littoral to deepest growing zone. Depending on number of transects, which are depending on size of lake and secchi depth

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Both hydrophytes and helophytes.
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Percent coverage  
**in relation to** Area  
**Unit** Both abundance and frequency are estimated at whole transect level. One value per transect.
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
Some of the questions are not very relevant for macrophytes.

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Finnish classification system for lake macrophytes was developed to take into account multiple pressures and humic rich waters. It is based on a multimetric index consisting of three metrics (Proportion of type specific taxa PTST, Percent Model Affinity PMA and Reference Index RI) supposed to meet the normative definitions of WFD
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
Median metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**  
**Number of sites:** 177 lakes  
**Geographical coverage:** Covering whole Finland  
**Location of sites:** See above,  
**Data time period:** 1970-2004  
**Criteria:**  
Reference lakes have been selected mostly based on pressure criteria. The main pressure criteria are: < 10% agriculture (in total catchment area), and no major point sources, mainly judged from visual observation of GIS land-use and population data. Further experts from local environmental centre were used in final determination.
- 3.08 Reference community description**  
Lake type specific description, e.g. in low alkalinity clear water lakes isoetids are typical.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** n.a.  
High-Good boundary is lower quartile of reference lakes metrics, other boundaries are evenly divided.
- 3.11 Boundary setting procedure**  
High-Good boundary is lower quartile of reference lakes metrics, other boundaries are evenly divided.
- 3.12 "Good status" community:** It is not described.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

Currently we are working uncertainty problem and developing reference conditions for our naturally eutrophic small lakes where reference conditions are difficult to find.

ID: 13

FMI

## 1. General information

- 1.01 GIG:** Northern  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Macrophytes
- 1.04 Country:** Ireland
- 1.05 Specification:** none
- 1.06 Method name:** *Free Macrophyte Index*
- 1.07 Original name:** *Free Macrophyte Index*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication

***Has the pressure-impact-relationship been tested?***

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Yes, with eutrophication pressure using the FMI. The  $r^2$  between the FMI and log transformed TP was 0.59 ( $p = 0.0001$ ,  $n = 93$ ). See Figures 5.16 and 5.17 in Free et al (2006). The metric was developed using data for lakes > 0.4 meq l<sup>-1</sup> Alkalinity.

- 1.10 Internet reference:** [https://www.epa.ie/downloads/pubs/research/water/Final%20Report%20\(2000-FS1-M1\).pdf](https://www.epa.ie/downloads/pubs/research/water/Final%20Report%20(2000-FS1-M1).pdf) pages 86 to 95

- 1.11 Pertinent literature of mandatory character:**

[https://www.epa.ie/downloads/pubs/research/water/Final%20Report%20\(2000-FS1-M1\).pdf](https://www.epa.ie/downloads/pubs/research/water/Final%20Report%20(2000-FS1-M1).pdf)

- 1.12 Scientific literature:**

Free, G., R. Little, D. Tierney, K. Donnelly & R. Caroni, 2006. A reference based typology and ecological assessment system for Irish lakes. Preliminary investigations. EPA, Wexford, Ireland.

- 1.13 Method developed by**

Gary Free  
c.plant@epa.ie, g.free@epa.ie  
Environmental Protection Agency, McCumiskey House,  
Richview, Clonskeagh Road, Dublin 14, Ireland

- 1.14 Method reported by**

Caroline Plant  
c.plant@epa.ie  
Irish Environmental Protection Agency

- 1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**

Environmental Protection Agency Internal Standard Operating Procedure for the Sampling of Lake Macrophytes.

- 2.02 Short description**

The first transect position is chosen randomly after which transect sites should be evenly spaced around the lake perimeter. Macrophyte surveys should consist of a minimum of four transects per lake in order to adequately generate a representative list of species occurrences and abundances. The number of transects selected for sampling is determined by the size of the lake, the complexity of the shoreline i.e. the lake perimeter and the number of inlets and sheltered bays and the number of km grid squares intersected by the shoreline. Transects should not be located adjacent to inflows or outflows. Examination of a bathymetric map and satellite pictures may aid selection of transect locations. If a lake has been surveyed in the past for macrophytes then all attempts should be made to revisit these locations to aid examination of annual variation. At each transect location the boat is landed and a shoreline investigation (Point 0) of littoral macrophytes is carried out 10m either side of the transect starting location. This may be aided by the use of a bathyscope. The dominant substrate at the shoreline and in the overall transect are recorded. From the shoreline site a transect is followed along a compass heading approximately perpendicular to the shoreline. Samples are taken at 0, 2.5, 5.0, 7.5, 10, 25, 50, 75 and 100 m from the shoreline. At each position the anchor is dropped and the depth and grid reference are recorded. The depth is measured using a portable echo sounder. At each position the lake bottom is viewed with a bathyscope and four rake samples are taken. The DAFOR scale is applied on the basis of the occurrence of the collected species on all four rakes. Macrophytes are often found at the transect position that are not necessarily captured by the rake. Such plants may be better seen by the bathyscope and must also be recorded and given an appropriate DAFOR score. If the shoreline is fringed by reeds, the species comprising the reed bed and their distance inshore is recorded. The outer limit of the reed bed should be considered the start of the transect (i.e. Point 0), and the survey continues at 5m, 10m, 25m, 50m, 75m and 100m from the reed bed, where possible. Where the shore is very flooded or lake levels are unusually high, it will be necessary to use expert judgement to estimate the true shore-line (Point 0) by looking for typical shore plants and starting the survey at this point. If plants still occur at the 100m mark, the survey should continue until a maximum depth of colonisation has been recorded. This is obtained by travelling slowly beyond the 100m mark, in line with the transect, and continually taking depth readings with the portable eco-sounder. Where the depth changes by at least 0.5m, the rake is re-thrown and the depth, species present and the distance from shore should be noted. If no plants are found, the transect is deemed to be complete, and the maximum depth recorded on the transect is used as maximum depth of colonisation. If, however, plants are recorded, the survey proceeds further, until another depth change of at least 0.5m is recorded, and the survey continues as outlined.

- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** Rake  
Rake head is used as a grapnel
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** June to August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Minimum of four transects (replicates) at each lake ( minimum of 36 sampling positions)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Minimum of 400 metres per lake

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** n/a
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Some mosses and most algae are only identified to Genus level, all others to species level where possible.
- 2.15 Record of abundance:** Relative abundance  
DAFOR scale is applied  
**in relation to** n.a.  
The DAFOR scale is applied to macrophytes collected from a rake sample of lake bed.  
Unit DAFOR
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** Where surveys are carried out on small lakes or narrow bays, it is possible that a single 100m transect will stretch from one side of the lake/bay to the other. This situation is avoided where possible by relocating transects or facing the transect perpendicular to shore instead of horizontally across bays, however, in unavoidable cases, the survey will stop at the point where shore-line plants from the opposite shore begin to influence the transect. This may be accompanied by a sharp decrease in depth on approach to the opposite shore, and expert judgement is required to determine the exact stopping point.
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
(i) Maximum depth of colonisation (Zc);(ii) Mean depth of presence ;(iii) Percent relative frequency of Chara;(iv) Percent relative frequency of Elodeids;(v) Plant trophic index; and,(vi) Percent relative frequency of tolerant taxa. The result of applying each of the above metrics is awarded a score ranging from 1 to 0.1, descending with increasing TP concentration (See Table 5.14 Free et al 2006 for scores and further details). The scores are averaged to produce an index value.
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**

**Number of sites:** 46  
**Geographical coverage:** Lakes in 11 counties out of 26 in the Republic of Ireland, the majority on the Western side of the country  
**Location of sites:** Counties Galway, Donegal, Mayo, Kerry, Clare, Wicklow, Leitrim, Roscommon, West Meath, Sligo  
**Data time period:** Three years

**Criteria:**

Reference sites were selected by expert opinion and also for some lakes through palaeolimnological validation: Leira M, Jordan P, Taylor D, Dalton C, Bennion H, Rose N, Irvine K. 2006. Assessing the ecological status of candidate reference lakes in Ireland using palaeolimnology. *Journal of Applied Ecology* 43: 816–827.

**3.08 Reference community description**

A description of reference conditions is provided on pages 84-85 and Table 5.11 of Free et al (2006). Essentially indicator values were calculated using indicator species analysis (Dufrene and Legendre, 1997) for the most common taxa occurring in a set of reference lakes for 7 lake types. This provides a succinct statistical description of the affinity of taxa for a type in reference condition. A more general description would be: High alkalinity - Chara sp. Low alkalinity - rosette species such as Isoetes lacustris and Lobellia dortamann. Medium alkalinity - Nitella sp. Myriophyllum alterniflorum

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise Using discontinuities in the relationship of anthropogenic pressure and the biological response.

**3.11 Boundary setting procedure**

The good/moderate boundary was placed through intercalibration in accordance with the NGIG. The national position for boundary setting was based on points of ecological change along a pressure gradient. For example where the depth of colonisation of the Charophytes is reduced by 24% from reference condition or where diversity declines with eutrophication pressure. See Free et al (2006) p216-227 for initial work on this issue.

**3.12 "Good status" community:** At good status the maximum depth of colonisation is greater and the plant trophic score is lower than that of the community at moderate status. Work at national level has indicated that good status is characterised by an initial increase of diversity. See Figures 9.1, 9.2 and 9.3 of Free et al. (2006). For lakes of alkalinity 0.4-2 meq/l typically from good to moderate status you tend to get a lower occurrence of isoetid taxa, an increase in Nymphaeids and a decline in Carophytes.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

For high alkalinity lakes > 2 meq l<sup>-1</sup> the decline in Carophytes can occur earlier with an increase in Lemnids and filamentous algae.

ID: 201

MACROPH-LAKE

## 1. General information

**1.01 GIG:** Northern  
L-N1+L-N8a; L-N2a, 2b, 5; L-N3a, 6a; L-N3b(6b)

**1.02 Category:** Lakes

**1.03 BQE:** Macrophytes  
All species within the distribution range

**1.04 Country:** Sweden

**1.05 Specification:**

**1.06 Method name:** *Assessment system for lakes using macrophytes*

**1.07 Original name:** *Bedömningsgrunder för makrofyter i sjöar*

**1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)

**1.09 Detected pressure(s):** Eutrophication

*Has the pressure-impact-relationship been tested?*

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Macrophyte data and Tot-P data from >300 lakes were analyzed to establish a pressure-impact relationship between the macrophyte metric (trophic index) and Tot-P. The correlation (spearman rank correlation coefficient) was significant (>0.6) for all three national typology groups.

**1.10 Internet reference:** <http://www.naturvardsverket.se/Documents/publikationer/620-0148-3.pdf>

**1.11 Pertinent literature of mandatory character:**

Naturvårdsverket, 2008. Naturvårdsverkets föreskrifter och allmänna råd om klassificering och miljö kvalitetsnormer avseende ytvatten. Naturvårdsverkets författningssamling. NFS 2008:1. ISSN 1403-8234.

**1.12 Scientific literature:**  
n.a.

**1.13 Method developed by**

Frauke Ecke

Frauke.Ecke@vatten.slu.se

Department of Aquatic Sciences and Assessment, SLU

**1.14 Method reported by**

Frauke Ecke

Frauke.Ecke@vatten.slu.se

Department of Aquatic Sciences and Assessment, SLU

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

New guidelines are under development. The so far applied method did not follow any guidelines, but macrophytes were sampled at a presence/absence scale using a whole-lake survey.

**2.02 Short description**

Macrophytes are sampled at a presence/absence scale using a whole-lake survey by mainly raking from boat. The survey is stopped when all macrophyte species are recorded

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Dredge, Grapnel, Rake  
snorkling

**2.05 Specification:** Mostly applied: common garden rake

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** June to August, depends on the geographical position of the lakes

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Since whole lake survey and only presence/absence, N=1

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Impossible to assess

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** mosses

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Abundance classes  
Only presence/absence

in relation to n.a.

Not related to anything since only presence/absence

Unit -

2.16 Quantification of biomass: n.a.

2.17 Other biological data: none

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

Trophic Macrophyte index (TMI) = SUM of (Indicator value for species i \* Weighting factor for species i)/Sum of Weighting factors

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: Not relevant

##### 3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

#### Reference conditions

3.05 Scope of reference conditions: Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Historical data, Least Disturbed Conditions

##### 3.07 Reference site characterisation

Number of sites: 49

Geographical coverage: Entire country, however only few reference lakes from the Swedish mountain range

Location of sites: Sites are scattered all over Sweden

Data time period: 1926-2006. Only one point in time per lake

##### Criteria:

Proportion of clear-cuts within the lakes catchments <10%, that of agricultural land <10% and that of urban areas <0.1%. No lowering of water level. Tot-P <12.5 microgram, Tot-N <300 microgram, pH >6.0

##### 3.08 Reference community description

Reference lakes are generally characterized by the occurrence of one or several species of isoetids (Isoetes, Lobelia, Subularia etc).

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: High-good boundary derived from metric variability at near-natural reference sites Using discontinuities in the relationship of anthropogenic pressure and the biological response.

##### 3.11 Boundary setting procedure

Class boundaries (between good and poorer status) were determined with classification trees using Tot-P values of species typical for the different classes of ecological status. The species used for classification were those showing sudden drops in their occurrence beyond the 75% percentile. For the high/good boundary, the 5th percentile of the reference lakes was taken.

3.12 "Good status" community: At good status stands of the sensitive taxa (large isoetids, Littorella, Lobelia, Isoetes in low alkalinity lakes or Chara spp. in high alkalinity lakes) occur, but significantly decrease at good-moderate boundary ("sudden drop") and are replaced by tolerant taxa

#### Uncertainty

3.13 Consideration of uncertainty: No (to be done)

##### 3.14 Comments:

none

ID: 209

FINLAKPHY

## 1. General information

**1.01 GIG:** Northern chlorophyll-a has been intercalibrated, but not total biomass or %-cyanobacteria

**1.02 Category:** Lakes

**1.03 BQE:** Phytoplankton

**1.04 Country:** Finland

**1.05 Specification:**

**1.06 Method name:** *Lake ecological status assessment using phytoplankton*

**1.07 Original name:** *Järvien ekologinen luokittelu kasviplanktonin avulla*

**1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)

**1.09 Detected pressure(s):** Eutrophication

***Has the pressure-impact-relationship been tested?***

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Lepistö 1999 Monogr. Boreal Env. Res. 16; Lepistö et al. 2006 Boreal Env. Res. 11:35-44; Ptacnik et al. 2008 Aquat. Ecol. 42:227-236. The pressure-impact relationship well known and presented e.g. in the text books of limnology, i.e. the positive regression between chlorophyll-a and total-P, and phytoplankton biomass and total-P.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Vuori, K.-M., S. Mitikka & H. Vuoristo (eds), 2009. Pintavesien ekologisen tilan luokittelu. Osa I: Vertailuolot ja luokan määrittäminen Osa II: ihmistoiminnan ympäristövaikutusten arvio. Suomen ympäristökeskuksen ohjeita. 88 p.

**1.12 Scientific literature:**

Alahuhta, J., K.-M. Vuori, S. Hellsten, M. Järvinen, M. Olin, M. Rask, A. Palomäki & P.K. Korhonen, 2009. Defining the ecological status of small forest lakes using multiple biological quality elements and paleolimnological analysis. *Fundamental and Applied Limnology* 175 (3): 203-216.

**1.13 Method developed by**

Liisa Lepistö, Anna-Liisa Holopainen, Sari Mitikka & Marko Järvinen  
marko.jarvinen@ymparisto.fi  
Finnish Environment Institute (SYKE), Water Centre, Monitoring and Assessment Unit

**1.14 Method reported by**

Marko Järvinen  
marko.jarvinen@ymparisto.fi  
Finnish Environment Institute (SYKE), Water Centre, Monitoring and Assessment Unit

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Sampling guidelines of the regional environment centres. Certified staff to take the samples. Chlorophyll analysis and phytoplankton analysis according to CEN standards (for phytoplankton quantitative analysis CEN 15204). More about phytoplankton quality control presented in: Lepistö, L., K. Vuorio, A.-L. Holopainen, A. Palomäki, M. Järvinen & M. Huttunen, 2009. Quality control in phytoplankton analysis [Kasviplanktonin laadunvarmistus]. *Suomen ympäristö* 40. 31 pp.

**2.02 Short description**

For phytoplankton: samples are taken from the agreed stations using the Limnos or tube sampler at 0-2 m depth. 3-5 replicate samples are taken from different sides of the boat and integrated in the container into one sample. A darkened glass bottle of 200 ml with 0.5 ml of acid Lugol's iodine is filled with the sample water. For chlorophyll: sampling as above; water samples are taken into dark 1-2 L plastic bottles.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Limnos water sampler, Tube sampler (0-2 m)

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
open water, plankton

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** for chlorophyll: May to September; for biomass and species composition: mostly June to August

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

one or more (up to 12) sampling occasions every 1-3-6 years

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

typically 3-5 subsamples are integrated from each sampling station; in large water bodies >1 sampling stations

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

A pooled sample of 200 mL from 0-2 m depth, representing 3-5 subsamples with a total volume of >10 L of water

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** > 2 um (in phytoplankton microscopy); >0.5 um (for chlorophyll glass-fibre filters)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Phytoplankton quantitative analysis according to CEN15204 standard (Utermöhl method) and accredited in-house method. Different sized taxa/counting units counted using different microscope magnifications.
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
In the analysis the target identification level is the species level in all algal groups. As some taxa (in preserved samples) cannot be identified into species level, they are identified to the highest possible resolution (genus, order or class level), or in some cases remain unidentified.
- 2.15 Record of abundance:** Individual counts  
**in relation to** Volume  
**Unit** chlorophyll-a: ug chl-a /l; phytoplankton biomass mg/l; percentage of harmful cyanobacteria: % of total biomass
- 2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
chlorophyll-a concentration, total phytoplankton biomass, percentage of harmful cyanobacteria
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
Median metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** 172 lakes  
**Geographical coverage:** covering whole Finland  
**Location of sites:** covering whole Finland  
**Data time period:** chlorophyll-a: 1976-2006, May-Sept; phytoplankton biomass 1980-2006 June-Aug; cyano-% 1980-2006 July-Aug  
**Criteria:**  
Reference lakes have been selected mostly based on pressure criteria. The main pressure criteria are: <10% agriculture (of the total catchment area), and no major point sources, mainly judged from visual observation of the GIS land-use and population data. In addition, experts from the regional environment centres were used in final decision making.
- 3.08 Reference community description**  
low phytoplankton biomass/chl-a, low percentage of harmful cyanobacteria of the total biomass
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure**  
H/G boundary: 75% of the median values of the reference lakes. G/M boundary: 95 % of the median values of the reference lakes + the reference values/2. M/P boundary: 2 x G/M. P/B boundary: 2 x M/P (for cyanobacteria, also boundary values

derived from IC-1 were used)

**3.12 "Good status" community:** n.a.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

Uncertainty to be dealt with in the Wisser project; reference conditions will be re-checked when more reference data available.

ID: 115

CHL-SE

## 1. General information

**1.01 GIG:** Northern  
LN2a, LN3a, LN5a, LN6a, LN8a

**1.02 Category:** Lakes

**1.03 BQE:** Phytoplankton

**1.04 Country:** Sweden

**1.05 Specification:** none

**1.06 Method name:** *Ecological Assessment methods of lakes, quality factor phytoplankton, parameter chlorophyll*

**1.07 Original name:** *Bedömningsgrunder för sjöar, kvalitetsfaktor växtplankton, parameter klorofyll*

**1.08 Status: Method is/will be used in** First RBMP (2009)

**1.09 Detected pressure(s):** Eutrophication

***Has the pressure-impact-relationship been tested?***

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Linear regression between chl and total phytoplankton biomass, the latter being first choice of parameter to measure. Linear regressions between chl and total P. 5038 observations of which 2774 from reference sites.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Naturvårdsverket, 2007. Status, potential och kvalitetskrav för sjöar, vattendrag, kustvatten och vatten i övergångszon. Handbok 2007:4. (Swedish EPA). Part A Bedömningsgrunder för sjöar och vattendrag. <http://www.naturvardsverket.se/Documents/publikationer/620-0147-6.pdf> Sonesten, L. & A. Wilander, 2006. Underlag och förslag till reviderade bedömningsgrunder för klorofyll. Rapport 2006:6 SLU Department of Aquatic Sciences and Assessment.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Lars Sonesten  
lars.sonesten@vatten.slu.se  
Department of Aquatic Sciences and Assessment, Swedish  
University of Agricultural Sciences

**1.14 Method reported by**

Stina Drakare  
stina.drakare@vatten.slu.se  
Department of Aquatic Sciences and Assessment, Swedish  
University of Agricultural Sciences

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Swedish Standard SS 02 81 46-1 and SS 02 81 70.

**2.02 Short description**

Chl a samples are taken at 0.5 m depth together with samples for chemical analyses.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Tube sampler or Ruttner sampler

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
Pelagic, usually 0.5-1 m depth

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** July to August

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

at least 1 time per year and use 3 year average

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

In small lakes usually one, very large lakes have several sampling stations

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

1 litre

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** Sampled: no minimum limit. Processed: pore size or Whatman GF/C filter

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

Sample is filtered. Depending of total amount of organisms either all organisms of the sample are collected on the filter or a subsample of known volume is used.

**2.14 Level of taxonomical identification:** Other

This is not a species composition analysis. It is a measure of the amount of chl a as a proxy for phytoplankton biomass.

**2.15 Record of abundance:** n.a.

In the evaluation of the chl method it was compared to total biovolume of phytoplankton

**in relation to** n.a.

**Unit** Amount of chl a per liter of water ( $\mu\text{g/L}$  or  $\text{mg/m}^3$ )

**2.16 Quantification of biomass:** Chlorophyll-a concentration**2.17 Other biological data:** none**2.18 Special cases, exceptions, additions:** none**2.19 Comments**

none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Chl parameter is only used as an assessment tool if other phytoplankton parameters are not available (total biomass, trophic plankton index (TPI) or % of cyanobacteria. If status with Chl parameter gets moderate or worse a full phytoplankton analysis and assessment are recommended.

**3.02 Does the metric selection differ between types of water bodies?** No**3.03 Combination rule for multi-metrics:** Not relevant**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites

**3.07 Reference site characterisation**

**Number of sites:** 63 lakes in Sweden

**Geographical coverage:** Sweden

**Location of sites:** All over Sweden

**Data time period:** 1996-2005, 1-8 times per year

**Criteria:**

Reference sites have to pass pressure criteria: agriculture <10% of catchment, no major point sources, urbanised area <0,1% of catchment, annual mean pH  $\geq 6$  and total P <10 $\mu\text{g/L}$  (if appropriate corrected for total P bound to humic substances).

**3.08 Reference community description**

Low concentration of chl a

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites  
Intercalibrated against Phytoplankton total biovolume and to the intercalibration exercise**3.11 Boundary setting procedure**

Intercalibration of the Chl a was mainly done against Phytoplankton total biovolume, and these intercalibrated boundaries were if possible calibrated to the results from the intercalibration exercise.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

The uncertainty has been estimated by the variation at mainly reference sites. At the moment there were not sufficient data available for a more elaborate estimation, which will be done when amount of data is sufficient.

**3.14 Comments:**

none



ID: 118

TPI

## 1. General information

- 1.01 GIG:** Northern  
LN2a, LN3a, LN5a, LN6a, LN8a
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Ecological assessment methods for lakes, quality factor phytoplankton, parameter Trophic plankton index*
- 1.07 Original name:** *Bedömningsgrunder för sjöar, kvalitetsfaktor växtplankton, parameter Trofiskt planktonindex*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
273 reference lakes was compared to 207 lakes not passing the reference filter. 75% for tested parameter compared to non-reference lakes were set as a border for high status.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Status, potential and kvalitetskrav för sjöar, vattendrag, kustvatten och vatten i övergångszon. Handbok 2007:4. Naturvårdsverket (Swedish EPA). Part A Bedömningsgrunder för sjöar och vattendrag. <http://www.naturvardsverket.se/Documents/publikationer/620-0147-6.pdf>
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Eva Willén  
eva.willen@vatten.slu.se  
Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences
- 1.14 Method reported by**  
Stina Drakare  
stina.drakare@vatten.slu.se  
Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique). Swedish standard SS-EN 15204:2006 from the Swedish Standards Institute.
- 2.02 Short description**  
Depth integrated samples are mixed in a bucket and a subsample are taken and immediately fixed with Lugols solution.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** 2 m tube sampler
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Pelagic, epilimnetic, representing c. 75% of epilimnion
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** July to August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per year, but use the average of at least 3 years of data
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Small lakes (<1 km<sup>2</sup>) composite sample from 5 sites at the center of the lake, large lakes 1 central sampling station
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Depth intervals of 2 m are sampled representing at least 75% of epilimnion, sample from each depth interval are mixed

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** Sampling: no minimum limit. Processing: approximately 1 µm, smaller cells are hard to classify in the microscope
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Sample are mixed and transferred to a sedimentation chamber of known volume (2-100 ml) depending on lake.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups

Most organisms with a trophic index value are possible to identify to species or genus level.

**2.15 Record of abundance:** Individual counts

**in relation to** Volume

**Unit** Abundance is transferred to biovolume after measuring each organism, unit mm<sup>3</sup>/L

**2.16 Quantification of biomass:** Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Trophic plankton index (TPI) is used together with other phytoplankton parameters: total biomass and % cyano.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites

**3.07 Reference site characterisation**

**Number of sites:** 273 lakes in Sweden

**Geographical coverage:** whole of Sweden

**Location of sites:** whole of Sweden

**Data time period:** national monitoring data from 1970-2003

**Criteria:**

Reference sites have to pass pressure criteria: agriculture <10% of catchment, no major point sources, urbanised area <0,1% of catchment, annual mean pH > or = 6 and total P <10µg/L.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

Determination of type 2 error frequency using independent data.

**3.14 Comments:**

none

ID: 119

BM

## 1. General information

- 1.01 GIG:** Northern  
LN2a, LN3a, LN5a, LN6a, LN8a
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Ecological assessment methods for lakes, quality factor phytoplankton, parameter total biomass*
- 1.07 Original name:** *Bedömningsgrunder för sjöar, kvalitetsfaktor växtplankton, parameter Totalbiomassa*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
273 reference lakes was compared to 207 lakes not passing the reference filter. 75% for tested parameter compared to non-reference lakes were set as a border for high status.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Status, potential och kvalitetskrav för sjöar, vattendrag, kustvatten och vatten i övergångszon. Handbok 2007: 4. Naturvårdsverket (Swedish EPA). Part A Bedömningsgrunder för sjöar och vattendrag. <http://www.naturvardsverket.se/Documents/publikationer/620-0147-6.pdf>
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Eva Willén  
eva.willen@vatten.slu.se  
Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences
- 1.14 Method reported by**  
Stina Drakare  
stina.drakare@vatten.slu.se  
Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences
- 1.15 Comments**  
A scientific publication in English is on its way.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique). Swedish standard SS-EN 15204:2006 from the Swedish Standards Institute.
- 2.02 Short description**  
Depth integrated samples are mixed in a bucket and a subsample are taken and immediately fixed with Lugol's solution.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** 2 m tube sampler
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Pelagic, epilimnetic, representing c. 75% of epilimnion
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** July to August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per year, but use the average of at least 3 years of data
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Small lakes (<1 km<sup>2</sup>) composite sample from 5 sites at the center of the lake, large lakes 1 central sampling station
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Depth intervals of 2 m are sampled representing at least 75% of epilimnion, sample from each depth interval are mixed and a subsample are taken from this mixed sample

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** Sampling: no minimum limit. Processing: approximately 1 µm, smaller cells are hard to classify in the microscope
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Sample are mixed and transferred to a sedimentation chamber of known volume (2-100 ml) depending on lake.
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups

Most often species level, some taxa are hard to separate with the chosen method. For these a less detailed level is chosen. Diatoms and several small flagellates are often grouped at less detailed level.

**2.15 Record of abundance:** Individual counts

**in relation to** Volume

**Unit** Abundance is transferred to biovolume after measuring each organism, unit mm<sup>3</sup>/L

**2.16 Quantification of biomass:** Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Total biomass are used together with other phytoplankton parameters: trophic plankton index (TPI) and % of cyanobacteria.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites

**3.07 Reference site characterisation**

**Number of sites:** 273 lakes in Sweden

**Geographical coverage:** whole of Sweden

**Location of sites:** whole of Sweden

**Data time period:** national monitoring data from 1970-2003

**Criteria:**

Reference sites have to pass pressure criteria: agriculture <10% of catchment, no major point sources, urbanised area <0,1% of catchment, annual mean pH > or = 6 and total P <10µg/L.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

Determination of type 2 error frequency using independent data.

**3.14 Comments:**

none

ID: 117

Artantal

## 1. General information

- 1.01 GIG:** Northern  
n.a.
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Ecological assessment methods for lakes, quality factor phytoplankton, parameter Number of species*
- 1.07 Original name:** *Bedömningsgrunder för sjöar, kvalitetsfaktor växtplankton, parameter Artantal*
- 1.08 Status: Method is/will be used in** neither first nor second RBMP
- 1.09 Detected pressure(s):** Acidification
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Status, potential och kvalitetskrav för sjöar, vattendrag, kustvatten och vatten i övergångszon. Handbok 2007:4. Naturvårdsverket (Swedish EPA). Part A Bedömningsgrunder för sjöar och vattendrag. <http://www.naturvardsverket.se/Documents/publikationer/620-0147-6.pdf>
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Eva Willén  
eva.willen@vatten.slu.se  
Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences
- 1.14 Method reported by**  
Stina Drakare  
stina.drakare@vatten.slu.se  
Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique). Swedish standard SS-EN 15204:2006 from the Swedish Standards Institute.
- 2.02 Short description**  
Depth integrated samples are mixed in a bucket and a subsample are taken and immediately fixed with Lugol's solution.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** 2 m tube sampler
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Pelagic, epilimnetic, representing c. 75% of epilimnion
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** July to August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per year, but use the average of at least 3 years of data
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Small lakes (<1 km<sup>2</sup>) composite sample from 5 sites at the center of the lake. Large lakes 1 central sampling station
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Depth intervals of 2 m are sampled representing at least 75% of epilimnion, sample from each depth interval are mixed

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** Sampling: no minimum limit. Processing: approximately 1 µm, smaller cells are hard to classify in the microscope
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Sample are mixed and transferred to a sedimentation chamber of known volume (2-100 ml) depending on lake.
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
Most often species level, some taxa are hard to separate with the chosen method. For these a less detailed level is chosen. Diatoms and several small flagellates are often grouped at less detailed level.

**2.15 Record of abundance:** Individual counts

**in relation to** Volume

**Unit** Abundance is transferred to biovolume after measuring each organism, unit mm<sup>3</sup>/L

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

For acidification this is the only parameter used within BQE Phytoplankton

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites

**3.07 Reference site characterisation**

**Number of sites:** 273 lakes in Sweden

**Geographical coverage:** whole of Sweden

**Location of sites:** whole of Sweden

**Data time period:** national monitoring data from 1970-2003

**Criteria:**

Reference sites have to pass pressure criteria: agriculture <10% of catchment, no major point sources, urbanised area <0,1% of catchment, annual mean pH > or = 6 and total P <10µg/L.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

Determination of type 2 error frequency using independent data.

**3.14 Comments:**

none

ID: 116

%cyano

## 1. General information

- 1.01 GIG:** Northern  
LN2a, LN3a, LN5a, LN6a, LN8a
- 1.02 Category:** Lakes
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Ecological assessment methods for lakes, quality factor phytoplankton, parameter Proportion of cyanobacteria*
- 1.07 Original name:** *Bedömningsgrunder för sjöar, kvalitetsfaktor växtplankton, parameter Andel cyanobakterier*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
273 reference lakes was compared to 207 lakes not passing the reference filter. 75% for tested parameter compared to non-reference lakes were set as a border for high status.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Status, potential and kvalitetskrav för sjöar, vattendrag, kustvatten och vatten i övergångszon. Handbok 2007:4. Naturvårdsverket (Swedish EPA). Part A Bedömningsgrunder för sjöar och vattendrag. <http://www.naturvardsverket.se/Documents/publikationer/620-0147-6.pdf>
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Eva Willén  
eva.willen@vatten.slu.se  
Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences
- 1.14 Method reported by**  
Stina Drakare  
stina.drakare@vatten.slu.se  
Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique). Swedish standard SS-EN 15204:2006 from the Swedish Standards Institute.
- 2.02 Short description**  
Depth integrated samples are mixed in a bucket and a subsample are taken and immediately fixed with Lugols solution.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** 2 m tube sampler
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Pelagic, epilimnetic, representing c. 75% of epilimnion
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** July to August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per year, but use the average of at least 3 years of data
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Small lakes (<1 km<sup>2</sup>) composite sample from 5 sites at the center of the lake. Large lakes 1 central sampling station
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Depth intervals of 2 m are sampled representing at least 75% of epilimnion, sample from each depth interval are mixed

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** Sampling: no minimum limit. Processing: approximately 1 µm, smaller cells are hard to classify in the microscope
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Sample are mixed and transferred to a sedimentation chamber of known volume (2-100 ml) depending on lake.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups

Most often species level, some taxa are hard to separate with the chosen method. For these a less detailed level is chosen.  
Very small cyanobacteria (pico-sized) often classified with less detail

**2.15 Record of abundance:** Individual counts

**in relation to** Volume

**Unit** Abundance is transferred to biovolume after measuring each organism, unit mm<sup>3</sup>/L

**2.16 Quantification of biomass:** Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

% cyano is used together with other phytoplankton parameters: total biomass and trophic plankton index (TPI).

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites

**3.07 Reference site characterisation**

**Number of sites:** 273 lakes in Sweden

**Geographical coverage:** whole of Sweden

**Location of sites:** whole of Sweden

**Data time period:** national monitoring data from 1970-2003

**Criteria:**

Reference sites have to pass pressure criteria: agriculture <10% of catchment, no major point sources, urbanised area <0,1% of catchment, annual mean pH > or = 6 and total P <10µg/L.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

Determination of type 2 error frequency using independent data

**3.14 Comments:**

none

ID: 91

SM

## 1. General information

- 1.01 GIG:** Baltic  
n.a.
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Angiosperms, Macroalgae  
Zostera marina, Potamogeton pectinatus, P.filiformis, Myriophyllum spicatum
- 1.04 Country:** Poland
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment system for coastal and transitional waters using macrophytes*
- 1.07 Original name:** *Metoda określania jakości ekologicznej wód przybrzeżnych i przejściowych na podstawie makrofitów.*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication  
*Has the pressure-impact-relationship been tested?*  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Lidia Kruk-Dowgiallo  
lidia.kruk-dowgiallo@im.gda.pl  
Maritime Institute in Gdańsk, Department of Ecology
- 1.14 Method reported by**  
Paulina Brzeska  
paulina.brzeska@im.gda.pl  
Maritime Institute in Gdansk, Department of Ecology
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Andrulewicz, E., L. Kruk-Dowgiallo & A. Osowiecki, 2004. Phytobenthos and macrozoobenthos of the Slupsk Bank stony reefs. Hydrobiologia 514 (1-3): 163-170. [Guidelines for monitoring of phytobenthic plant and animal communities in the Baltic Sea](#). 1999. Annex for HELCOM COMBINE Manual.
- 2.02 Short description**  
Using DAK frame (diver collects four samples (by means of DAK device) at one station (its central point is marked by anchor at the bottom). Four samples are located 2m away from the anchor in the directions: North, South, East and West. Additionally diver collects one sample for qualitative analyses, estimates vegetation cover (%) and substrate cover in the vicinity of the anchor).
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.  
frame DAK, Bernatowicz grab
- 2.05 Specification:**  
1. Frame DAK (small frame with collection bag and cuff which covers the upper part of the frame - Andrulewicz E., L. Kruk - Dowgiallo
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)  
soft bottom (Angiosperms), hard bottom (macroalgae)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** June and September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
two per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
5 samplings at each of at least 3 stations in one water body
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
0,04 m2 (actually covered by samplin device DAK), 0,16 m2 (actually covered by sampling device Bernatowicz grab)

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 200 units as in the example below (mesh size)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Genus level: Enteromorpha, Acrochaetium. Species level: other macroalgae, Angiosperms.

- 2.15 Record of abundance:** Percent coverage  
dry biomass  
**in relation to** n.a.  
biomass  
**Unit** gram of dry weight of individual taxon per square-metre
- 2.16 Quantification of biomass:** n.a.  
Determination of dry weight of taxon (Guidelines for monitoring of phytobenthic plant and animal community).
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
SM = biomass of "positive taxa / biomass of "negative" taxa
- 3.02 Does the metric selection differ between types of water bodies?** Yes
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time  
Data from single spatial replicate
- Reference conditions**
- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** 2  
**Geographical coverage:** Northern Poland: one transitional water body and one coastal water body  
**Location of sites:** transitional waters (Puck Bay - inner and outer part), coastal waters (coastal waters between Jarosławiec and Rowy)  
**Data time period:** inner Puck Bay: 1956-2009 (June - September), outer Puck Bay: 2008-2009 (June and September)  
**Criteria:**  
absence/low biomass of species regarded as "eutrophication indicators", negative taxa, i.e.: *Pilayella littoralis*, *Ectocarpus siliculosus*, *Cladophora glomerata*, *Chaetomorpha linum*, *Enteromorpha* sp.
- 3.08 Reference community description**  
high biomass of "positive" taxa, i.e. angiosperms and/or macroalgae (except ones mentioned in c-12)
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
expert judgment
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
This questionnaire was approved by Chief Inspectorate for Environmental Protection, a government body responsible for running monitoring and assessment of ecological status.

ID: 87

LT-BI-CT

## 1. General information

- 1.01 GIG:** Baltic  
n.a.
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Lithuania
- 1.05 Specification:** to all water bodies except heavily modified WB - Klaipeda strait (harbour)
- 1.06 Method name:** *Assessment system for transitional and coastal waters using macrozoobenthos*
- 1.07 Original name:** *Tarpinių ir pakrantės vandenų ekologinės būklės vertinimo sistema pagal makrozoobentosą*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Daunys, D., S. Olenin, R. Paškauskas, P. Zemlys, I. Olenina & M. Bučas, 2007. Typology and Classification of Ecological Status of Lithuanian Coastal and Transitional Waters: An Update of Existing System. Technical Report for Transition Facility project No. 2004/016-925-04-06: Procurement of services for the Institutional building for the Nemunas River Basin management, 66 pp. In Lithuanian: Baseinių valdymo plano požeminio vandens dalies Nemuno upių baseinų rajonui parengimas ir integravimas į bendrąjį valdymo planą, pirkimo nr. 62298, 2009. Olenin, S. & D. Daunys, 2006 Technica Note XIII: Benthic macrofauna of the Curonian Lagoon and the Lithuanian coastal waters of the Baltic Sea. Implementation of the EU Water Framework Directive, Lithuania.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Doc. dr. Darius Daunys and Prof. Habil. Dr. Sergej Olenin  
darius@corpi.ku.lt, sergej@corpi.ku.lt  
Coastal Research and Planning institute, Klaipeda University
- 1.14 Method reported by**  
Nijole Remeikaite-Nikiene  
n.nikiene@jtc.am.lt  
Center of Marine Research
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
HELCOM COMBINE programme LST EN ISO 9391: 2000. Water quality - Sampling in deep waters for macro-invertebrates - Guidance on the use of colonization, qualitative and quantitative samplers (ISO 9391:1993). ISO 5667-14:1998 Guidance on quality assurance of environmental water sampling and handling.
- 2.02 Short description**  
Benthic invertebrates samples are taken at each monitoring station. Station net is covering all types of waters. 2-3 grab samples are sieved at stations, invertebrates are preserved by formaline.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Grab  
scuba diving
- 2.05 Specification:** Van Veen Grab 0,1 m<sup>2</sup>, 75 kg (for sea), 25 kg (for the Curonian lagoon). Petersen grab (0,025 m<sup>2</sup>) was used as well.
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Soft bottom, hard bottom, shelly bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** May
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
2-3 replicates per station/2-3 stations per water body
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
2-3 replicates per station (0,2 or 0,3 m<sup>2</sup>) /12 stations = 2,4 - 3,6 m<sup>2</sup> (in transitional and coastal waters)

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 500 µm mesh-size of sieving net
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups

Mainly to species level; some: Ostracoda, Oligochaets - to class level; Hydrobia, Gammarus – to genus level; Chironomides - family level.

**2.15 Record of abundance:** Individual counts

**in relation to** Area

**Unit** Number of individuals per one square-metre

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Additionally, diversity indexes are used to estimate species diversity (Margalef index), evenness (Pielou index) and a common measure of both evenness and species richness (Shannon-Wiener) in bottom macrofauna communities. They are supplementary to descriptions only and should not be used individually.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

**3.05 Scope of reference conditions:** Habitat-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data

**3.07 Reference site characterisation**

**Number of sites:** In total 420 and 188 samples were taken in the Curonian Lagoon and in the coastal zone of the Baltic Sea respectively.

**Geographical coverage:** Curonian lagoon stations and stations along the Lithuanian coast

**Location of sites:** Lithuanian northern and southern coasts, northern and central parts of the Curonian lagoon

**Data time period:** Data on benthic macrofauna was collected in period from 1980 to 2003.

**Criteria:**

Reference conditions are described using community description (see above) approach and average number of species per sample.

**3.08 Reference community description**

Main features: Baltic Sea sandy coast - Biomass dominant species: *Macoma baltica*, *Mya arenaria*, *Cerastoderma lamarcki*. Dense colonies of *Pygospio elegans* are common. Comparatively high number of other species. High abundance of juvenile forms in summer time. Baltic Sea stony coast - Bottom macrofauna community includes a variety of hard bottom species. *Mytilus edulis* is a biomass dominant macrofauna species, however within euphotic zone its biomass does not exceed that of the red algae *Furcellaria lumbricalis*. Curonian Lagoon (transitional waters) - Communities of native unionids as biomass dominant species, clusters of *Dreissena polymorpha*. High species diversity. Several species sensible to organic pollution (Ephemeroptera, Trichoptera) are present.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** n.a.

mainly expert judgement

**3.11 Boundary setting procedure**

The reference conditions in the Curonian lagoon were partly derived from the historical studies (Gasiūnas, 1959, unpublished monitoring data collected since 1980's) and based on distribution limits of dominant species (*Valvata piscinalis*, *Dreissena polymorpha*, *Nereis diversicolor*). Deviations from high status were determined using data collected during various surveys and monitoring programs in period from 1980's to 2003. In contrast to the Curonian Lagoon, at the open coast some locations might be considered as only slightly disturbed and thus representing high status of benthic communities. The comparable historical data are not available, except study of distribution of the red algae *Furcellaria lumbricalis* stock at the

Lithuanian coast in the late 1968 (Blinova, Tolstikova, 1972). Description of "good", "moderate" and "bad" status was based on several benthic community indexes, bottom characteristics, characteristics and biomass dominant/subdominant species, while separation between these categories was mainly based on expert judgement.

- 3.12 "Good status" community:** Main features: Baltic Sea sandy coast ☐Biomass dominant species: *Macoma baltica*, *Mya arenaria*. Subdominants: *Marenzelleria viridis*, *Nereis diversicolor*, *Mesidothea entomon*. Characteristic species: *Pygospio elegans* (usually dominant in abundance), *Corophium volutator*, *Bathyporeia pilosa* (in the upper sublittoral).☐High abundance of juvenile forms in summer time. ☐☐  
Baltic Sea stony coast ☐Biomass dominant species: *Mytilus*. Subdominant: *Balanus improvisus*. Characteristic species: *Nereis diversicolor*, *Fabricia sabella*, *Corophium volutator*, *Bathyporeia pilosa*, *Jaera albifrons*, *Gammarus zaddachi*, *G. salinus*, *Theodoxus fluviatilis*. Species richness and related indexes are the highest on sites where *Mytilus* co-occurs with *Furcellaria*☐within the euphotic zone. Beneath euphotic zone all stones are occupied by dense colonies of *Mytilus* with associated fauna. ☐☐Curonian Lagoon - Muddy and sandy bottoms with shell deposits. Dense clusters of *Dreissena polymorpha* on native unionids. Biomass dominant species - *Dreissena* and unionids. High species richness. Characteristic organisms: molluscs -*Viviparus*, *Bithynia*, *Valvata*, *Pisidium*; larvae of mayflies☐and caddis flies.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 89

LT-AL-CT

## 1. General information

- 1.01 GIG:** Baltic  
n.a.
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Macroalgae
- 1.04 Country:** Lithuania
- 1.05 Specification:** Only northern coastal waters and Plume of the Curonian Lagoon in the Baltic Sea
- 1.06 Method name:** ***Assessment system for transitional and coastal waters using macroalgae (maximum depth limit of *Furcellaria lumbricalis*)***
- 1.07 Original name:** *Tarpinių ir pakrantės vandenų ekologinės būklės vertinimo sistema pagal makrodumblius (maksimalus *Furcellaria lumbricalis* tyrimo gylis)*
- 1.08 Status: Method is/will be used in:** RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, Riparian habitat alteration  
**Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Daunys, D., S. Olenin, R. Paškauskas, P. Zemlys, I. Olenina & M. Bučas, 2007. Typology and Classification of Ecological Status of Lithuanian Coastal and Transitional Waters: An Update of Existing System. Technical Report for Transition Facility project No. 2004/016-925-04-06: Procurement of services for the Institutional building for the Nemunas River Basin management, 66 pp.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Dr. Martynas Bucas, Doc. dr. Darius Daunys  
martynas@corpi.ku.lt, darius@corpi.ku.lt  
Coastal Research and Planning institute, Klaipeda University
- 1.14 Method reported by**  
Nijole Remeikaite-Nikiene  
n.nikiene@jtc.am.lt  
Center of Marine Research
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
HELCOM COMBINE Annex C-9 Guidelines for monitoring of phytobenthic plant and animal communities in the Baltic Sea.
- 2.02 Short description**  
Diving survey along the transect
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.  
visual
- 2.05 Specification:** Diver transect
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Sublittoral hard bottoms
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** May-August
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling event
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1 transect per water body
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups
- 2.15 Record of abundance:** Percent coverage  
in relation to Area  
Unit

- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
n.a.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

- 3.05 Scope of reference conditions:** Habitat-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data
- 3.07 Reference site characterisation**
- Number of sites:** Single note from literature
- Geographical coverage:** Only sites from exposed part of the eastern Baltic considered
- Location of sites:** talasological reserve (south-eastern Baltic)
- Data time period:** 1968

**Criteria:**

Northern coastal waters. The reference condition are defined according to the maximum depth record of these red algae at the Lithuanian coast late 1950s: 19 m (Kireeva,1960). Similar depth limit (20 m) was recorded during the same time period at Blekinge, south-eastern coast of Sweden (Essays on biological productivity in the Baltic Sea, 1984). Thus, >20 m depth limit is using as reference conditions at coastal waters. Suggested classification of water quality according to maximum depth of *Furcellaria lumbricalis* in the plume of the Curonian lagoon in the Baltic Sea is based on comparison of historical data with patterns of recent distribution of *F. lumbricalis*. The reference conditions are defined according to the maximum depth records of these red algae south off Palanga in late 1950s, when the species was found in depths of 17 m (Kireeva, 1960). Thus, >18 depth limit is using as reference conditions at the transitional waters (plume of the Lagoon in the Baltic Sea).

**3.08 Reference community description**

The reference condition for coastal waters are defined according to the maximum depth record of these red algae at the Lithuanian coast late 1950s: 19 m (Kireeva,1960). Similar depth limit (20 m) was recorded during the same time period at Blekinge, south-eastern coast of Sweden (Essays on biological productivity in the Baltic Sea, 1984). The reference conditions for transitional waters are defined according to the maximum depth records of these red algae south off Palanga in late 1950s, when the species was found in depths of 17 m (Kireeva, 1960).

- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
based on comparison of historical data with patterns of recent distribution of *F. lumbricalis*

**3.11 Boundary setting procedure**

Same approach was used for boundary setting procedure for coastal and transitional waters. Coastal waters: The reference condition are defined according to the maximum depth record of these red algae at the Lithuanian coast late 1950s: 19 m (Kireeva,1960). Similar depth limit (20 m) was recorded during the same time period at- Blekinge, south-eastern coast of Sweden (Essays on biological productivity in the Baltic Sea, 1984). Good status is defined by the maximum depth record within the 15-19 m, a range which generally corresponds with recent distribution of *F.lumbricalis*. This limit did not change significantly during the recent five decades (Olenin et al., 2003; Bučas et al., in press; unpublished data cited by Daunys D., et al., 2007). Moderate status is defined by the maximum depth record within the 9-15 m range. It is suggested that decline of depth limit up to 15 m will result in subsequent reduction of the most valuable, dense overgrowths at lower depths, which may be interpreted as habitat alteration. If the maximum distribution depth will decline to 9 m it is unlikely that the dense overgrowths at lower depth will survive. Poor status is defined by the maximum depth record within the 5-9 m range,

which is classified as a critical limit determining loss of dense overgrowths at lower depths. Bad status is defined by the maximum depth limit at less than 5 m, which is considered as a very high risk of *F. lumbricalis* extinction at the Lithuanian coast due to competition with opportunistic filamentous algae, reduced area of available substrate and strong wave effect.

- 3.12 "Good status" community:** Good status is defined by the maximum depth record within the 15-19 m, range for northern coastal waters between 14 to 17 m. Range for transitional waters which generally corresponds with recent distribution of *F. lumbricalis*. This limits did not change significantly during the recent five decades.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)

- 3.14 Comments:**  
none

ID: 90

LT-PP-CT

## 1. General information

- 1.01 GIG:** Baltic  
n.a.
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Lithuania
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment system for transitional and coastal waters using phytoplankton indicators*
- 1.07 Original name:** *Tarpinių ir pakrantės vandenų ekologinės būklės vertinimo sistema naudojant fitoplanktono rodiklius*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Ecological data from 5 stations at coastal waters and 5 stations at transitional waters were examined. The relationship between phytoplankton metric (chlorophyll a) and TP (summer) at coastal waters showed significant correlation (Coefficient of Determination 0,48). The relationship between chlorophyll a and TN (summer) at transitional waters showed significant correlation (Coefficient of Determination ranging 0,76-0,83).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

In Lithuanian: Baseinų valdymo plano požeminio vandens dalies Nemuno upių baseinų rajonui parengimas ir integravimas į bendrąjį valdymo planą, pirkimo nr. 62298, 2009.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

Doc. dr. Darius Daunys  
darius@corpi.ku.lt  
Coastal Research and Planning institute, Klaipeda University

**1.14 Method reported by**

Nijole Remeikaite-Nikiene  
n.nikiene@jtc.am.lt  
Center of Marine Research

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

HELCOM COMBINE programme, ISO 5667-14: 1998. Guidance on quality assurance of environmental water sampling and handling. LST EN 15204:2007 Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)(EN 15204:2006).

**2.02 Short description**

In the Baltic sea phytoplankton as well as chlorophyll a samples are taken at monitoring stations 3-4 times per year. Phytoplankton: 1 integrated sample from water layers 1m, 2.5 m, 5 m, 7.5 m, 10 m. Chlorophyll a additional samples from surface layer (1 m), 5 m, 10 m. In the Curonian lagoon samples are taken 12 times per year - once per month, from surface water layer.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Plastic bathometer 5 l

**2.06 Sampled/surveyed habitat:** n.a.

**2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone

**2.08 Sampling/survey month(s):** June-September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Much as possible in period June-September; Now – 1-4 sampling occasions

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

16 stations (2-3 stations per water body).

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

n.a.

### Sample processing

**2.12 Minimum size of organisms sampled and processed:**

Phytoplankton: Using microscopes with magnification x400. Organisms length is approximately from 2 µm.  
Chlorophyll a concentration: filters, GF/F pore size 0,7 µm

- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
For phytoplankton microscopy analysis 5, 10 or 25 ml (in winter could be used 50 ml) of subsample is taken from 100 ml sample which was made of mixing 1L from each layer.
- 2.14 Level of taxonomical identification:** Genus, Other, Species/species groups  
Most algae to species level, some (Aphanizomenon, Eutreptiella, Anabaena, etc.) - to genus level, Gymnodiniales - to order.
- 2.15 Record of abundance:** Individual counts  
**in relation to** Volume  
**Unit**
- 2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
n.a.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** 5 stations at transitional and 5 at coastal waters  
**Geographical coverage:** Curonian lagoon stations and stations along Lithuanian coast  
**Location of sites:** Lithuanian northern and southern coast, northern and central parts of the Curonian lagoon  
**Data time period:** Monitoring data obtained at Sea from 1992 (August); monitoring data obtained at the Curonian Lagoon from 1984 (June-September); historical cyanobacteria data in years 1951, 1954 and 1955 (July) at the Lagoon, Modeled long-term maximum of average chlorophyll  
**Criteria:**  
There is no reference sites in the Lithuanian coastal and transitional waters. Chlorophyll a summer concentration is used for ecological status assessment (for coastal waters <2µg/l; for transitional waters <26.4 and <37 µg/l (for northern and central parts of the Curonian Lagoon)). Phytoplankton biomass will be used as well.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Using discontinuities in the relationship of anthropogenic pressure and the biological response.  
using statistical approaches and defined blooming classes (Reimers,1990)
- 3.11 Boundary setting procedure**  
Coastal waters: 1) Modeled long-term maximum of average chlorophyll a concentrations (2.1 µg/l) for summer months in the south-eastern Baltic (Schernewski, Neuman, 2005) was used to define reference conditions for chlorophyll a. 2) Threshold between poor and bad water quality classes was defined using a lower limit of intensive phytoplankton bloom 10 mg/l (Reimers,1990). This value was recalculated into average summer phytoplankton concentration using empirical relationship between maximum (PHmax) and mean (PHmean) phytoplankton biomass (national monitoring data from the period 1993-2007 obtained at 5 stations).  $PH_{mean} (mg/l) = 0.796 \times PH_{max} (mg/l) - 0.0295$  ( $R^2 = 0.93$ ). 3) Reference chlorophyll a (Chl a)

concentration was transformed into phytoplankton biomass (PHbiom) using empirical relationship:  $\text{Chl a (mg/l)} = 0.3001 \times \text{PHbiom (mg/l)} + 0.188$  ( $R^2 = 0.43$ ). 4) Boundaries between reference and bad classes (good, moderate and poor) for phytoplankton biomass were estimated using equal proportion principle and back calculated to chlorophyll a boundaries. 5) Total phosphorus (TP) reference concentration and water quality class boundaries were estimated using empirical relationship between total phosphorus and chlorophyll a mean summer concentration.  $\text{TP (mg/l)} = 3.9403 \times \text{Chl a (}\mu\text{g/l)} + 11.1881$  ( $R^2 = 0.48$ ). Lower limits of 95% confidence interval of predicted TP values were used for class boundaries. 6) Reference TN concentration was fixed according to the average pristine N:P ratio of 20 for the eastern Baltic Sea (Schernewski, Neumann, 2005). Using expert judgment boundary for poor and bad water quality classes was set to 600  $\mu\text{g/l}$  and overall interval was divided into classes following equal proportions. 2) Transitional waters: 1) Maximum July abundance of cyanobacteria in years 1951, 1954 and 1955 in two water bodies of the Curonian lagoon was used to derive reference conditions. First of all maximum summer cyanobacteria abundance (CYAabund) was related to the mean summer abundance (using long-term monitoring data from the period 1980-2007) and the later was used to estimate reference values for chlorophyll a (chl a) and total phytoplankton biomass (PHbiom) according to empirical relationships:  $\text{PHbiom (}\mu\text{g/l)} = 0.1719 \text{CYAabund (x103 cells/l)}$   $\text{Chl a (}\mu\text{g/l)} = 1.2655 \times \text{PHbiom (}\mu\text{g/l)} + 20.82$  ( $R^2 = 0.62$ ) (northern Curonian lagoon)  $\text{Chl a (}\mu\text{g/l)} = 1.2007 \times \text{PHbiom (}\mu\text{g/l)} + 30.14$  ( $R^2 = 0.77$ ) (central Curonian lagoon) 2) Threshold between poor and bad water quality classes was defined using a lower limit of phytoplankton hyperbloom biomass 100 mg/l. This value was recalculated into average summer phytoplankton using empirical relationships between maximum and mean phytoplankton biomass. 3) Boundaries between good, moderate and poor water quality classes for phytoplankton biomass were estimated dividing range between reference and bad classes into equal intervals. 4) Reference for summer average concentration of total nitrogen (TN) and water quality class boundaries were estimated using empirical relationship between total nitrogen and chlorophyll a mean summer concentration. 5) Negligible relationships were found between phytoplankton biomass, cyanobacteria abundance and total phosphorus (TP) in the transitional waters, therefore description of reference TP concentration was based on historical data (Jurevicius, 1959) for dissolved inorganic phosphorus (DIP). Maximum DIP values for July from 1956 and 1957 in the central and northern parts of the Curonian lagoon were used (Jurevicius, 1959). TP was calculated deriving phytoplankton P from biomass values according to stoichiometric C:N:P ratio and adding obtained phytoplankton P amount to the available historical DIP concentrations. The estimated TN and TP boundary values resulted in N:P ratio of 35 both in the central and northern parts reflecting phosphorus limitation and conditions not favourable for cyanobacteria blooms. 6) Estimation of TP boundaries between water quality classes was estimated on

**3.12 "Good status" community:** n.a.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

Classification rules are used to assess confidence level of the whole assessment system (not for separate methods).

ID: 26

PL-div-CT

## 1. General information

- 1.01 GIG:** Baltic  
neither of the metrics has been intercalibrated
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Poland
- 1.05 Specification:** not for phytoplankton biomass; chlorophyll-a is already legally approved
- 1.06 Method name:** **Monitoring and classification methods of biological quality elements for the assessment of ecological status of transitional and coastal marine water bodies**
- 1.07 Original name:** *Metodyka badania i klasyfikacji elementów biologicznych w procedurze oceny stanu ekologicznego części wód powierzchniowych*
- 1.08 Status: Method is available in the following languages:** Polish
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, General degradation, Habitat destruction, Heavy metals, Impact of alien species, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).
- 1) Chlorophyll-a: Monitoring data from the period 1999-2005; from 10-60 data from 1-3 monitoring stations per area (water body); the relationship between chlorophyll-a and DIP, TP and TN showed significant correlation (Spearman correlation coefficient ranging from 0.29 to 0.76).  
2) Total phytoplankton biomass (mean in summer [VI-IX] months): Monitoring data from the period 2002-2008; from 19-54 data from 1-3 monitoring stations per area (water body); the relationship between biomass and DIP, TP, DIN, TN and SiO<sub>4</sub> showed significant correlation (Spearman correlation coefficient ranging from 0.34 to 0.96).
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Chlorophyll-a: Decree of the Minister of Environment from 20 August 2008 concerning classification of unit water bodies (Rozporządzenie ministra Środowiska z dnia 20 sierpnia 2008 r. w sprawie klasyfikacji stanu jednolitych części wód powierzchniowych. Dz.U. Nr 162, poz. 1008, 8654-8681 [in Polish])  
Phytoplankton biomass none yet.
- 1.12 Scientific literature:**  
HELCOM, 2009. Eutrophication in the Baltic Sea - an integrated thematic assessment of the effects of nutrient enrichment and eutrophication in the Baltic Sea region. Balt. Sea Environ. Proc. No 115 B: 148 pp. Lysiak-Pastuszek, E., W. Krzysiński & L. Lewandowski, 2009. Development of tools for ecological quality assessment in Polish marine areas according to the water Framework Directive. Part II - Chlorophyll-a. Oceanological and Hydrobiological Studies 38 (3): 101-1122.
- 1.13 Method developed by**  
Elżbieta Łysiak-Pastuszek  
Elzbieta.Lysiak-Pastuszek@imgw.pl  
Institute of Meteorology and Water Management, Maritime Branch, Al. Waszyngtona 42, 81-342 Gdynia, Poland
- 1.14 Method reported by**  
Elżbieta Łysiak-Pastuszek  
Elzbieta.Lysiak-Pastuszek@imgw.pl  
Institute of Meteorology and Water Management, Maritime Branch, Al. Waszyngtona 42, 81-342 Gdynia, Poland
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
HELCOM COMBINE Manual [http://www.helcom.fi/manual/en\\_GB/cover/](http://www.helcom.fi/manual/en_GB/cover/)
- 2.02 Short description**  
1) Chlorophyll-a: at stations up to 10 m depth, water samples are collected with water sampler at 0, 2.5, 5.0, 7.5 m depths and 0.5 m above bottom and chlorophyll-a concentrations are determined at each depth (at shallower stations, sampling depths are adjusted accordingly); and at stations >10m depth, water for chlorophyll-a determination is sampled with a "hose" (polyvinyl tube) from 2 layers 0-10 m and 10-20 m; water volume for filtration depends on phytoplankton development and region (in lagoons usually not more than 0.2-0.5 l).  
2) Phytoplankton analysis: at stations <10 m depth a sample is integrated of water from 0, 2.5, 5.0 etc. to attain 200 cm<sup>3</sup> altogether; at stations >10 m depth 2 samples are taken from 0-10 m and from 10-20 m layer; always a qualitative sample is taken with plankton net of the mesh 25 µm. Samples (200 cm<sup>3</sup>) are treated with Lugol solution.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Plankton net, Water sampler
- 2.05 Specification:** Niskin water sampler, plankton net mesh size 25 µm
- 2.06 Sampled/surveyed habitat:** n.a.
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** March to November

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

at least 3 per season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1-3 monitoring stations per water body

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

n.a.

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:** n.a.

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

Samples are subdivided depending on phytoplankton abundance. Division is performed according HELCOM COMBINE manual.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts

in relation to Volume

Unit: number of counts (cells/units/colonies) per one cubic meter

**2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique

Utermöhl technique with Olenina modifications (Olenina, I., Hajdu, S., Andersson, A., Edler, L., Wasmund, N., Busch, S., Göbel, J., Gromisz, S., Huseby, S., Huttunen, M., Jaanus, A., Kokkonen, P., Ledaine, I., Niemi, E., 2006. Biovolumes and size-classes of phytoplankton in the Baltic Sea. Baltic Sea Environment Proceedings No.106, 144pp. Printed Paper is available: <http://www.helcom.fi/stc/files/Publications/Proceedings/bsep106.pdf>

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

**3. Data evaluation**

**Evaluation**

**3.01 List of biological metrics**

1) chlorophyll-a, mean concentration of summer months (VI-IX) 2) total phytoplankton biomass, mean of summer months (VI-IX)

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

mean EQR

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

**Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge

statistical method

**3.07 Reference site characterisation**

**Number of sites:** 1-3 sites per water body

**Geographical coverage:** 1) chlorophyll-a: all transitional and coastal water bodies in Poland; 2) phytoplankton biomass: selected water bodies in the Gulf of Gdańsk and along the central Polish coast

**Location of sites:** as C-08

**Data time period:** months VI-IX, chlorophyll-a 1999-2006, phytoplankton biomass 2002-2008

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** n.a.  
1) chlorophyll-a: statistical calculation of percentiles and expert judgment; 2)  
phytoplankton biomass: Jenks@Caspall (1970) method of natural breaks

**3.11 Boundary setting procedure**  
n.a.

**3.12 "Good status" community:** n.a.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 192

SE-PP-CT

## 1. General information

- 1.01 GIG:** Baltic
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Sweden
- 1.05 Specification:** Baltic proper. In other areas fixed type-specific reference values and boundaries chlorophyll and biomass have been determined
- 1.06 Method name:** *Assessment system for coastal and transitional waters: Phytoplankton*
- 1.07 Original name:** *Bedömningsgrunder för kustvatten och vatten i övergångszon: Växtplankton*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Correlations between summer tot-N and chlorophyll and phytoplankton biomass.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Naturvårdsverket Handbok 2007: 4 - bilaga B, Bedömningsgrunder för kustvatten och vatten i övergångszon. <http://www.naturvardsverket.se/Documents/publikationer/620-0149-0.pdf> Larsson, U., J. Walve, S. Hajdu, A. Andersson, P. Larsson & L. Edler, 2006. Bedömningsgrunder för kust och hav – växtplankton, näringsämnen, klorofyll, siktdjup. Report to Naturvårdsverket.
- 1.12 Scientific literature:**  
n.a.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Ulf Larsson/Jakob Walve<br>ulf.larsson@ecology.su.se, jakob.walve@ecology.su.se<br>Department of Systems ecology, Stockholm University | <b>1.14 Method reported by</b><br>Jakob Walve<br>jakob.walve@ecology.su.se<br>Department of Systems Ecology, Stockholm University |
|---|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Naturvårdsverkets Handbok för miljöövervakning Helcom monitoring guidelines Comment: Recommended in method to take Chl a samples at 0m depth rather than 0-10m.
- 2.02 Short description**  
Phytoplankton (for total biomass determination): 200 ml of hose sample 0-10m preserved with acidified Lugol's solution. Chlorophyll a: 1-2L of water sample from surface filtered through GF/F-filter.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Water sampler  
Water sampler=Hose 0-10m fo
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** n.a.
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** June-August (Comment: Evaluation of the probably better period July-August is about to begin)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
recommended 3-5/year and minimum 3 years
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
1 if representative position.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** single-celled phytoplankton < ca 2-3 µm are not included in counts (but included in chl a= GF/F filtration)
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
Phytoplankton: Sub-sample is sedimented and counted by conventional microscopic methods (HELCOM 1988 combine guidelines)

- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
**in relation to** Volume  
**Unit** number/ml
- 2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique  
Divided into size classes with defined volumes
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Chlorophyll a concentration, Phytoplankton biomass (if available)
- 3.02 Does the metric selection differ between types of water bodies?**
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

#### Reference conditions

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Historical data, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** historical Secchi depth data from the Baltic Sea, combined with totN - Secchi and totN - chl and totN-biomass correlations  
**Geographical coverage:** Baltic proper  
**Location of sites:** n.a.  
**Data time period:** ca 1900-1930 for Secchi depth data  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
Comment: The reference totN for the open coastal sea areas was derived from mentioned historical data and regressions. Reference values for totN in inner coastal areas are adjusted according to salinity and a simple mixing model. In the mixing model, fresh water has certain background totN conc (in turn estimated from SMHI watershed models), and high salinity sea water has reference value as above. Chl a and biomass reference values are also adjusted using empirical correlations with totN
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure**  
Status of open coastal areas assumed to be below good-moderate boundary (based on general agreement that current status is not acceptable).
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 74

SE-AN-CT

## 1. General information

- 1.01 GIG:** Baltic, North-East-Atlantic  
NEA 8, 9, 10 and Baltic B0, B2, B3 and B12
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Angiosperms, Macroalgae  
Rooted submerged phanerogams of many freshwater taxa and seagrasses
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment of Biological Quality Elements in coastal and transitional waters - macrovegetation*
- 1.07 Original name:** *Bedömningsgrunder för biologiska kvalitetsfaktorer i kustvatten och övergångsvatten - makrovegetation*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).  
Data from a large number of sites along the coast were examined to establish impact of eutrophication i.e. chl a nutrients, TN and TP and Secchi depth. The variance was very large for all pressure -impact factors and only Secchi depth was found to be significantly correlated with depth distribution.
- 1.10 Internet reference:** <http://www.naturvardsverket.se/sv/Arbete-med-naturvard/Vattenforvaltning/Lagstiftning-och-vagledning/Vagledning/Handbok-20074/>
- 1.11 Pertinent literature of mandatory character:**  
Naturvårdsverkets författningssamling, NFS2008: 1.
- 1.12 Scientific literature:**  
n.a.
- |  |  |
|--|--|
| <b>1.13 Method developed by</b><br>Lena Kautsky, Hans Kautsky<br>lena.kautsky@botan.su.se, hassek@ecology.su.se<br>Department of Botany, Stockholm university, Department of Systems Ecology, Stockholm university | <b>1.14 Method reported by</b><br>Lena Kautsky<br>lena.kautsky@botan.su.se<br>Department of Botany, Stockholm university, Sweden |
|--|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
Transects should have rocky bottoms to a depth so that not substrate but light is limiting depth distribution. For each type a set of common easily identified macrophyte species have been selected for which the max depth distribution is determined.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying, Stratified sampling/s
- 2.04 Sampling/survey device:** Scraper  
Diving surveys, investigating c
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** Mainly July- September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
During one time period/occasion per year - when the vegetation has its max development
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Depends on how homogenous the water body is, but at least three transects per water body are recommended and they should be placed on rocky substrates, at intermediate wave exposure
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
At each transect an area of 3-5 meters on each side is covered to find the deepest growing individuals of the selected species.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts  
Determination of the deepest growing individuals  
**in relation to** n.a.

**Unit**

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** For seagrass species the shoot density has been used - same method as developed in Denmark

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**  
n.a.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

Index is calculated for each transect. For the quality assessment of a water body, the data from several transects in that water body are used to calculate the EQR

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** n.a.

**Location of sites:** n.a.

**Data time period:** Two data sets from the 1940-ties, one in the Gräsö region, Åland Sea and one in Gullmaren, Swedish west coast.

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

**3.14 Comments:**

none

ID: 83

SE-BI-CT

## 1. General information

- 1.01 GIG:** Baltic, North-East-Atlantic  
NEA 8, 9, 10 and Baltic B0, B2, B3 and B12
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Sweden
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment of Biological Quality Elements in coastal and transitional waters - benthic invertebrate fauna*
- 1.07 Original name:** *Bedömningsgrunder för biologiska kvalitetsfaktorer i kustvatten och vatten i övergångszon - bottenfauna*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Examples in Josefson et al Marine Pollution Bulletin 58 (2009) 1263–1277 "Assessment of marine benthic quality change in gradients of disturbance: Comparison of different Scandinavian multi-metric indices"
- 1.10 Internet reference:** <http://www.naturvardsverket.se/sv/Arbete-med-naturvard/Vattenforvaltning/Lagstiftning-och-vagledning/Vagledning/Handbok-20074/>
- 1.11 Pertinent literature of mandatory character:**  
Naturvårdsverkets författningssamling, NFS2008:1.
- 1.12 Scientific literature:**  
Leonardsson, K., M. Blomqvist & R. Rosenberg, 2009. Theoretical and practical aspects on benthic quality assessment according to the EU-Water Framework Directive- examples from Swedish waters. Marine Pollution Bulletin 58: 1286–1296. Rosenberg, R., M. Blomqvist, H.C. Nilsson, H. Cederwall & A. Dimming, 2004. Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. Marine Pollution Bulletin 49: 728–739.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Mats Blomqvist, Hans Cederwall, Kjell Leonardsson, Rutger Rosenberg<br>mb@hafok.se, hlc@ecology.su.se,<br>Kjell.Leonardsson@vfm.slu.se, rutger.rosenberg@marecol.gu.se<br>Hafok AB; Department of Systems Ecology Stockholm University;<br>Department of Wildlife, Fish, and Environmental Studies,<br>Swedish University of Agricultural Sciences; Department of<br>Marine Ecology, University of Gothenburg | <b>1.14 Method reported by</b><br>Mats Blomqvist<br>mb@hafok.se<br>Hafok AB |
|--|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
Standard marine benthic sampling, 0.1 m<sup>2</sup> grab, 1 mm sieve, conservation in formaldehyde or ethanol, sorting at six times magnification.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying, Stratified sampling/s
- 2.04 Sampling/survey device:** Grab
- 2.05 Specification:** van Veen Grab, Smith-McIntyre Grab
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** mainly may-june
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Depends on homogeneity of water body, rule of thumb at least 5 stations per water body (one grab per station)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
In general 0.5 - 2 square meters per water body (5 to 20 grabs)

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 1 mm
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to Area  
Unit number of individuals per grab
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Benthic Quality index (BQI) based on sensitivity of species, number of species and total abundance. See formula in Leonardsson et al 2009 Marine Pollution Bulletin 58 1286–1296.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single spatial replicate  
Index is calculated for each grab. For the quality assessment of a water body, the data from several sites in the water body is used to calculate the lower confidence limit, which is then compared with the status boundaries.

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** n.a.  
see description in Leonardsson et al 2009 Marine Pollution Bulletin 58 1286–1296.
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** Yes
- 3.14 Comments:**  
Difficult to answer the last questions. Please see descriptions on our principles for boundary setting, handling of uncertainty etc in Leonardsson et al 2009 Marine Pollution Bulletin 58 1286–1296.

ID: 178

AMBI

## 1. General information

- 1.01 GIG:** Black Sea  
n.a.
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Romania
- 1.05 Specification:** Romanian Black Sea waters
- 1.06 Method name:** *Assessment method for coastal waters using macrozoobenthos*
- 1.07 Original name:** n.a.
- 1.08 Status: Method is/will be used in** n.a.

**1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction

*Has the pressure-impact-relationship been tested?*

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

Borja, A. & I. Muxica, 2005. Guidelines for the use of AMBI (AZTI's Marine Biotic Index) in the assessment of the benthic ecological quality. Marine Pollution Bulletin 50: 787-789. [Borja, A., I. Muxica & J. Franco, 2003. The application of a marine biotic index to different impact sources affecting soft-bottom communities along European coasts. Marine Pollution Bulletin 46: 835-845. Borja, A., J. Franco & V. Perez, 2000. A Marine Biotic Index to establish the ecological quality of soft bottoms within European estuarine and coastal environments. Marine Pollution Bulletin 40 \(12\): 1100-1114.](#)

**1.13 Method developed by**

n.a.

n.a.

**1.14 Method reported by**

Camelia Dumitrache and Valeria Abaza

iulia@alpha.rmri.ro, abaza@alpha.rmri.ro

Department of Ecology and Environmental Protection, National Institute for Environmental Protection R&D

**1.15 Comments**

This method was applied using the list of benthic invertebrates found in the Romanian Black Sea waters.

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Todorova, V. & T. Konsulova, 2005. Manual for quantitative sampling and sample treatment of marine soft-bottom macrozoobenthos. [www.blacksea-commission.org](http://www.blacksea-commission.org)

**2.02 Short description**

Single-sampling is carried out. A sample consists of 24 "sampling units" taken from all soft-bottom habitat types. A "sampling unit" is a stationary sampling performed with Van Veen Grab long arm from an area of 0.25 x 0.2 m.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Grab

**2.05 Specification:** Van Veen Grab long arm

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** February, April to July, October-November

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

24 replicates (one per sampling site)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Sum of 24 spatial replicates a 0.05 square-metres = 1.2 square-metres of soft-bottom in total

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 0.250 mm

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Genus, Other, Species/species groups

Molluscs, Polychaeta, amphipods, isopods, decapods, tanaides, cumaceans are identified to species/genus level. Others,

like oligochaetes, chironomids, Harpacticoida, nematodes to level of order.

**2.15 Record of abundance:** Individual counts

**in relation to** Area

**Unit** Number of individuals per one square-metre

**2.16 Quantification of biomass:** n.a.

Direct weighting for bigger groups and using weight tables for the others

**2.17 Other biological data:** Length of individual specimens for molluscs and decapods

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

List of species, abundance of taxa and biomass (wet weight) of taxa

**3.02 Does the metric selection differ between types of water bodies?** 0

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

Data from single spatial replicate

#### Reference conditions

**3.05 Scope of reference conditions:** Habitat-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data

**3.07 Reference site characterisation**

**Number of sites:** 11 sites at the Romanian Black Sea

**Geographical coverage:** All Romanian Black Sea shore between Sulina (in North) and Vama Veche (in South)

**Location of sites:** Sulina, Mila 9, Sf. Gheorghe, Portita, Chituc, Gura Buhaz, Constanta, Eforie South, Costinesti, Mangalia, Vama Veche

**Data time period:** Historical data between 1960s and 1980s

**Criteria:**

The soft bottom communities suffered in the last four decades different modifications due to ecological changes occurrence. In the last decade, nutrient inputs from the inland waters (Danube River) significantly decreased, allowing to sensitive species (mentioned above at C-11) to develop stable and abundant populations.

**3.08 Reference community description**

Soft bottom communities with high number of sensitive taxa, and high abundance, the most characteristic species being different according to grain size: *Donacilla cornea*, *Donax trunculus*, *Lentidium mediterraneum*, *Spio filicornis* and others.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

Macroinvertebrates were placed into five groups in relation with their sensitivity to an increasing stress gradient. These five groups are as follows: (I). Species very sensitive to organic enrichment and present under unpolluted conditions (initial state) (II). Species indifferent to enrichment (III). Species tolerant to excess organic matter enrichment (IV). Second-order opportunistic species (V). First-order opportunistic species. Boundaries for HG and GM were determined from this relationship. The HG boundary was identified as the point at which all tolerant species were on average <10% of cover. The GM boundary was the point at which the lower confidence limits of the sensitive and upper confidence limit of the tolerant species intersect. At this point there is still a high probability of having >50% cover of sensitive species and no more than 50% cover of tolerant species. This would be indicative of slight change, the community could still easily recover to its original status. The highly sensitive species are still present (10-50% cover) and highly tolerant (undesirable) species would be <20% cover. The MP boundary was set where the lower confidence limit of the sensitive and upper confidence limit of the tolerant species intersect. At this point there is a low probability that sensitive species would be at 50% cover, but a high probability that tolerant species would be at 50% cover. Very sensitive species are still present, but the community has thus undergone a moderate change. The PB boundary is a point at which highly sensitive species are extinct and there are very

few sensitive species. Here the community is dominated by tolerant species.

**3.12 "Good status" community:** At good status stands of the sensitive taxa are well developed, but significantly decreasing at good-moderate boundary and replaced by tolerant taxa.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 191

RO-PP-CT

## 1. General information

- 1.01 GIG:** Black Sea
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Romania
- 1.05 Specification:**
- 1.06 Method name:** *Assessment system for coastal and transitional waters using phytoplankton*
- 1.07 Original name:** n.a.
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** Eutrophication, General degradation
- Has the pressure-impact-relationship been tested?*
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**
- 1.14 Method reported by**  
Laura Boicenco  
boicenco@alpha.rmri.ro  
Department of Environmental Protection, National Institute for  
Marine Research and Development "Grigore Antipa" (NIMRD)
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Bodeanu, N., 1987-88. Structure et dynamique de l'algoflore unicellulaire dans les eaux du littoral roumain de la mer Noire. Cercetari marine, IRCM Constanta, 20/ 21: 19 - 250. Edler, L., 1979. Recommendations on methods for Marine Biological Studies in the Baltic Sea. Phytoplankton and Chlorophyll. Baltic Marine Biologists Publication No. 5, pp. 38. Moncheva, S., 2005. Standard operating procedures for phytoplankton sampling and analysis in the Black Sea, pp. 22. Morozova-Vodianitkaia, N.V., 1954. Fitoplankton Cernogo Moria. II. Tr. Sev. Biol. St. 9: 11-99. Uteromohl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt int. Verein. theor. angew. Limnol. 9: 1-38.
- 2.02 Short description**  
Phytoplankton samples have been collected with Nansen bottles (enabling more than 500 ml of water per sample) and immediately treated with a formaldehyde solution of 4% concentration, followed by further processing based on sedimentation method.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** Nansen bottles (enabling 500 ml of water per sample)
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** March - November
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one sampling per spring and autumn season and monthly sampling during the summer season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
no replicates per samples are taken
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** microalgae size between 5 - 50 µm
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
- samples are processed by sedimentation method (Morozova, Vodianitzkaya, 1956, Bodeanu, 1987/88) - two weeks sedimentation and then concentration (at 15-20ml concentrate) - the determination and counting of the cells by species

in the 0.1 ml fraction of the analyzed sample was carried out at plankton inverted microscopes, using objective lens of 40x, 63x for small forms (less than 5-20 µm) and of 10x, 16.3x or 20x for those exceeding those sizes.

**2.14 Level of taxonomical identification:** Genus, Species/species groups

**2.15 Record of abundance:** Individual counts  
in relation to Volume

Unit number of cells per litre

**2.16 Quantification of biomass:** Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**  
abundance (cells/l) and biomass (mg/m<sup>3</sup>) determination for the total phytoplankton

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Site-specific

**3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data

**3.07 Reference site characterisation**

**Number of sites:** 5 transects from the Romanian Black Sea coastal marine waters

**Geographical coverage:**

**Location of sites:** Romanian

**Data time period:** Reference data from 1960-1970 period used for define the "high" quality status; for the "bad" quality status we used the data from the period 1986-1997 (known as maximum eutrophication period to the north-western Black Sea)

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 172

M-AMBI

## 1. General information

- 1.01 GIG:** Black Sea, Mediterranean, North-East-Atlantic  
several
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Spain
- 1.05 Specification:** NEA coastal regions (Basque Country, Cantabria, Asturias, Galicia, Andalucía, Canary Islands) and TW in Basque Country
- 1.06 Method name:** *Multivariate AMBI*
- 1.07 Original name:** *Multivariate AMBI*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Heavy metals, Hydromorphological degradation, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter  
Dredging

### **Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

It has been tested by different authors, under many different pressures and geographical areas, you can see next references: Borja, A., A. B. Josefson, A. Miles, I. Muxika, F. Olsgard, G. Phillips, J. G. Rodr guez, B. Rygg, 2007. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. *Marine Pollution Bulletin*, 55: 42-52. Muxika, I., A. Borja, J. Bald, 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. *Marine Pollution Bulletin*, 55: 16-29. Bigot, L., A. Gremare, J.-M. Amouroux, P. Frouin, O. Maire, J. C. Gaertner, 2008. Assessment of the ecological quality status of soft-bottoms in Reunion Island (tropical Southwest Indian Ocean) using AZTI marine biotic indices. *Marine Pollution Bulletin*, 56: 704-722. Borja, A., D. Dauer, R. Diaz, R. J. Llans s, I. Muxika, J. G. Rodr guez, L. Schaffner, 2008. Assessing estuarine benthic quality conditions in Chesapeake Bay: A comparison of three indices. *Ecological Indicators*, 8: 395-403. Bouchet, V. M. P., P.-G. Sauriau, 2008. Influence of oyster culture practices and environmental conditions on the ecological status of intertidal mudflats in the Pertuis Charentais (SW France): A multi-index approach. *Marine Pollution Bulletin*, 56: 1898-1912. de Paz, L., J. Patr cio, J. C. Marques, A. Borja, A. J. Laborda, 2008. Ecological status assessment in the lower Eo estuary (Spain). The challenge of habitat heterogeneity integration: A benthic perspective. *Marine Pollution Bulletin*, 56: 1275-1283. Teixeira, H., F. Salas, J. M. Neto, J. Patr cio, R. Pinto, H. Ver ssimo, J. A. Garc a-Charton, C. Marcos, A. P rez-Ruzafa, J. C. Marques, 2008. Ecological indices tracking distinct impacts along disturbance-recovery gradients in a temperate NE Atlantic Estuary - Guidance on reference values. *Estuarine, Coastal and Shelf Science*, 80: 130-140. Bakalem, A., T. Ruellet, J. C. Dauvin, 2009. Benthic indices and ecological quality of shallow Algeria fine sand community. *Ecological Indicators*, 9: 395-403. Borja, A., I. Muxika, J. G. Rodr guez, 2009. Paradigmatic responses of marine benthic communities to different anthropogenic pressures, using M-AMBI, within the European Water Framework Directive. *Marine Ecology*, 30: 214-227. Lavesque, N., H. Blanchet, X. de Montaudouin, 2009. Development of a multimetric approach to assess perturbation of benthic macrofauna in *Zostera noltii* beds. *Journal of Experimental Marine Biology and Ecology*, 368: 101-112. Pinto, R., J. Patricio, A. Baeta, B. D. Fath, J. M. Neto, J. C. Marques, 2009. Review and evaluation of estuarine biotic indices to assess benthic condition. *Ecological Indicators*, 9: 1-25. Simonini, R., V. Grandi, G. Massamba-N'Siala, M. Lotti, G. Montanari, D. Prevedelli, 2009. Assessing the ecological status of the North-western Adriatic Sea within the European Water Framework Directive: a comparison of Bentix, AMBI and M-AMBI methods. *Marine Ecology*, 30: 241-254. Tomassetti, P., E. Persia, I. Mercatali, D. Vani, V. Marussso, S. Porrello, 2009. Effects of mariculture on macrobenthic assemblages in a western mediterranean site. *Marine Pollution Bulletin*, 58: 533-541.

**1.10 Internet reference:** <http://www.azti.es>

### **1.11 Pertinent literature of mandatory character:**

ORDEN ARM/2656/2008, de 10 de septiembre, por la que se aprueba la instrucci n de planificaci n hidrol gica. BOE 229, 22 septiembre 2008.

### **1.12 Scientific literature:**

Borja, A., A.B. Josefson, A. Miles, I. Muxika, F. Olsgard, G. Phillips, J.G. Rodr guez & B. Rygg, 2007. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. *Marine Pollution Bulletin* 55: 42-52. Borja, A., J. Franco, V. Valencia, J. Bald, I. Muxika, M.J. Belzunce & O. Solaun, 2004. Implementation of the European Water Framework Directive from the Basque Country (northern Spain): a methodological approach. *Marine Pollution Bulletin* 48: 209-218. Borja, A., J. Mader, I. Muxika, J.G. Rodr guez & J. Bald, 2008. Using M-AMBI in assessing benthic quality within the Water Framework Directive: Some remarks and recommendations. *Marine Pollution Bulletin* 56: 1377-1379. Borja, A., I. Muxika & J.G. Rodr guez, 2009. Paradigmatic responses of marine benthic communities to different anthropogenic pressures, using M-AMBI, within the European Water Framework Directive. *Marine Ecology* 30: 214-227. Muxika, I., A. Borja & J. Bald, 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. *Marine Pollution Bulletin* 55: 16-29.

### **1.13 Method developed by**

Angel Borja  
aborja@azti.es  
AZTI-Tecnalia; Marine Research Division

### **1.14 Method reported by**

Angel Borja  
aborja@azti.es  
AZTI-Tecnalia; Marine Research Division

### **1.15 Comments**

none

## 2. Data acquisition

### **Field sampling/surveying**

#### **2.01 Sampling/Survey guidelines**

Standards protocol: ISO 16665.

## 2.02 Short description

2-6 sampling locations are visited per water body once a year in winter. At each location 3 van Veen grab replicates are taken (0.1 square-metres each), and sieved on board by 1 mm mesh.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Grab

**2.05 Specification:** Van Veen Grab

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft-bottom

**2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones

**2.08 Sampling/survey month(s):** winter

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Once a year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

3 replicates per station (2-6 stations per water body)

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

0.3 square-metres (each replicate has 0.1 square-metres)

## Sample processing

**2.12 Minimum size of organisms sampled and processed:** 1 mm mesh

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

Some groups can be identified to higher taxonomical levels.

**2.15 Record of abundance:** Individual counts

in relation to Area

Unit Number of individuals per one square-metre

**2.16 Quantification of biomass:** n.a.

dry weight

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

## 3. Data evaluation

### Evaluation

#### 3.01 List of biological metrics

Composition of the species is needed to calculate AMBI. Then also richness and Shannon's diversity is calculated. From here M-AMBI is derived. Exists a free software to calculate it from Excel tables including species composition and abundance. The web page is <http://www.atzi.es>.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

Is a multivariate analysis (Factor Analysis) which calculates vectorial distances to reference conditions

#### 3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

AMBI is calculated per replicate and then averaged by sampling station, richness and diversity by station.

### Reference conditions

**3.05 Scope of reference conditions:** Habitat-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data, Modelling (extrapolating model results)

#### 3.07 Reference site characterisation

**Number of sites:** no specific number

**Geographical coverage:** Northern Spain

**Location of sites:** Basque Country

**Data time period:** 1995-2005

**Criteria:**

Virtual locations, see: Muxika, I., A. Borja, J. Bald, 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. Marine Pollution Bulletin, 55: 16-29.

**3.08 Reference community description**

See: Borja, A., Aguirrezabalaga, F., Martinez, J., Sola, J.C., Garciaarberas, L., & Gorostiaga (2003). Benthic communities, biogeography and resources management. In: Borja, A. & Collins, M. (Ed.). Ocenaography and Marine Environment of the Basque Country, Elsevier Oceanography Series n. 70: 27-50.

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise

**3.11 Boundary setting procedure**

See: Borja A, Josefson AB, Miles A, Muxika I, Olsgard F, Phillips G, Rodríguez JG, Rygg B (2007) An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. Marine Pollution Bulletin 55:42-52.

**3.12 "Good status" community:** Borja A, Josefson AB, Miles A, Muxika I, Olsgard F, Phillips G, Rodríguez JG, Rygg B (2007): An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. Marine Pollution Bulletin 55:42-52.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 232

EI

## 1. General information

- 1.01 GIG:** Mediterranean
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Angiosperms, Macroalgae
- 1.04 Country:** Greece
- 1.05 Specification:**
- 1.06 Method name:** *Ecological Evaluation Index*
- 1.07 Original name:** *Ecological Evaluation Index*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Pollution by organic matter
- Has the pressure-impact-relationship been tested?*
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
  
sorfanid@inale.gr
- 1.14 Method reported by**  
Sotiris Orfanidis  
sorfanid@inale.gr  
National Agricultural Research Foundation (NAGREF)-Fisheries  
Research Institute (FRI)

### 1.15 Comments

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Boudouresque, C.F., 1971. Méthodes d' étude qualitative et quantitative du benthos (en particulier du phytobenthos). Téthys (1): 79-104.
- 2.02 Short description**  
Sampling follows a nonaligned block design, in which a sample is located randomly within a representative permanent cell of dimensions 10m × 10 m. The sampling is destructive, using for coastal lagoons a metal hand-held box corer (17 cm17 cm15 cm; lengthwidthheight), which is vertically pushed through the benthic vegetation and sediment. From each sample the existing vegetation (seaweeds, seagrasses leaves and roots, Cyanobacteria colonies) was carefully removed and placed individually in airtight plastic bags, where it was fixed in 4–5% formalin in sea water for a few seconds. The excess formalin solution was later removed from the plastic bag, which was then sealed, labelled, and stored in a plastic box. A similar procedure is followed in coastal waters using a metallic frame of 25x25cm.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.
- 2.05 Specification:** Metallic frame for coastal waters, Box corer for TWs
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Soft bottom for coastal lagoons (benthic macrophytes), hard bottom for coastal waters
- 2.07 Sampled/surveyed zones in area (as well as tidal influence):** Subtidal zone
- 2.08 Sampling/survey month(s):** All year, preferably from April to November
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Two or three sampling occasions
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3 to 5 replicates per sampling occasion per site
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Sum of at least 9-12 spatial replicates: ca. 0.289 sqm for coastal lagoons, 0.625 sqm for coastal waters

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** all down to light microscope scale
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Other, Species/species groups  
All plants are classified at a species and a functional group (sensu Orfanidis et al. 2001) level.

- 2.15 Record of abundance:** Percent coverage  
     **in relation to** Area  
     **Unit** % coverage
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
% coverage of Ecological State Group I, II
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Habitat-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**
- Number of sites:** 62 Aegean sites in the Mediterranean Sea
- Geographical coverage:** n.a.
- Location of sites:** Greek Aegean Islands
- Data time period:** One year
- Criteria:**

1. Macroalgal communities of high diversity should be dominated quantitatively by brown algae mainly of the order Fucales in high irradiance sites and red algal Corallinales in vertical cliffs. 2. Dense well-developed macroalgal communities thriving in the upper infralittoral zone with most characteristic species belonging to the genera *Cystoseira*, *Sargassum*, *Lithophyllum*, *Peyssonnelia*, *Corallina* and *Padina*. Other common species belong to the genera *Halopteris*, *Stypocaulon*, *Dictyota*, *Dictyopteris*, *Laurencia*, *Cladophora* and *Jania*. 3. In shadow zones (exposed steep vertical cliffs) *Lithophyllum byssoides* develops, forming important organogenic structures (trottoir). In marine caves with scarce light conditions a sciaphilic vegetation of red and green algae dominant. 4. Spatio-temporal variability of the community's composition and abundance affected by hard substrata availability, intense and frequency of natural disturbances, e.g. hydrodynamism, grazing, by seasonal cycle of light period and intense, and by limiting factors like nutrients.

#### **3.08 Reference community description**

For the description of macroalgal community of the rocky upper infralittoral zone reference conditions in Greek coastal waters 62 samples from 26 putatively pristine Aegean sites dominated by *Cystoseira cf. crinita* community as part of the Hellenic "NATURA 2000" data-base (see Panayotidis et al., 2001) in combination with the biotic index Ecological Evaluation-EEI Index (Orfanidis et al., 2001; 2003) were used. The aim was (1) to develop an objective and statistically valid "virtual" list of the most common algal species in the Aegean under undisturbed conditions, and (2) to test the conceptual model and the EEI recently developed by Orfanidis et al. (2001, 2003) for the implementation of Water Framework Directive (2000/60/EC) in Greek coasts. In total 113 taxa (73 Rhodophyceae, 25 Phaeophyceae, 15 Chlorophyceae) were identified in *Cystoseira cf. crinita* community of the Aegean Sea (Panayotidis et al., 2007). Nine (9) major taxa (except *C. cf. crinita*) contributed cumulatively by 90% in the community: *Haliptilon virgatum*, *Cystoseira compressa*, *Jania rubens*, *Padina pavonica*, *Herposiphonia secunda*, *Corallina elongata*, *Cladophora* spp., *Sphacelaria cirrosa* and *Titanoderma cystoseirae*. Moreover, 34 taxa contributed cumulatively by 99%. Under-storey layer considerably dominated to the community with most common representatives the red coralligenous algae *Haliptilon virgatum*, *Corallina elongata* and *Jania rubens*, and the brown alga *Padina pavonica*. It was followed by *C. crinita* epiphytes distinguished in: 1) filamentous green (*Cladophora* spp.), brown (*Sphacelaria cirrosa*) and red (*Herposiphonia secunda*) algae, and 2) in encrusting red algae (*Titanoderma cystoseirae* and *Hydrolithon* spp.). *Cystoseira compressa* contributed significantly (23.08%) to *C. crinita* community indicating that these species share common habitat resources in the Aegean Sea.

- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** There are slight changes in the composition and abundance of macroalgal taxa compared to the type-specific communities. Such changes do not indicate any accelerated growth of phytobenthos or higher forms of plant life resulting in undesirable disturbance to the balance of organisms present in the water body or to the physicochemical quality of the water. This condition corresponds with slightly polluted sites (unbalanced). At the good status as is indicated by the EEI, the ESG I group may range from 30 to 60% while the ESG II from 0 to 30% of the macroalgae coverage, or the combination may thus that ESG I accounts for over 60% and ESG II between 30 and 60% of the total macroalgae coverage.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 131

DE-AN-CT

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
Note: Site specific boundaries in the northern Wadden Sea (not intercalibrated at the present)
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Angiosperms  
Zostera marina, Zostera noltii, saltmarsh vegetation - see separately
- 1.04 Country:** Germany
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment tool for intertidal seagrass in coastal and transitional waters*
- 1.07 Original name:** *Verfahren zur Bewertung der eulitoralen Seegrassbestände in Küsten- und Übergangsgewässern*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, Habitat destruction, Hydromorphological degradation  
*Has the pressure-impact-relationship been tested?*  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt\\_Makrophyten.html](http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt_Makrophyten.html)
- 1.11 Pertinent literature of mandatory character:**  
Dolch, T., C. Buschbaum & K. Reise, 2008. Seegrass-Monitoring im Schleswig-Holsteinischen Wattenmeer. AWI, Sylt. Jaklin, S., B. Petersen, W. Adolph, G. Petri & W. Heiber, 2007. Aufbau einer Matrix für die Gewässertypen nach EG-WRRL im Küstengebiet der Nordsee, Schwerpunkt Flussgebietseinheiten Weser und Elbe. Abschlussbericht Teil A: Nährstoffe, Fische, Phytoplankton, Makrophyten (Makroalgen und Seegrass). Berichte des NLWKN 2007. Kolbe, K., 2007. Intercalibration Report (NEA GIG). Assessment of German Coastal Waters (NEA1/26, NEA3/4) and Transitional Waters (NEA11) by Macroalgae and Angiosperms. NLWKN Wilhelmshaven.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Karsten Reise, Kerstin Kolbe, Sandra Jaklin, Winny Adolph  
Kerstin.Kolbe@nlwkn-ny.niedersachsen.de,  
Karsten.Reise@awi.de  
Lower Saxony Water Management, Coastal Defense and Nature Conservation Agency (NLWKN, Lower Saxony); State Agency for Agriculture, Environment and Rural Areas (LLUR - Schleswig-Holstein)
- 1.14 Method reported by**  
Wilfried Heiber  
Wilfried.Heiber@nlwkn-bra.niedersachsen.de  
Lower Saxony Water Management, Coastal Defense and Nature Conservation Agency (NLWKN)
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Monitoring-Handbuch des Bund-Länder-Messprogramms Meeresumwelt (MHB). [http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt\\_Makrophyten.html](http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt_Makrophyten.html)
- 2.02 Short description**  
Throughout the flight the areas covered by seagrass are recorded on topographic maps (Schleswig-Holstein); aerial photography 1:20 000. Field mapping: crossing the seagrass fields along transects and noting species composition and density
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** n.a.  
Aerial mapping in combination
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)  
Only habitats within the NEA types CW 2 (26) and 4 ,and TW 11;
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Intertidal zone
- 2.08 Sampling/survey month(s):** July to September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season (one sampling season per every six years); some selected monitoring sites will be mapped
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
n.a.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Total area surveyed

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** -----
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Abundance classes, Percent coverage  
**in relation to** Area  
**Unit** Portion of intertidal (in percent) covered by seagrass fields; density of seagrass within the seagrass field
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
The sub-component 'seagrass' (with its metric 'acreage/bed extent of intertidal seagrass' and the combined metric 'species composition/density') is part of the assessment tool for the quality component Macrophytes ; other sub-components are 'saltmarsh vegetation' and macroalgae (specific metrics look there). Note: Macroalgae only in coastal waters.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores, Weighted average metric scores  
Note: Average metric scores (Lower Saxony); Weighted average metric scores (Schleswig-Holstein)
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** German Wadden Sea  
**Location of sites:** n.a.  
**Data time period:** Historical quantitative data and qualitative descriptions from the 1920s to the 1970s; Schleswig-Holstein: data mainly before the 1950s, Niedersachsen mainly data since the 1950s and 1960s  
**Criteria:**  
Absence of eutrophication and mechanical disturbance. Hydromorphological stress within the natural scale. According to this pressure at the present no reference site situation in the Wadden Sea.
- 3.08 Reference community description**  
Seagrass beds cover up to 30% of the intertidal area. Both species are present. Within the seagrass fields the sediment is densely covered by seagrass.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Southern German Wadden Sea: boundaries taken over from the intercalibration exercise. Northern German Wadden Sea: Geometric row.
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** Within the good status the abundance (parameters: bed extent, density of seagrass within the beds) does not fall below 70 % of the abundance (southern Wadden Sea) respectively 50 % of the abundance (northern Wadden Sea) in the reference situation.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

It will be checked how the sub-component 'Seagrass' might be completed by integrating a metric which focuses on sublittoral seagrass. The differences in the boundary setting between Schleswig-Holstein and Niedersachsen which are existing actually will be checked.

ID: 130

DE-AN-CT

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Angiosperms  
saltmarsh vegetation
- 1.04 Country:** Germany
- 1.05 Specification:** Southern German Wadden Sea, estuaries of the rivers Ems and Weser
- 1.06 Method name:** *Tool the assessment of saltmarsh vegetation coastal and transitional waters*
- 1.07 Original name:** *Verfahren zur Bewertung der Salzwiesen in Küsten- und Übergangsgewässern*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation  
*Has the pressure-impact-relationship been tested?*  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt\\_Makrophyten.html](http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt_Makrophyten.html)
- 1.11 Pertinent literature of mandatory character:**  
Adolph, W., G. Petri, S. Janklin, B. Petersen & W. Heiber, 2007. Aufbau einer Matrix für die Gewässertypen nach EG-WRRL im Küstengebiet der Nordsee, Schwerpunkt Flussgebietseinheiten Weser und Elbe. Abschlussbericht Teil B: Makrophyten (Röhrichte, Brack- und Salzmarschen). Berichte des NLWKN 2007. [Arens, S., 2006. Bewertungssystem nach WRRL für die Angiospermen der Übergangs- und Küstengewässer der FGE Weser und für das Küstengewässer der FGE Elbe. Berichte des NLWKN 2006.](#) [Arens, S., 2009. Erfassung und Bewertung der Röhrichte, Brack- und Salzmarschen \(Makrophyten/Angiospermen\) im Rahmen eines Praxistests zur Umsetzung der EG-WRRL in den Übergangsgewässern von Weser und Ems. Berichte des NLWKN 2009.](#)
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Sabine Arens, Winny Adolph  
Plantagis@web.de  
Lower Saxony Water Management, Coastal Defense and Nature Conservation Agency (NLWKN, Lower Saxony);
- 1.14 Method reported by**  
Wilfried Heiber  
Wilfried.Heiber@nlwkn-bra.niedersachsen.de  
Lower Saxony Water Management, Coastal Defense and Nature Conservation Agency (NLWKN)
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Monitoring-Handbuch des Bund-Länder-Messprogramms Meeresumwelt (MHB). [http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt\\_Makrophyten.html](http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt_Makrophyten.html)
- 2.02 Short description**  
Mapping the extent of the saltmarsh area by aerial photography. Mapping the zonation of the saltmarsh by aerial photography combined with field mapping.
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** n.a.  
Aerial mapping in combination
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Intertidal zone
- 2.08 Sampling/survey month(s):** June to September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season (one sampling season per every six years); some selected monitoring sites might be
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
n.a.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Total area surveyed
- 2.12 Minimum size of organisms sampled and processed:** -----
- 2.13 Sample treatment:** n.a.

- 2.14 Level of taxonomical identification:** Other
- 2.15 Record of abundance:** Percent coverage, Relative abundance  
**in relation to** Area  
**Unit** Portion of the saltmarsh area within the water body; portion of the specific saltmarsh zone within the saltmarsh area
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
The sub-component 'saltmarsh' (with it's metrics 'extent of saltmarsh area' and 'zonation of saltmarsh') is part of the assessment tool for the quality component Macrophytes ; other sub-components are 'seagrass' and macroalgae (specific metrics look there). Note: in transitional waters without macroalgae, but additionally with brackish marsh metrics for the oligo- to mesohaline zone of the estuary
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** Southern German Wadden Sea and estuaries  
**Location of sites:** n.a.  
**Data time period:** Historical quantitative data from 1860 (saltmarsh area extent); quantitative data from 1950s to 1990s (zonation)  
**Criteria:**  
Absence of eutrophication; mechanical and hydromorphological disturbance within the natural scale.
- 3.08 Reference community description**  
The saltmarsh area corresponds to the specific natural potential of the specific water body; all saltmarsh zone are present and well developed.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** Within the good status the salt marsh extent does not fall below 75 % of the water body specific extent in the reference situation. The occurrence of the different salt marsh zones shows only slight deviations from a balanced zonation.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
It is intended to develop the assessment tool further in cooperation with Schleswig-Holstein and Hamburg.

ID: 103

Seagrass

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Angiosperms  
Zostera noltii and Zostera marina (sensu angustifolia)
- 1.04 Country:** Ireland
- 1.05 Specification:** none
- 1.06 Method name:** *Intertidal Seagrass*
- 1.07 Original name:** *Intertidal seagrass: abundance (areal extent and density) and species composition*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, Hydromorphological degradation, Impact of alien species  
*Has the pressure-impact-relationship been tested?*  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Foden, J. & D.J. de Jong, 2007. Assessment metrics for littoral seagrass under the European Water Framework Directive; outcomes of UK intercalibration with the Netherlands. *Hydrobiologia* 583 (1): 187-197. Foden J. & D.P. Brazier, 2007. Seagrass within the EU Water Framework Directive; a UK perspective. *Marine Pollution Bulletin* 55: 181-195.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Robert Wilkes<br>r.wilkes@epa.ie<br>Environmental Protection Agency | <b>1.14 Method reported by</b><br>Robert Wilkes<br>r.wilkes@epa.ie<br>Environmental Protection Agency |
|--|---|
- 1.15 Comments**  
Tool will be used in second RMBP as no background data existed for first round

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
Ensure the fieldwork is carried out at the time of peak biomass, i.e. before overwintering wildfowl arrive and graze the intertidal Zostera. The procedure involves mapping the perimeter of each of the sea grass beds encountered. If the survey is subsequent to previous surveys of the same bed of Zostera ensure all coordinates and maps of the bed in question are on site during fieldwork. The area is mapped with GPS. The boundary of the Zostera bed is determined as the level at which there is 5% or greater cover of Zostera sp. on the sediment. In areas of patchiness, the extent of the bed was strictly kept to the 5% cover level. Distinct patches were isolated and identified separately. Transects are run across each sea grass bed (maximum of 4 transects/bed depending on the size of the bed). Quadrats (0.25m<sup>2</sup>) are randomly placed along each transect. The percentage of Zostera within each quadrat is recorded.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.
- 2.05 Specification:** In situ shore survey
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Intertidal seagrass beds
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Intertidal zone
- 2.08 Sampling/survey month(s):** August to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season over several years
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
all beds in WB assessed, adequate quadrats taken for biomass assessment
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
All intertidal seagrass beds in waterbody assessed

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n/a

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Percent coverage  
**in relation to** Area

**Unit** Shoot density as percent cover

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** shoot density

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

1 Change in spatial extent 2 Change in shoot density 3 Change in number of species

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

##### 3.04 From which biological data are the metrics calculated?

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Expert knowledge, Historical data

##### 3.07 Reference site characterisation

**Number of sites:** still to be decided

**Geographical coverage:** Historical data and recent data from unimpacted sites

**Location of sites:** Various sites around Ireland

**Data time period:** still to be decided

**Criteria:**

still to be established

##### 3.08 Reference community description

Spatial extent, species composition and shoot density stable.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient

##### 3.11 Boundary setting procedure

n.a.

**3.12 "Good status" community:** No change in species composition, only slight decrease in shoot density or spatial extent.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

##### 3.14 Comments:

none

ID: 85

UK-AN-CO

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Angiosperms  
Zostera nolti, Z. marina; Z.marina (var angustifolia), Ruppia spp.
- 1.04 Country:** United Kingdom
- 1.05 Specification:** none
- 1.06 Method name:** *Seagrass*
- 1.07 Original name:** *Seagrass*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/angiosperms](http://www.wfduk.org/bio_assessment/bio_assessment/angiosperms)
- 1.11 Pertinent literature of mandatory character:**  
[http://www.wfduk.org/bio\\_assessment/bio\\_assessment/angiosperms](http://www.wfduk.org/bio_assessment/bio_assessment/angiosperms) UKTAG Transition an coastal water assessment methods angiosperms Seagrass (Tostera) bed assessment (Draft). To be read in conjunction with contracting parties Ministerial Directions.
- 1.12 Scientific literature:**  
Foden, J. & D.J. de Jong, 2007. Assessment metrics for littoral seagrass under the European Water Framework Directive; outcomes of the Auk intercalibration with the Netherlands. *Hydrobiologia* 579: 187-197. Foden, J. & D.P. Braizier, 2007. Angiosperms (seagrass) within the EU water framework directive: a UK perspective. *Marine Pollution Bulletin* 55: 181-195.
- 1.13 Method developed by** through UK & RoI Marine Plants Task Team, Chair Mike Best, lead developer Jo Foden  
mike.best@environment-agency.gov.uk, Jo.Foden@cefas.co.uk  
UK & RoI MPTT, funding via Environment Agency, Scottish and Northern Ireland Forum for Environmental Research (SEPA, EANI), CEFAS
- 1.14 Method reported by** Mike Best  
mike.best@environment-agency.gov.uk  
Environment Agency (for England & Wales)
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
WFD tool paper and sampling guidelines converted into competent authorities standard operating procedures (SOPs) eg Operational instruction 214\_07 Intertidal seagrass monitoring for Water Framework Directive (WFD) purposes and Operational instruction 202-07 Technical reference material: mapping marine plants for Water Framework Directive (WFD).
- 2.02 Short description**  
This monitoring protocol applies to littoral seagrass beds in CWs and TWs. The three key monitoring metrics are: • Taxonomic composition: seagrass species present • Bed extent – area cover in m<sup>2</sup> of the continuous bed (deemed to be >5% shoot density) and, where possible, the whole bed (<5% shoot density). This may be determined from aerial survey or the perimeter walked with a GPS • Shoot density – estimated percentage cover of seagrass using 1m<sup>2</sup> quadrats in a sampling grid
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying
- 2.04 Sampling/survey device:** n.a.  
field survey with quadrat
- 2.05 Specification:** 0.25m<sup>2</sup> or 1m<sup>2</sup> quadrat, GPS, aerial photography (if applicable for bed extent)
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** June-September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
once per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
All seagrass beds should be sampled
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
The area of all intertidal beds in a waterbody is measured and stratified random quadrats (3 or more dependant on bed size) are deployed in the beds for % cover measurements

## **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** part of individual seagrass plants
- 2.13 Sample treatment:** Organisms of the complete sample are identified.  
Note: beds tend to be dominated by 1 taxa. The quadrats aim to access the percentage cover of the seagrass.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
For intertidal beds two taxa occur in UK waters *Zostera noltii* and *Z. angustifolia*, rarely *Ruppia* spp. is found in very brackish waters. There is some debate over *Z. angustifolia* being an intertidal form of *Zostera marina*
- 2.15 Record of abundance:** Percent coverage  
in relation to Area  
Unit % cover in m<sup>2</sup> quadrats
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** % cover or shoot density, aerial extent of beds, number of seagrass taxa,
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Change in Taxonomic composition from reference seagrass taxa: Seagrass bed spatial extent – % loss of area from reference condition  
Seagrass shoot density – exemplar metric scores for % loss of density from reference condition (annual or 5 year rolling mean)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates  
All beds sampled in water body every year over the reporting period in order to calculate 5 year rolling mean

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** UK  
**Location of sites:** Least impacted areas  
**Data time period:** Historical data from 1900 to present. [Note some of this is anecdotal, and may not be representative before the "wasting disease of the 1930's"]

#### **Criteria:**

Sites free from obvious pressures and correspond to reference conditions (there was rarely sufficient data over the right time period to make this assessment) [Note. No reference sites for seagrass were identified in the water bodies of participating MSs. Seagrass beds are naturally highly variable in extent, abundance, species composition and biomass, dependant on a variety of factors; e.g. geographical location, substrate, hydrodynamic regimes. It was not possible to identify with certainty reference sites on which to base reference conditions for every combination of these factors, for each water type. Each MS set its reference conditions for they are found are similar. The UK approach establishes reference condition values using historic data, where such data exist. Expert judgement then refines the reference conditions to be the maximum potential seagrass species, area and abundance, in natural hydrodynamic and physicochemical state, for individual water bodies (Foden & Brazier, 2006).]

#### **3.08 Reference community description**

1. All reference condition taxa present  
The UK Reference conditions are based on the maximum number of taxa (species) historically recorded in a water body, which will be between 1 and 4. The level of deviation from reference conditions determines ecological status for taxonomic composition. 2. Less than 10% loss of bed extent from reference area  
Reference conditions for bed extent in UK water bodies are based on historic data (whenever such data exist) and expert judgement. Rather, reference conditions have been established for individual water bodies using historic data representing its healthiest previously recorded condition, and modified by expert judgement if this is unrealistic. A bed's

current extent is then compared against these reference conditions and the level of deviation establishes the status class for bed extent. 3. Less than 5% loss of % cover of seagrass over a 5 year rolling mean [ with limited data Less than 10% annual loss Seagrass shoot density (% cover)] Natural variability in density of seagrass is high in shallow water where populations are disturbed by physical parameters and average values across geographic regions do not adequately describe growth regulation by resources. UK water bodies, therefore, is that seagrass density data are not compared across geographic regions, as naturally occurring, local events and physical parameters may cause significant natural change. Rather, an individual bed's current density is compared against historic data representing its healthiest previously recorded condition. There is no division made between the different seagrass species that may comprise the bed. As with other seagrass metrics, ecological status reflects the degree of deviation from reference condition. Duarte & Kirkman (2001) found the time frame to determine real changes brought about by most human disturbance may take 5–10 years, unless disturbance is catastrophic such as habitat removal for coastal redevelopment. Strong fluctuations in area and density of seagrass are possible due to climatic (and apparently coincidental) circumstances, in particular in littoral eelgrass (*Z. angustifolia*) (de Jong, 2004). Classification status for density is determined by the underlying trend over a period of 5–6 years, where data exist (Foden & Brazier, 2006). This will significantly reduce noise created by natural variability. The trend for an individual bed and the loss or gain, as compared with reference conditions, can be used as a supporting parameter to the other metrics to identify whether the seagrass bed is in a state of degradation or recovery.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient

#### **3.11 Boundary setting procedure**

Data from known sea grass beds was compared with risk assessments from the Water body characterisation process, particularly for pressure that would disturb sea grass beds (eg dredging, port works, flood defence, flow changes, dumping of waste and eutrophication). However there is much natural variability depending on the local hydrodynamic regime and sediment type. The literature was also searched for current information on natural variability and recovery rates. Where present, beds should be healthy, with no loss of bed extent or density (# shoots m<sup>-2</sup> or % cover). Natural variability may be up to 30% (Krause-Jensen et al., 2000) and this defines the Good/Moderate boundary. Where data sets allow, a 5-year rolling mean should be used to reduce noise and identify longer term trends. A 30%-reduction when using a 5-year rolling mean will mask underlying trends. Therefore 15% is considered as tolerable evidence of natural variation and decreases in extent of > 15% should be viewed suspiciously. The lower boundaries moderate /poor and poor /bad are set to proportionate losses. These will be reviewed in the next reporting round when more data are available.

**3.12 "Good status" community:** 1. Usually no loss of taxa (1/4 to 1/3 loss). 2. Less than 30% loss of bed extent. 3. Less than 15% loss of % cover (shoot density) on a 5 year rolling mean (30% on single annual figure).

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

#### **3.14 Comments:**

none

ID: 41

IQI

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
Types NEA1/26 and NEA7
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Ireland
- 1.05 Specification:** none
- 1.06 Method name:** *Infaunal Quality Index*
- 1.07 Original name:** *Infaunal Quality Index*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** General degradation, Heavy metals, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Limited testing to date. Unpublished studies relating the IQI to organic enrichment and metals (sewage sludge disposal ground - 178 samples: 20 years x ~10 samples) and physical smothering (mine waste - 214 samples: 5 years x ~43 samples) pressure gradient data. Pearson Correlation coefficients between the IQI and contaminant data of 0.80 and 0.62 respectively. Further validation required.
- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/trac\\_iqi](http://www.wfduk.org/bio_assessment/bio_assessment/trac_iqi) (Coastal Waters only)
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Graham Phillips<br>graham.phillips@environment-agency.gov.uk<br>Environment Agency of England and Wales | <b>1.14 Method reported by</b><br>Graham Phillips<br>graham.phillips@environment-agency.gov.uk<br>Environment Agency of England and Wales |
|--|---|
- 1.15 Comments**  
Further validation of the IQI is required (effectiveness over a range of pressures and habitats) revisions in the IQI reference conditions in 2009.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
ISO, 2005. Water quality - Guidelines for quantitative sampling and sample processing of marine soft-bottom macrofauna, ISO 16665.
- 2.02 Short description**  
Sampling design variable according to UK and Ireland monitoring authority. Samples taken from soft bottom habitats, either i) spread as single samples or ii) taken as replicates at one or more stations. Surveys are undertaken either i) annually or ii) once in a reporting cycle according to monitoring authority. Biological samples require an associated sediment field sample for particle size analysis and supporting depth and salinity information.
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying, Stratified sampling/surveying
- 2.04 Sampling/survey device:** Corer, Grab
- 2.05 Specification:** Van Veen Grab (0.1m<sup>2</sup>), Day Grab (0.1m<sup>2</sup>), Hand Core (0.01m<sup>2</sup>)
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Soft bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** February to May (current recommended target months)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Minimum of one occasion for classification (varies between 1-3 for UK and Ireland monitoring authorities)
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Variable according to habitat, number of years/stations, methodology and required confidence.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Variable according to habitats, number of years/stations, methodology and required confidence.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 500µm (Transitional Waters) & 1000µm (Coastal Waters)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups

- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per area of sample
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** Presence/absence recorded where taxa are unsuitable for quantification (e.g. colonial taxa). Truncation rules are applied to the data to exclude non-benthic and non-invertebrate fauna from the IQI assessment.
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
AZTI Marine Biotic Index (AMBI), Simpsons Evenness Index ( $1-\lambda'$ ) and Taxa number (S). Combined in the formula:  $IQI = (((0.38 \times ((1-(AMBI/7))/(1-(AMBI/7)Ref)) + (0.08 \times ((1-\lambda')/(1-\lambda' Ref))) + (0.54 \times ((S/S Ref)^{0.1}))) - 0.4)/0.62)$  Ref = Expected sub-metric value under reference conditions.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

#### Reference conditions

- 3.05 Scope of reference conditions:** Habitat-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** >1000 sites from UK and Ireland  
**Geographical coverage:** All available data from UK and Ireland used to model IQI sub-metric reference condition values.  
**Location of sites:** Extensive list of locations across UK and Ireland.  
**Data time period:** Data from 1979 to 2003  
**Criteria:**  
Reference condition samples were identified as being from least disturbed conditions, selected on the basis of a) expert judgement and b) from impact gradient study control sites. Reference condition values for AMBI, Simpsons and taxa number were identified from the data. Data was used from sites with low levels of natural disturbance and outliers (e.g. those with anomalously high taxa numbers in contrast to the remaining data) were identified according to expert judgement and excluded.
- 3.08 Reference community description**  
Reference condition macrobenthic communities are dominated by pollution sensitive taxa (AMBI Ecological Group (EG) I taxa), have low relative abundance of indifferent (EG II) and tolerant (EG III) taxa and negligible relative abundance of opportunist (EG IV) and pollution indicator (EG V) taxa. High numbers of taxa with an even abundance distribution throughout the community is also indicative of reference conditions.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise
- 3.11 Boundary setting procedure**  
AMBI ecological group proportions were established for samples over a sewage sludge disposal pressure gradient. Initially, equidistant class boundaries were set and each AMBI EG proportion was calculated for i) the overall status and ii) the lower and upper quartiles of the data in each status. Where the AMBI EG proportions did not conform to those interpreted from the WFD Normative Definitions, the status boundary was adjusted towards the quartile that gave a more accurate representation. Boundaries were further optimised during Intercalibration Phase I.
- 3.12 "Good status" community:** Taxa number and Simpsons evenness are slightly reduced in comparison to values under reference conditions. Whilst variable according to habitat, community abundance (as assessed

by AMBI) are slightly unbalanced: sensitive taxa (EG I) abundance may range from high sub-dominant to absent; indifferent taxa (EG II) are of low sub-dominant abundance; tolerant taxa (EG III) of dominant abundance; abundance of opportunistic (EG IV) and indicator taxa (EG.V) may range from negligible or low to comparable abundance with indifferent Taxa (EG II).

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

The variability of the IQI scores within an assessment is estimated and, in combination with the number of samples/stations/years, the Standard Error (SE) is derived. Uncertainty is established by comparing the average IQI from the samples (with associated SE) to each status boundary. This allows the estimation of the probability that the average IQI (from the samples in the assessment) lies within a different status class than the true IQI. Uncertainty is not incorporated within the IQI itself.

**3.14 Comments:**

none

ID: 190

KRW-maatlatten

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
CW-NEA1, CW-NEA3, CW-NEA4, CW-NEA26, TW-NEA11
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Netherlands
- 1.05 Specification:**
- 1.06 Method name:** *WFD-metrics for natural watertypes*
- 1.07 Original name:** *KRW-maatlatten voor natuurlijke watertypen*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, Flow modification, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Pollution by organic matter  
fishery (mussel seed; mussels; cockles);

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

3 levels of metrics as part of the assessment (see C-01). Level 1: metric at ecosystem level. pressure-impact relationship assessed is the system primary production vs benthic invertebrate biomass. (n = 18; R<sup>2</sup> 0,68). See Herman et al (1999). This level metric is not part of the intercalibration. Level 2: metric at habitat level. Not part of intercalibration. The metric is straightforward (ratio of actual habitat size divided by reference habitat size). Level 3: metric at community level. Part of intercalibration. Reference situation for the 4 parameters are determined with permutation tests with replication (bootstrapping) from a large reference database including temporal and spatial variation. Qualitative pressure-impact assessments are available in several documents (see A-22). Quantitative pressure-impact relationships are not yet available and may be difficult to obtain in the multi-pressure situations. The impact for pressures on the benthic invertebrate community is assessed in the Netherlands, but not in relation to the WFD assessment method. For more reading on pressure-impact relations: see chapter 1.4 in Ysebaert T, De Mesel I, Herman P (2008). Water Framework Directive - background document salt water benthic invertebrates. report C076/08

- 1.10 Internet reference:** [http://themas.stowa.nl/thema/ecologische\\_boordeling/krw-maatlatten.aspx?mld=7213&rld=817](http://themas.stowa.nl/thema/ecologische_boordeling/krw-maatlatten.aspx?mld=7213&rld=817)

**1.11 Pertinent literature of mandatory character:**

Besluit Kwaliteitseisen en Monitoring Water, 2009. Ministry of Housing, Spatial Planning and the Environment (presently under public consultation).

**1.12 Scientific literature:**

Most relevant literature is available at the BEQI-website. <http://www.beqi.eu/background.php>

**1.13 Method developed by**

Diederik van der Molen and Willem van Loon, RWS Waterdienst  
willem.van.loon@rws.nl  
Rijkswaterstaat Waterdienst

**1.14 Method reported by**

Roel Knoben, Willem van Loon  
r.knoben@royalhaskoning.com / willem.van.loon@rws.nl  
Royal Haskoning / Rijkswaterstaat Waterdienst

**1.15 Comments**

WFD assessment method for benthic invertebrates is based on the Benthic Ecosystem Quality Index (BEQI). For more information: [www.beqi.eu](http://www.beqi.eu). The Benthic Ecosystem Quality Index (BEQI) is based on an ecosystem functioning approach which aims to give an indication about ecosystem structure and functioning, and about biological relationships. BEQI evaluates at the scale of a whole water body, contrary to methods applied by other member states that evaluate the ecological status per sampling station.

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

STOWA, 2009. Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009)

STOWA, NN. Quality Handbook Hydrobiology (in prep).

**2.02 Short description**

The assessment method for benthic invertebrates in transitional and coastal waters has 3 levels (see C-01). Level 1: primary production (mostly expert judgement). Level 2: Aerial photos, digital terrain maps and modelling results (e.g. hydrodynamic models) are combined in a Geographical Information System. Level 3: The four variables are easily estimated from samples of the macrobenthic benthic community. Normally sediment cores are collected at sampling stations with a sampling core at low tide on the intertidal flats or with a device like the Reineck Box corer or Van Veen grab operated from a ship for subtidal stations. The sediment is washed through a 1mm mesh. Specimens are sorted from the residue, identified to the species level, counted and weighed. Biomass is most accurately measured by the difference between dry weight and ash weight, the ash free dry weight AFDW.

- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying, Stratified sampling/surveying

- 2.04 Sampling/survey device:** Corer

- 2.05 Specification:** corer tube; box corer (e.g. Reineck Box corer); flushing sampler (only in saline lakes 0 -2 m)

- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
for level 3 metric at present only a single habitat type per water body is assessed. The most
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both habitat types with respect to habitat size and total biomass.  
We are currently expanding the number of habitats at level 3 to 3 or 4 per water body in order to obtain better spatial coverage.
- 2.08 Sampling/survey month(s):** Water types with intertidal areas (NEA11, NEA4) Fall: August 15th to november 1st.  
Coastal water types (NEA1, NEA3): spring: March 1st to June 15th.
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Minimum one survey per year (preferably fall), and scores and classification preferably averaged over three years.
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
It has been calculated by Van Hoeij et al (2007) that the following minimum sampling areas are necessary for the following ecotopes in the Westerschelde: Saline lowdyn midlitoral muddy = 0.21 m<sup>2</sup>; highdyn brackish gully = 0.63 m<sup>2</sup>; hydyn salt gully = 2.97 m<sup>2</sup>; highdyn salt litoral = 0.65 m<sup>2</sup>; lowdyn sandy midlitoral = 0.33 m<sup>2</sup>. These necessary minimum sample areas may be the combined area over a period of 3 years.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
For the currently used and reported ecotope, the Saline lowdyn midlitoral muddy; the necessary 0.21 m<sup>2</sup> has been sampled over a period of 3 years.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 1 mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to Area  
Unit #/m<sup>2</sup>
- 2.16 Quantification of biomass:** n.a.  
ash free dry weight (AFDW)
- 2.17 Other biological data:** primary production and total benthic production (for metric level 1). Surface area of large scale habitats (for metric level 2)
- 2.18 Special cases, exceptions, additions:** Similarity index used as part of the level 3 metric is the Bray-Curtis index. Biomass in ash free dry mass is either estimated through mass length relationships in bivalves, conversion of wet weight to ash free dry mass (AFDW) in polychaetes or by the weight difference after drying and after incinerating.
- 2.19 Comments**  
The present Dutch surveillance monitoring can be split up in 3 areas, based on differences in sampling strategy, namely (1) the Delta in the southwestern part of the Netherlands, (2) the Dutch coast and (3) the Waddenzee & Eems-Dollard. Although the macrobenthic fauna monitoring activities in the coastal waters are all unified in the BIOMON program and under the responsibility of one agency (but different offices) there are some small taxonomic differences in the methodology. Since these differences also exist in the reference data sets, it is expected that the impact of these small inconsistencies on the EQR-scores are very small. The present (2008) monitoring effort for coastal waters NEA1, NEA3) is not yet sufficient for overall assessment with the WFD method.

## 3. Data evaluation

### Evaluation

#### 3.01 List of biological metrics

3 levels of metrics as part of the assessment. level 1: metric at ecosystem level. Relationship between the system averaged benthic invertebrate biomass (AFDW/m<sup>2</sup>) and the system primary production (g C/m<sup>2</sup> year). level 2: metric at habitat level. Surface area coverage of specific large scale habitats compared to the reference situation. The quotient is the score for this level's metric. level 3: metric at community level. The level 3 metric evaluates the state of the benthic invertebrates within a habitat based on four parameters: number of species, density, biomass and species composition changes. calculating score for level 3:  $(2 \times [\text{density}] + 2 \times [\text{biomass}] + 2 \times [\text{number of species}] + 1 \times [\text{similarity}]) / 7$  final score: if level 2 score can be determined:  $\text{EQR} = 1 * (\text{score level 1} + 2 * (\text{score level 2}) + 2 * (\text{score level 3}) / 5$  If score for level 2 can not be determined:  $\text{EQR} = 1 * (\text{score level 1} + 2 * (\text{score level 3}) / 3$  The assessment with the level 3 metric is only suitable for one habitat type in each water body. The choice is made per water type. E.g. for water type K1 (NEA3) this is the muddy fine sand community.

further reading (in dutch): Ysebaert T, De Mesel I, Herman P (2008). Water Framework Directive - background document salt water benthic invertebrates. report C076/08

- 3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Weighted average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

**3.05 Scope of reference conditions:** Site-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** Typically the same as for the assessment data set. The number of sites per water body approximately are: Westerschelde circa 300 stations; Oosterschelde 120 stations; Eems-Dollard 120 stations; Lake Veere 60 stations; Lake Grevelingen 60 stations; North

**Geographical coverage:** level 1: (historical) data from relative undisturbed well mixed transitional and coastal waters from western Europe and North America. Level 2: maps for the water bodies from beginning 20th century. level 3: data from Netherlands.

**Location of sites:** level 1: western Europa and North America. level 2 & 3: historical data from the water bodies (Westerschelde, Oosterschelde, Eems-Dollard, Grevelingenmeer, Waddenzee, coastal waters North Sea)

**Data time period:** level 2: first half 20th century. level 3: 1970's

**Criteria:**

n.a.

**3.08 Reference community description**

level 1: ratio benthic invertebrate biomass / system primary production close to 1/10. level 2: Surface area coverage is specific for each individual water body. Surface area coverage of habitats comparable to situation early 20th century. level 3: reference community description is specific for each individual water body. Reference conditions based on historical data from 1970's. Furthermore a general description is given (in Dutch) in: STOWA (2009) Referenties en maatlaten voor natuurlijke watertypen. report 2007-32

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient  
statistical techniques (permutation test, bootstrap test)

**3.11 Boundary setting procedure**

Level 1: Ratio 1/10 is the reference (or high boundary). Lower boundaries are based on literature, historical data, modelling, field observations, assuming linearity of the relationships between the primary production and the benthic invertebrate biomass. e.g. the good-moderate ratio's are 1:15 en 2:15. Level 2: not directly related to pressures but calculated as the quotient actual surface coverage/reference situation. Results in a score on a 0-1 scale. Level 3: For the different parameters (biomass, number of species etc), the reference value to be expected in the case of a good status corresponds with the 5th percentile value out of the permutation distribution of each parameter (permutation distribution = at random 2000 samples are drawn with replacement from the reference database). The 5th percentile is a statistically accepted level which is not too restrictive and which accounts for the variability within the reference data. The moderate/poor and poor/bad boundaries are scaled in equally intervals relative to the number of species measured for the good/moderate boundary and are respectively 2/3 and 1/3 of the number of species of the good/moderate boundary.

**3.12 "Good status" community:** n.a.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Level 3 metric is based on the statistical distribution of the reference datasets and statistical limits for classes are used (see C-15). Precision and uncertainty is regarded in Van Herpen, van Tongeren, Knobben, Baggelaar, van Loon (2009). Quick scan precision and confidence of KRW assessment (in Dutch). This study resulted in a statistical method to assess the level of precision and confidence monitoring results and status classifications (including identifying outliers and estimates for missing values). The confidence of a status classification is expressed as the probability of exceeding a chemical limit value or the biological status classification moderate/good. Recommendations from this study are incorporated in the Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) (see question B.0).

**3.14 Comments:**

none

ID: 40

IQI

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
Types NEA1/26 and NEA7
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** United Kingdom
- 1.05 Specification:** none
- 1.06 Method name:** *Infaunal Quality Index*
- 1.07 Original name:** *Infaunal Quality Index*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** General degradation, Heavy metals, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Limited testing to date. Unpublished studies relating the IQI to organic enrichment and metals (sewage sludge disposal ground - 178 samples: 20 years x ~10 samples) and physical smothering (mine waste - 214 samples: 5 years x ~43 samples) pressure gradient data. Pearson Correlation coefficients between the IQI and contaminant data of 0.80 and 0.62 respectively. Further validation required.
- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/trac\\_iqi](http://www.wfduk.org/bio_assessment/bio_assessment/trac_iqi) (Coastal Waters only)
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Graham Phillips<br>graham.phillips@environment-agency.gov.uk<br>Environment Agency of England and Wales | <b>1.14 Method reported by</b><br>Graham Phillips<br>graham.phillips@environment-agency.gov.uk<br>Environment Agency of England and Wales |
|--|---|
- 1.15 Comments**  
Further validation of the IQI is required (effectiveness over a range of pressures and habitats) revisions in the IQI reference conditions in 2009.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
ISO, 2005. Water quality - Guidelines for quantitative sampling and sample processing of marine soft-bottom macrofauna, ISO 16665.
- 2.02 Short description**  
Sampling design variable according to UK and Ireland monitoring authority. Samples taken from soft bottom habitats, either i) spread as single samples or ii) taken as replicates at one or more stations. Surveys are undertaken either i) annually or ii) once in a reporting cycle according to monitoring authority. Biological samples require an associated sediment field sample for particle size analysis and supporting depth and salinity information.
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying, Stratified sampling/surveying
- 2.04 Sampling/survey device:** Corer, Grab
- 2.05 Specification:** Van Veen Grab (0.1m<sup>2</sup>), Day Grab (0.1m<sup>2</sup>), Hand Core (0.01m<sup>2</sup>)
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Soft bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** February to May (current recommended target months)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Minimum of one occasion for classification (varies between 1-3 for UK and Ireland monitoring authorities)
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Variable according to habitat, number of years/stations, methodology and required confidence.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Variable according to habitats, number of years/stations, methodology and required confidence.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 500µm (Transitional Waters) & 1000µm (Coastal Waters)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups

- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per area of sample
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** Presence/absence recorded where taxa are unsuitable for quantification (e.g. colonial taxa). Truncation rules are applied to the data to exclude non-benthic and non-invertebrate fauna from the IQI assessment.
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
AZTI Marine Biotic Index (AMBI), Simpsons Evenness Index ( $1-\lambda'$ ) and Taxa number (S). Combined in the formula:  $IQI = (((0.38 \times ((1-(AMBI/7))/(1-(AMBI/7)Ref)) + (0.08 \times ((1-\lambda')/(1-\lambda' Ref))) + (0.54 \times ((S/S Ref)^{0.1}))) - 0.4)/0.62)$  Ref = Expected sub-metric value under reference conditions.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time  
Data from single spatial replicate

#### Reference conditions

- 3.05 Scope of reference conditions:** Habitat-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** >1000 sites from UK and Ireland  
**Geographical coverage:** All available data from UK and Ireland used to model IQI sub-metric reference condition values.  
**Location of sites:** Extensive list of locations across UK and Ireland.  
**Data time period:** Data from 1979 to 2003  
**Criteria:**  
Reference condition samples were identified as being from least disturbed conditions, selected on the basis of a) expert judgement and b) from impact gradient study control sites. Reference condition values for AMBI, Simpsons and taxa number were identified from the data. Data was used from sites with low levels of natural disturbance and outliers (e.g. those with anomalously high taxa numbers in contrast to the remaining data) were identified according to expert judgement and excluded.
- 3.08 Reference community description**  
Reference condition macrobenthic communities are dominated by pollution sensitive taxa (AMBI Ecological Group (EG) I taxa), have low relative abundance of indifferent (EG II) and tolerant (EG III) taxa and negligible relative abundance of opportunist (EG IV) and pollution indicator (EG V) taxa. High numbers of taxa with an even abundance distribution throughout the community is also indicative of reference conditions.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise
- 3.11 Boundary setting procedure**  
AMBI ecological group proportions were established for samples over a sewage sludge disposal pressure gradient. Initially, equidistant class boundaries were set and each AMBI EG proportion was calculated for i) the overall status and ii) the lower and upper quartiles of the data in each status. Where the AMBI EG proportions did not conform to those interpreted from the WFD Normative Definitions, the status boundary was adjusted towards the quartile that gave a more accurate representation. Boundaries were further optimised during Intercalibration Phase I.
- 3.12 "Good status" community:** Taxa number and Simpsons evenness are slightly reduced in comparison to values under reference conditions. Whilst variables according to habitat, community abundance (as assessed

by AMBI) are slightly unbalanced: sensitive taxa (EG I) abundance may range from high sub-dominant to absent; indifferent taxa (EG II) are of low sub-dominant abundance; tolerant taxa (EG III) of dominant abundance; abundance of opportunistic (EG IV) and indicator taxa (EG.V) may range from negligible or low to comparable abundance with indifferent Taxa (EG II).

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

The variability of the IQI scores within an assessment is estimated and, in combination with the number of samples/stations/years, the Standard Error (SE) is derived. Uncertainty is established by comparing the average IQI from the samples (with associated SE) to each status boundary. This allows the estimation of the probability that the average IQI (from the samples in the assessment) lies within a different status class than the true IQI. Uncertainty is not incorporated within the IQI itself.

**3.14 Comments:**

none

ID: 101

OGA

## 1. General information

**1.01 GIG:** North-East-Atlantic  
n.a.

**1.02 Category:** Coastal Waters, Transitional Waters

**1.03 BQE:** Macroalgae

**1.04 Country:** Ireland

**1.05 Specification:** none

**1.06 Method name:** *Opportunistic Green Macroalgal Abundance*

**1.07 Original name:** *Opportunistic Green Macroalgal Abundance*

**1.08 Status: Method is/will be used in** First RBMP (2009)

**1.09 Detected pressure(s):** Eutrophication

*Has the pressure-impact-relationship been tested?*

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

<http://www.environ.ie/en/Legislation/Environment/Water/FileDownload,20824,en.pdf>

**1.12 Scientific literature:**

Scanlan, C., J. Foden, E. Wells & M.A. Best, 2007. The monitoring of opportunistic macroalgal blooms for the Water Framework Directive. Marine Pollution Bulletin 55: 162-171.

**1.13 Method developed by**

Robert Wilkes

r.wilkes@epa.ie

Environmental Protection Agency

**1.14 Method reported by**

Robert Wilkes

r.wilkes@epa.ie

Environmental Protection Agency

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

n.a.

**2.02 Short description**

Within a selected waterbody all sediment-based shores are investigated for the presence of mats of opportunistic green algae. Areas representing 5% or greater of the water-body area were mapped with a GPS. The presence of other algal mat areas (<5%) of the waterbody are also noted. Within the mapped areas, transect lines are placed across the algal bed. 0.25m<sup>2</sup> quadrats are randomly placed along each transect (a minimum of 5 quadrats/transect) Within each quadrat the following is carried out: Percentage cover of algae to the nearest 5% within the quadrat is estimated. A photograph of each quadrat is taken. Position on GPS. The contents of each quadrat is removed, rinsed to remove sand particles/mud and weighed to the nearest gram (squeeze dry method). The average biomass of opportunistic algae in g/m<sup>2</sup> is calculated.

Mapped areas are plotted on GIS software and the extent of the areas calculated. In addition the area of the intertidal substrate available for macroalgal settlement is also calculated using Admiralty Charts/or other suitable methods

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** n.a.

**2.05 Specification:** In situ shore survey

**2.06 Sampled/surveyed habitat:** Single habitat(s)

intertidal sediments capable of supporting green algal growth

**2.07 Sampled/surveyed zones in areas with tidal influence:** Intertidal zone

**2.08 Sampling/survey month(s):** June to September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

entire available intertidal habitat assessed, adequate quadrats taken for biomass assessment

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

entire available intertidal habitat in waterbody assessed

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** n/a

**2.13 Sample treatment:** Organisms of the complete sample are identified.

- 2.14 Level of taxonomical identification:** Genus  
Taxonomical information not needed for assessment
- 2.15 Record of abundance:** Relative abundance  
Biomass recorded as g/m<sup>2</sup>  
**in relation to** Area  
Biomass recorded as g/m<sup>2</sup>  
**Unit** Biomass recorded as g/m<sup>2</sup>
- 2.16 Quantification of biomass:** Utermöhl technique  
wet weight of algae from each quadrat
- 2.17 Other biological data:** spatial cover in WB
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
1 Total % cover 2 Total affected area 3 Average biomass in AIH 4 Average biomass in affected area 5 percent of quadrats with entrained algae
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
No specific reference sites have been identified in the UK or IE coastal and transitional waters. There are a number of sedimentary intertidal sites particularly located within transitional waters around the coast of IE that can be considered to be of reference conditions due to the lack of macroalgae blooms, some data is becoming available for these areas.
- 3.08 Reference community description**  
Opportunistic macroalgal blooms of anthropogenic origin should be absent or if present should cover less than 5% of the available intertidal habitat. Total area coverage of opportunist macroalgae should be no greater than 100 hectares. Generally directed at intertidal sedimentary shores in both transitional and coastal waters.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
Degradation through coastal morphological change or increased pressure, specifically dredging activity causing increased sedimentation and excess deposition with restriction of light and limiting growth to opportunist or tolerant species only.  
Increased nutrients inputs from both direct and indirect sources such as sewage outfalls and land run-off contribute to eutrophication problems. These may exacerbate the growth of opportunist species or extend their growth or peak season. Freshwater run-off or outflows reducing salinity can also lead to a dominance of more tolerant species such as the opportunist macroalgae. Increased morphological pressure leading to loss or complete removal of coastal habitats can cause a shift in community structure from long lived perennial species to ephemeral, opportunist species which can dominate the community and restricted continued growth of other faunal and floral species. Excess suspended particulate matter

increases turbidity leading to smothering and light limitation causing a dominance of tolerant macroalgae species and restricting growth of less tolerant species. Increasing nutrients lead to excess algal growth and the production of opportunist macroalgae blooms which in turn may smother the understory and prevent bird feeding due to restricted access to benthic fauna and causing an undesirable anoxic layer. Increased and persistent growth of opportunist macroalgae can eventually lead to the complete anoxia of underlying sediment with rotting algal causing pungent odours and causing general disruption to the natural environment. Reduction in salinity can lead to the removal of sensitive species and promote the growth of tolerant opportunist species especially in the vicinity of freshwater run-off. Excessive growth of opportunist macroalgae species or 'blooms' is generally considered a nuisance to the surrounding environment causing a general shift in the natural community.

**3.12 "Good status" community:** High Status The taxonomic composition corresponds totally or nearly totally with undisturbed conditions and disturbance sensitive taxa are present. Also there are no detectable changes in macroalgae abundance due to anthropogenic activities. The species composition is unaltered from reference conditions. The area and total % cover of opportunist macroalgae species in a waterbody is at its minimum taking into account seasonal fluctuation and variations in growth. Any presence of macroalgae is minimal and shows no persistence. The total area and % cover of opportunist macroalgae within the water body is at or close to reference conditions. Good status Most disturbance-sensitive macroalgae associated with undisturbed conditions are present. The level of macroalgal cover shows slight signs of disturbance. There is a slight deviation from the reference conditions. There may be minimal presence of opportunist macroalgae growth but still shows no persistence.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**  
none

ID: 24

MAB

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Macroalgae
- 1.04 Country:** United Kingdom
- 1.05 Specification:** none
- 1.06 Method name:** **Macroalgal Bloom Assessment (Opportunistic macroalgae)**
- 1.07 Original name:** *Macroalgal Bloom Assessment (Opportunistic macroalgae)*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/Opportunistic](http://www.wfduk.org/bio_assessment/bio_assessment/Opportunistic)

**1.11 Pertinent literature of mandatory character:**

UKTAG Transitional & Coastal Waters Assessment Method Macroalgae: Macroalgal Bloom Assessment (Opportunistic Macroalgae).

**1.12 Scientific literature:**

Scanlan, C.M., J. Foden, E. Wells & M. Best, 2007. The monitoring of opportunistic macroalgal blooms for the Water Framework Directive. Marine Pollution Bulletin 55 (1-6): 162-171. Wells, E., M. Best, C. Scanlan, S. Holt & J. Foden, 2007. WFD Tools Paper, Draft v2. Opportunistic Macroalgae, Abundance. MPTT/MAT01.

**1.13 Method developed by**

WFD UK & RoI Marine Plants Task Team - leads were Mike Best, Emma Wells, Clare Scanlan, Jo Foden  
mike.best@environment-agency.gov.uk,  
emma@wellsmarine.org, clare.scanlan@sepa.org.uk,  
jo.foden@uea.ac.uk

Mike Best (Environment Agency, England & Wales); Emma Wells (Wells Marine); Clare Scanlan (SEPA); Jo Foden (CEFAS)

**1.14 Method reported by**

Dr. Clare Scanlan  
clare.scanlan@sepa.org.uk

Scottish Environment Protection Agency

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Agency-specific standard operating procedures, as derived from the MPTT tool paper.

**2.02 Short description**

Sites are selected based on previous knowledge and possibly with the benefit of aerial photographs or equivalent imagery to identify areas of algae presence. The available intertidal habitat (AIH) area is derived from maps, GIS or aerial imagery. The extent of algal mats is assessed either by mapping this on foot in the field, or from imagery which has been ground-truthed. The spatial extent of the algal cover is assessed from multiple quadrats within patches; the number of quadrats should be proportional to patch size and variability and estimates made to the nearest 5%. Samples are collected for biomass estimation (after percentage cover has been assessed) by removing the surface layer of algae from within the quadrat area. Samples are then washed to remove excess mud and animals, and then wet weighed. Whether or not the algae are entrained into the mud is assessed in the field. All data are recorded at the time of collection either electronically or on paper. The principal genus/genera within patches is/are identified.

- 2.03 Method to select the sampling/survey site or area:** n.a.

- 2.04 Sampling/survey device:** n.a.

Quadrats

- 2.05 Specification:** 0.25square metre quadrat

- 2.06 Sampled/surveyed habitat:** Single habitat(s)

Soft sediment (mud/sand) primarily but may include mussel beds - not hard substrata such

- 2.07 Sampled/surveyed zones in areas with tidal influence:** Intertidal zone

- 2.08 Sampling/survey month(s):** June to September

- 2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Variable from site to site and varies with extent, patchiness and abundance of macroalgal blooms

- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Total intertidal area available or algal growth

### **Sample processing**

**2.12 Minimum size of organisms sampled and processed:** Not applicable

**2.13 Sample treatment:** n.a.

Neither description above applies adequately. Biomass samples are washed to remove excess mud and animals and the sample weighed wet. Percentage cover is assessed in the field.

**2.14 Level of taxonomical identification:** Genus

**2.15 Record of abundance:** n.a.

% cover and wet weight (g)

**in relation to** Area

**Unit** Percentage cover is expressed per patch and also aggregated to waterbody level (i.e. percentage cover of total AIH). Biomass is expressed as g/square metre for patches and averaged across the whole AIH.

**2.16 Quantification of biomass:** n.a.

g/m<sup>2</sup> wet weight

**2.17 Other biological data:** Whether algae are entrained into the sediment or living on the surface

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

## **3. Data evaluation**

### **Evaluation**

#### **3.01 List of biological metrics**

The method uses a multi-parameter index, the "Macroalgal Bloom Index" for the purpose of assessing the condition of the quality element, macroalgae. The Index is based on five parameters: total extent of macroalgal bed; cover of available intertidal habitat; biomass of opportunistic macroalgal mats; biomass over the available intertidal habitat; proportion of entrained algae. See Method Statement and tool paper for full explanation of calculations (summarised below). Reference values for each parameter

Parameter	Reference values
macroalgal bed (TE) (hectares)	10
Cover of available intertidal habitat (CAIH) (%)	100
Biomass of opportunistic macroalgal mats (BAA) (g.m <sup>-2</sup> )	100
Biomass over the available intertidal habitat (BAIH) (g.m <sup>-2</sup> )	100
Proportion of entrained algae (PEA) (% of quadrats)	100

Calculation of the ecological quality ratio for each parameter

The ecological quality ratio (EQRTE) for the parameter, total extent of macroalgal bed, should be calculated using the following equation:  $EQRTE = [551 - \text{observed value for parameter}] \div [551 - \text{reference value for parameter}]$

The ecological quality ratio (EQRCAIH) for the parameter, cover of available intertidal habitat, should be calculated using the following equation:  $EQRCAIH = [100 - \text{observed value for parameter}] \div [100 - \text{reference value for parameter}]$

The ecological quality ratio (EQRBAA) for the parameter, biomass of opportunistic macroalgal mats, should be calculated using the following equation:  $EQRBAA = [6000 - \text{observed value for parameter}] \div [6000 - \text{reference value for parameter}]$

The ecological quality ratio (EQRBAIH) for the parameter, biomass over the available intertidal habitat, should be calculated using the following equation:  $EQRBAIH = [6000 - \text{observed value for parameter}] \div [6000 - \text{reference value for parameter}]$

The ecological quality ratio (EQRPEA) for the parameter, proportion of entrained algae, should be calculated using the following equation:  $EQRPEA = [100 - \text{observed value for parameter}] \div [100 - \text{reference value for parameter}]$

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

**3.05 Scope of reference conditions:** Habitat-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data

**3.07 Reference site characterisation**

**Number of sites:** Not specified

**Geographical coverage:** United Kingdom

**Location of sites:** Various UK sites and from published literature

**Data time period:** Various

**Criteria:**

Actual reference sites were not defined as such. Rather, expert knowledge from within the tool development group, published literature and previous government sponsored technical group (DETR, 2001) was used alongside data from affected sites to establish reference and boundary criteria. Ref: Dept. of Environment, Transport & the Regions, Workshop report, 2001

**3.08 Reference community description**

Less than 5% cover of AIH of opportunistic macroalgae (defined as those with comparatively short generation times, and which can utilise excess nutrients to promote rapid growth, e.g. Enteromorpha, Ulva, Chaetomorpha, Cladophora, Ectocarpus, Pilayella, Porphyra.) Such plants may be present as normal parts of the flora, but should not exceed the stated criteria.

**3.09 Results expressed as EQR? Yes**

**Boundary setting**

**3.10 Setting of ecological status boundaries: Equidistant division of the EQR gradient**

**3.11 Boundary setting procedure**

Expert knowledge (from within the tool development group, published literature and previous government sponsored technical group) was used alongside data from affected sites to establish reference and boundary criteria. The H/G boundary was identified as 5% algal cover, 100g/m<sup>2</sup> biomass, 10ha total affected area, and 1% entrainment (percentage of quadrates containing entrained algae). The G/M boundary was identified as 15% cover, 50ha total affected area, 500g/m<sup>2</sup> biomass and 5% entrainment. The M/P boundary was identified as 25% cover, 100ha total affected area, 1,000g/m<sup>2</sup> biomass and 20% entrainment. The P/B boundary was identified as 75% cover, 250ha total affected area, 3,000g/m<sup>2</sup> biomass and 50% entrainment.

**3.12 "Good status" community: There is limited cover (<15%) of opportunistic macroalgae, low biomass (<500g/m<sup>2</sup>) and no growth of algae in the underlying sediment, i.e. no entrainment of algae.**

**Uncertainty**

**3.13 Consideration of uncertainty: Yes**

Confidence in class may be calculated for a single survey's data or for data from aggregated temporal surveys. A spreadsheet with embedded calculations is used to calculate the class, as per the standard equations and final EQR (face value class), but also calculates the probability of the water body being in each of the five WFD status classes. The face value class may not be the same as the most probable class given by the CofC assessment, because the EQR is constrained to be between 0 and 1. This typically occurs where the EQR is close to a boundary - the face value may be Good, but the CofC assessment may say there is a 40% chance of High, 50% of Good and 10% of Moderate. There is therefore 90% confidence of Good or better. The process allows the user to specify a relative standard deviation to represent the likely certainty (or error) in the measurement of parameters such as area of patch and AIH. Full details of the statistical methodology used in CAPTAIN are provided in the report "Confidence of Class for Marine Plant Tools".

**3.14 Comments:**

Full details of the statistical methodology used in CAPTAIN are provided in the report "Confidence of Class for Marine Plant Tools".

ID: 135

KRW-maatlatten

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
CW-NEA1/26, CW-NEA3, CW-NEA4, TW-NEA11
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Netherlands
- 1.05 Specification:** none
- 1.06 Method name:** *WFD-metrics for natural watertypes*
- 1.07 Original name:** *KRW-maatlatten voor natuurlijke watertypen*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation  
turbidity (e.g. caused by human activities as dredging etc)

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

metric for chlorophyll-a was extensively assessed during intercalibration. Principles based on OSPAR methodology. historical data and model results were used which had been previously developed within the context of the Aquatic Outlook for the AMOEBA documentation (Baptist & Jagtman, 1997). In addition to validation for earlier methods (AMOEBE, Ospar) also WFD metric was assessed. Validation has been performed with the aid of expert opinions relating to the Ems-Dollard and the Western Scheldt. This qualitative validation shows a good match between the calculated value and the estimation of the status of the system by 5 experts; only the Western Scheldt was assessed to be slightly poorer by the experts (n = 2) Further reading: Berg van den M.S., Pot R [eds] (2008): Background document on phytoplankton references and metrics for the Water Framework Directive (in dutch).

- 1.10 Internet reference:** [http://themas.stowa.nl/thema/ecologische\\_boordeling/krw-maatlatten.aspx?mld=7213&rld=817](http://themas.stowa.nl/thema/ecologische_boordeling/krw-maatlatten.aspx?mld=7213&rld=817)

**1.11 Pertinent literature of mandatory character:**

Besluit Kwaliteitseisen en Monitoring Water, 2009. Ministry of Housing, Spatial Planning and the Environment (presently under public consultation).

**1.12 Scientific literature:**

Baptist, H.J.M. & E. Jagtman, 1997. The AMOEBAS of the marine waters. Aquatic Outlook working group. Report RIKZ-97.027: 149.

**1.13 Method developed by**

development by national expert group commissioned by STOWA, Bas van der Wal & RWS Waterdienst, Diederik van der Molen  
b.van.der.wal@stowa.nl  
STOWA Foundation for Applied Water Management Research & Centre for Water Management (Rijkswaterstaat Waterdienst)

**1.14 Method reported by**

Roel Knobens  
r.knobens@royalhaskoning.com  
Rijkswaterstaat Waterdienst

**1.15 Comments**

Description of KRWmaatlatten in Dutch. Van der Molen, D.T., 2004. References and classification tools for transitional and coastal waters for the purpose of the Water Framework Directive

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) Quality Handbook Hydrobiology (in prep). 2009. STOWA.

**2.02 Short description**

abundance (chlorophyll): vertical sampling . Mixed water samples. species composition (Phaeocystis bloom): horizontal and vertical sampling. Mixed water samples.

**2.03 Method to select the sampling/survey site or area:** n.a.

**2.04 Sampling/survey device:** n.a.

CTD / Rosetta

**2.05 Specification:**

all stations: few metres below surface level; stratified stations at three depths: surface, thermocline and close to the bottom

**2.06 Sampled/surveyed habitat:** Single habitat(s)

pelagic; at stratified stations at three depths

**2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone

**2.08 Sampling/survey month(s):** March - September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

minimum 7 occasions per year ( March - September), but classification preferably averaged over three years.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

n.a.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

n.a.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** all organisms in sample are processed
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
abundance: chlorophyll a in ug/l. species composition: % of time (months/year) a Phaeocystis bloom is occurring. Blooms are identified by individual counts of Phaeocystis cells.  
**in relation to** Volume  
**Unit** species composition: # months with phaeocystis bloom ( $> 10^6$  cells/l). abundance: chlorophyll a in ug/l
- 2.16 Quantification of biomass:** Chlorophyll-a concentration
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** phaeocystis bloom defined as  $> 10^6$  cells/l
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
multimetric assessment: abundance and species composition metric abundance (chlorophyll-a). Chlorophyll a concentration the corresponding EQR-value can be calculated with a table given in the metrics (water type specific values) multimetric species composition: metric based on # months with Phaeocystis bloom ( $> 10^6$  cells/l) more reading: KRWmaatlaten or Van den Berg M.s., Pot R. [eds] (2008): Background document references and metrics phytoplankton for the Water Framework Directive.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
conditional average of both metrics; if metric for abundance (chlorophyll-a) has a score lower than metric for species composition then only the abundance metric is regarded.
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Modelling (extrapolating model results)  
historical data and model results were used which had been previously developed within the context of the Aquatic Outlook for the AMOEBA documentation (Baptist & Jagtman, 1997)
- 3.07 Reference site characterisation**  
**Number of sites:** There are no undisturbed reference areas in the Netherlands and in the North Sea eco-region  
**Geographical coverage:** no actual reference sites. Geographical coverage of the historical data: Netherlands  
**Location of sites:** abundance: Eems-Dollard; Dutch coastal waters, Wadden Sea, North Sea  
**Data time period:** reference situation is assumed for 1930. Situation for 1930 is predicted with models  
**Criteria:**  
Dutch sites were tested against reference criteria by Wasson (2006) and all rejected.
- 3.08 Reference community description**  
Low chlorophyll-a concentration, with not more than one month per year  $> 10^6$  cells/l for phaeocystis. Furthermore a general description is given (in Dutch) in: STOWA (2009) Referenties en maatlaten voor natuurlijke watertypen. report 2007-32
- 3.09 Results expressed as EQR?** Yes normalized EQR's

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise

modelling outcome

**3.11 Boundary setting procedure**

Ecological Quality Objectives of OSPAR and the historical data and model results were used which had been previously developed within the context of the Aquatic Outlook for the AMOEBA documentation (Baptist & Jagtman, 1997) provided the point of departure. Abundance: HG boundary is 90 percentile of the summer values from AMOEBA. Reference is 2/3 of the HG boundary (based on intercalibration). The GM boundary is situated at 1,5 times the upper boundary of the reference. This factor of 1.5 has been described in OSPAR. MP and PB boundaries are 2 times the value for the boundary above. Species composition (phaeocystis bloom): all boundaries based on expert judgement.

**3.12 "Good status" community:** Chlorophyll-a concentration maximal 1.5 times reference situation. Up to two months with bloom of Phaeocystis. Furthermore a general description is given (in Dutch) in: STOWA (2009) Referenties en maatlaten voor natuurlijke watertypen. report 2007-32

**Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Precision and uncertainty is regarded in Van Herpen, van Tongeren, Knobben, Baggelaar, van Loon (2009). Quick scan precision and confidence of KRW assessment (in Dutch). This study resulted in a statistical method to assess the level of precision and confidence monitoring results and status classifications (including identifying outliers and estimates for missing values). The confidence of a status classification is expressed as the probability of exceeding a chemical limit value or the biological status classification moderate/good. Recommendations from this study are incorporated in the Instructie; Richtlijn Monitoring Oppervlaktewater en Protocol Toetsen & Beoordelen (28 april 2009) (see question B.0).

**3.14 Comments:**

none

ID: 84

UK-PP-CO

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters, Transitional Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** United Kingdom
- 1.05 Specification:** none
- 1.06 Method name:** *Phytoplankton Toolkit*
- 1.07 Original name:** *Phytoplankton Toolkit*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

### *Has the pressure-impact-relationship been tested?*

Yes, with qualitative data (e.g. response at reference against impacted sites).

No reference sites but sites with identified low nutrient pressure too numerous to list. A risk index of waterbodies based on the level of nutrient enrichment and susceptibility of the waterbody to enrichment was established, allocating a risk factor to waterbodies within England and Wales only. The levels of risk (low, moderate or high) were selected based on nutrient loading and nutrient concentrations and the susceptibility of waterbody to nutrient enrichment. The risk index was calculated from a combination of nutrient enrichment, susceptibility (light availability) and physical conditions. For each national typology class groups were established either directly to nutrients or by ranking against the nutrient pressure risk assessment used in the water body characterisation process (see C-09); Outcomes from the risk assessment were used to test the boundary conditions suggested for index 1(chlorophyll biomass) and index 2 (elevated count index). Data from all the risk assigned waterbodies were then used to define ranges for each classification boundary

- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/LibraryPublicDocs/phytoplankton%20v2](http://www.wfduk.org/bio_assessment/LibraryPublicDocs/phytoplankton%20v2)

### **1.11 Pertinent literature of mandatory character:**

UKTAG coastal water assessment methods: Phytoplankton: Multi- Metric tool kit. ISBN: 978-1-906934-18-7, together with appropriate states ministerial directions (eg The River Basin Districts Surface Water and Groundwater Typology and Environmental Standards (Water Framework Directive) (England and Wales) Direction 2009).

### **1.12 Scientific literature:**

Devlin, M.J., J. Foden, D. Sivyer, D. Mills, R. Gowen & P. Tett, 2008. Relationships between suspended particulate material, light attenuation and Secchi depth in waters around the UK. *Estuarine, Coastal and Shelf Science* 79: 429-439. Devlin, M.J., J. Barry, S. Painting & M. Best, 2009. Extending the phytoplankton tool kit for the UK Water Framework Directive: indicators of phytoplankton community structure. *Hydrobiologia* 633: 151-168. Devlin, M.J., J. Foden, D. Sivyer, D. Mills, R. Gowen & P. Tett, 2008. Estimating the diffuse attenuation coefficient from optically active constituents in UK marine waters. *Estuarine, Coastal and Shelf Science* 79: 429-439. Devlin, M.J., M. Best, D.Coates, E. Bresnan, S. O'Boyle, R. Park, J. Silke, J. Skeats & J. Barry, 2007. Establishing boundary classes for classification of marine waters using phytoplankton communities - the first step in establishing a link between nutrient pressure and the marine plant community. *Marine Pollution Bulletin* 55 (1- 6): 91-10. Devlin, M.J., S.J. Painting & M. Best, 2006. Connecting nutrients with ecological functioning for the EU Water Framework Directive based on nutrient enrichment, primary production and undesirable disturbance. *Marine Pollution Bulletin* 55 (1- 6): 91-104. Painting, S.J., M. Devlin, S.J. Malcolm, E.R. Parker, D.K. Mills, C. Mills, P. Tett, A. Wither, A. Burt, R. Jones & K. Winpenny, 2006. Assessing the impact of nutrient enrichment in estuaries: susceptibility to eutrophication. *Marine Pollution Bulletin* 55: 74-91. Painting, S.J., M.J. Devlin, S.I. Rogers, D.K. Mills, E.R. Parker & H.L. Rees, 2005. Assessing the suitability of OSPAR EcoQOs for eutrophication vs ICES Criteria for England and Wales. *Marine Pollution Bulletin* 50 (12): 1569-1584.

### **1.13 Method developed by**

through UK & RoI Marine Plants Task Team, Chair Mike Best, lead developer Michelle Devlin  
mike.best@environment-agency.gov.uk,  
michelle.devlin@jcu.edu.au  
UK & RoI MPTT, funding via Environment Agency, Scottish and Northern Ireland Forum for Environmental Research (SEPA, EANI), CEFAS

### **1.14 Method reported by**

Mike Best  
mike.best@environment-agency.gov.uk  
Environment Agency (for England & Wales)

### **1.15 Comments**

none

## 2. Data acquisition

### ***Field sampling/surveying***

#### **2.01 Sampling/Survey guidelines**

WFD tool paper and sampling guidelines converted into competent authorities' standard operating procedures (SOPs) (eg Operational instruction 006\_07 Collecting and handling marine phytoplankton samples for Water Framework Directive and OSPAR. Laboratory analysis of Phytoplankton samples follows CEN standards and Lab SOPs.

#### **2.02 Short description**

Each water body will have had 3-10 samples allocated to it (depending on size and variability). Depending on the depth a surface water sample (<5m), or a 5m (5-10m depth), 10m (10-15m depth) or 15m integrated hose sample is taken and placed into a 10l bucket (several integrated hoses ay be taken to ensure enough sample for filtering for Chl and to provide a sample for taxa enumeration and for rinsing of equipment). The bucket sample is well mixed and a sub sample is filtered for Chl A analysis (the filtering is for 10 minutes or 3 l of sample). The filter papers are frozen and sent back to the lab for analysis using

cold acetone and spectrophometric detection. A 250ml subsample is preserved with Lugols Iodine and sent to the lab for enumeration following the utremol method.

- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Water sampler  
Integrated Hose (eg Lund Tube)
- 2.05 Specification:** Integrated hose (Lund Tube) or 5, 10, 15m lengths or surace sampler & top and bottom sample in shallow waters
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** Jan - Dec
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Ideally monthly (ie 12x per year)
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3-10 (depending on size of waterbody. note for Estuaries there are usually 2 salinity zones to be considered)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
up to 5 l of water is collected per sample of this upto 3 l will be filtered for Chl A analysis and 250ml for Phytoplankton analysis

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** approx 2  $\mu\text{m}$
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
The 250ml sub-sample is taken from a well mixed bucket sample (see B-13). This is then settled in a settling chamber and the whole plate counted under an inverted microscope
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
Identification aspires to species level but with routine Lugol's preserved samples this is not always possible, hence identification is to a mixed taxonomic level. A truncated taxa list has been developed for use where species cannot be easily identified, most taxa can be identified to genus but there are some diatoms and dinoflagellates that have to be determined at higher levels
- 2.15 Record of abundance:** Individual counts  
in relation to Volume  
Unit: cells per ml
- 2.16 Quantification of biomass:** Chlorophyll-a concentration
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

#### **3.01 List of biological metrics**

A. 90<sup>th</sup>ile Chlorophyll A in growing season (March-Oct), B. Elevated Counts - average of the percentage frequency at which 4 thresholds are exceeded: 1. Chl > 10  $\mu\text{g/l}$  (all year) 2. Any taxa over 250,000 cells per litre 3. Total taxa over  $10^6$  ( $10^7$  North of a line between Flamborough Head & the Solway) cells per litre 4. Phaeocystis over 106 cells per litre (not found in more northern UK waters) C. Seasonal Succession of Functional Groups The frequency that diatoms, dinoflagellates (and microflagellates, in Scotland) fall within local reference curves. The reference curves were constructed from long time series of data. The classification tool works by recording the number of occasions that the Z score (calculated by month) exceeds the upper envelope of monthly reference z scores for southern phytoplankton waters. Application of the 50% error to the reference curve for each Z score was applied to account for natural variability. The figure below show the shape of this reference curve for diatoms. A template graph was set up for the comparison of monthly Z score against the reference envelope (max values) \ plus 50%. Monthly Z scores are calculated for diatoms and dinoflagellates and/or monoflagellates from the monthly log mean (counts) against the mean and SD of the reference data for each waterbody and compared against the upper value of the reference envelope (+50%). Final calculation is the number of times the Z score falls under or equal to the reference upper value for diatoms and dinoflagellates (measured as a % for both functional groups). Total N = 12 (no of months) for each functional group. The two geographical zones (northern and southern phytoplankton communities) are defined with a line joining 550 North on the West Coast of Scotland to the Flamborough Front (approx Flamborough Head).

Estuarine Chlorophyll This tool is based on the calculation of a number of statistical metrics for chlorophyll to define the patterns of chlorophyll in variable transitional waters. Chlorophyll Data is separated into two salinity zones: "mixing zone" (1-25ppt), "coastal zone" (25-35ppt). Statistical measurements are calculated from the chlorophyll data within each zone, including mean (mixing threshold 15 $\mu\text{g/l}$ ; coastal threshold 10 $\mu\text{g/l}$ ), median (12 $\mu\text{g/l}$ ; 8), % of samples under 10 $\mu\text{g/l}$  (>70%; 75%), % of

samples under 20ug/l (>80%; 85%), % of samples over 50ug/l (<5%). Each statistical measurement is calculated from all the data over a 6 year reporting period. Each statistical measurement has a threshold associated for each zone and a 0 is awarded for exceedance and 1 for non exceedance. Final classification is based on a score out of 10 for the two combined salinity zones.

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

Aggregated data from multiple spatial replicates

### **Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data, Least Disturbed Conditions, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** All UK data used together with known nutrient data and risk assessment for nutrient pressures from water body condition assessments. There were about 350 sites used for different aspects of the tool kit

**Geographical coverage:** Sites considered high quality from Northern Scotland to the South of England and Wales, also Northern Ireland and Republic of Ireland (Eire) sites considered.

**Location of sites:** No reference sites but sites with identified low nutrient pressure too numerous to list. A risk index of waterbodies based on the level of nutrient enrichment and susceptibility of the waterbody to enrichment was established, allocating a risk factor to waterbodies within England and Wales only. The levels of risk (low, moderate or high) were selected based on nutrient loading and nutrient concentrations and the susceptibility of waterbody to nutrient enrichment. The risk index was calculated from a combination of nutrient enrichment, susceptibility (light availability) and physical conditions.

**Data time period:** Current data, recent data and historical data back to the 1980's (where available)

**Criteria:**

Sites that had absence or reduced pressures (or far offshore for Chlorophyll)

**3.08 Reference community description**

In "Atlantic" Waters Chlorophyll 90 percentile during the growing season will be less than 5ug/l whilst in "North Sea type" waters Chlorophyll will be less than 10ug/l. Blooms either of individual species / taxa of the total community will be infrequent and associated with the "natural" spring and autumn bloom period (<5% of occasions). The seasonal change between dominant functional groups should show the expected pattern (>80% within the expected envelope). In estuaries the chlorophyll metric should score better than 8 (all measures below thresholds), and normal seasonal and flow related blooms occur.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient

**3.11 Boundary setting procedure**

For each national typology class groups were established either directly to nutrients or by ranking against the nutrient pressure risk assessment used in the water body characterisation process (see C-09); Outcomes from the risk assessment were used to test the boundary conditions suggested for index 1 (chlorophyll biomass) and index 2 (elevated count index). Data from all the risk assigned water bodies were then used to define ranges for each classification boundary.

**3.12 "Good status" community:** In "Atlantic" Waters the 90th percentile of Chlorophyll concentration during the growing season will be less than 10ug/l whilst in "North Sea type" waters it will be less than 15ug/l. Blooms either of individual species / taxa of the total community will be occasional and associated with the "natural" spring and autumn bloom period (<20% of occasions). The seasonal change between dominant functional groups may deviate slightly from the expected pattern (>60% within the expected envelope). In estuaries the chlorophyll metric should score better than 5 (most measures below thresholds) and occasionally extra blooms to the normal seasonal and flow related blooms occur.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Partially done. The variability of the submetric scores within an assessment is estimated and, in combination with the number

of samples/stations/years, the Standard Error (SE) is derived. Uncertainty is established by comparing the average sub-metric score from the samples (with associated SE) to each status boundary. This allows the estimation of the probability that the average score (from the samples in the assessment) lies within a different status class than the true score. Uncertainty is not incorporated within the Phytoplankton toolkit itself. PUGWASH (Phytoplankton Uncertainty Gets Worked out And Statistically Handled) calculates confidence of class for the WFD TraC Phytoplankton Tool. Each sub-metric is computed using data for the water body as a whole over a six year reporting period. Each sub-metric score is converted into an EQR via a two-step normalisation process. The first step converts the sub-metric score to an EQR scale between 0 and 1, where the status class boundaries are not equidistant (for example, Bad = 0.0 – 0.27, Poor = 0.27 – 0.34, Moderate = 0.34 – 0.44 etc). The second step transforms these EQR values onto an equal-width class scale (Bad = 0.0 – 0.20, Poor = 0.20 – 0.40, Moderate = 0.40 – 0.60 etc). For simplicity, PUGWASH combines these two normalisation steps into one. The three sub-metric EQRs are then averaged to give a Final EQR between 0 and 1. (see WRC Ref: EA7954 12/03/2009. CONFIDENCE OF CLASS FOR WFD MARINE PLANT TOOLS)

**3.14 Comments:**

none

ID: 45

ELBO-approach

**1. General information**

- 1.01 GIG:** Baltic  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Angiosperms, Macroalgae, Phytoplankton  
Zostera marina, Ruppia, Myriophyllum
- 1.04 Country:** Germany
- 1.05 Specification:** Baltic coast
- 1.06 Method name:** **Ecological assessment approach for German Baltic coastal waters**
- 1.07 Original name:** *Ökologischer Gesamtansatz für die Bewertung der Küstengewässer an der Deutschen Ostseeküste entsprechend*
- 1.08 Status:** Method is ~~not~~ **used in** ~~WRL~~ **WRL**
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
0  
data from the monitoring program were tested for sensitivity against a synthetically degradation index consisting of 4 parameters linked to eutrophication (CCA)
- 1.10 Internet reference:** <http://www.biologie.uni-rostock.de/oekologie/RMB.htm> follow "RMB20"
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Gasiunaite, Z.R., A.C. Cardoso, A.S. Heiskanen, P. Henriksen, P. Kauppila, I. Olenina, R. Pilkaityte, I. Purina, A. Razinkovas, S. Sagert, H. Schubert & N. Wasmund, 2005. Seasonality of coastal phytoplankton in the Baltic Sea: Influence of salinity and eutrophication. *Estuarine, Coastal and Shelf Science* 65: 239-252. Heiskanen, A.-S., J. Carstensen, Z. Gasiunaite, P. Henriksen, A. Jaanus, P. Kauppila, E. Lysiak-Pastuszak & S. Sagert, 2005. Monitoring strategies for phytoplankton in the Baltic Sea coastal waters. JRC Technical report EUR 21583/EN: 1-4. Heiskanen, A.-S., S. Gromisz, A. Jaanus, P. Kauppila, I. Purina, S. Sagert & N. Wasmund, 2005. Developing reference conditions for phytoplankton in the Baltic coastal waters. Part I: Applicability of historical and long-term datasets for reconstruction of past phytoplankton conditions. JRC Technical report, EUR 21582/EN/1: 1-73. Krause-Jensen, D., S. Sagert, H. Schubert & C. Boström, 2008. Empirical relationships linking distribution and abundance of marine vegetation to eutrophication. *Ecological indicators* 8 (5): 515-529. Sagert, S., D. Krause-Jensen, P. Henriksen, T. Rieling & H. Schubert, 2005. Integrated ecological assessment of Danish Baltic Sea coastal areas by means of phytoplankton and macrophytobenthos. *Estuarine, Coastal and Shelf Science* 63: 109-118. Sagert, S., T. Rieling, A. Eggert & H. Schubert, 2008. Development of a phytoplankton indicator system for the ecological assessment of brackish coastal waters (German Baltic Sea coast). *Hydrobiologia* 611: 91-103. Schories, D., C. Pehlke & U. Selig, 2009. Depth limit distributions of *Fucus vesiculosus* Linnaeus and *Zostera marina* L. as criteria for implementing the European Water Framework Directive on the German Baltic coast. *Ecological Indicators* 9: 670-680. Schubert, H., M. Schubert & J.C. Krause, 2007. Reconstruction of XIXth century submerged vegetation of coastal lagoons of the German Baltic Sea. *Sea and Environment* 1 (14): 16-27. Selig, U., A. Eggert, D. Schories, M. Schubert, C. Blümel & H. Schubert, 2007. Ecological classification of macroalgae and angiosperm communities of inner coastal waters in the Southern Baltic Sea. *Ecological Indicators* 7: 665-678. Selig, U., A. Eggert, M. Schubert, T. Steinhardt, S. Sagert & H. Schubert, 2007. The influence of sediments on soft bottom vegetation in inner coastal waters of Mecklenburg-Vorpommern (Germany). *Estuarine, Coastal and Shelf Science* 71: 241-249.
- 1.13 Method developed by**  
Uwe Selig, Dirk Schories, Sigrid Sagert  
not employed at the university anymore  
Rostock University
- 1.14 Method reported by**  
hendrik schubert  
hendrik.schubert@uni-rostock.de  
Rostock University
- 1.15 Comments**  
none

**2. Data acquisition****Field sampling/surveying**

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
n.a.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.  
transect mapping / Scuba-divi
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** spring for phytoplankton
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**

macrophytes: twice per year in cases of Tolypella-occurrence;

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
site-specific

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
2m-corridors along transects until depth limit of macrophyte occurrence

### **Sample processing**

**2.12 Minimum size of organisms sampled and processed:** n.a.

**2.13 Sample treatment:** n.a.

**2.14 Level of taxonomical identification:** Family, Genus, Species/species groups  
altogether about 300 taxa are included in the analysis of phytoplankton data, for classification purposes at the moment less than 20 were found to be sensitive

**2.15 Record of abundance:** Individual counts, Percent coverage, Relative abundance  
**in relation to** Area, Volume  
**Unit** number of individuals per volume (phytoplankton)

**2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

**3.01 List of biological metrics**

[http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB\(20\)%2025-44.pdf](http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB(20)%2025-44.pdf)  
(macrophytes) @ [http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB\(20\)%2045-70.pdf](http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB(20)%2045-70.pdf)  
(phytoplankton)

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** n.a.

[http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB\(20\)%2025-44.pdf](http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB(20)%2025-44.pdf)  
(macrophytes) [http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB\(20\)%2045-70.pdf](http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB(20)%2045-70.pdf)  
(phytoplankton)

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

### **Reference conditions**

**3.05 Scope of reference conditions:** Habitat-specific

**3.06 Key source(s) to derive reference conditions:**  
Historical data, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** about 30

**Geographical coverage:** whole German Baltic coastline

**Location of sites:** n.a.

**Data time period:** starting in 1880

**Criteria:**  
n.a.

**3.08 Reference community description**

[http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB\(20\)%2025-44.pdf](http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB(20)%2025-44.pdf)

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Using discontinuities in the relationship of anthropogenic pressure and the biological response.

**3.11 Boundary setting procedure**

Changes in vegetation association structure

**3.12 "Good status" community:** A given site-specific association is asked for: [http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB\(20\)%2025-44.pdf](http://www.biologie.uni-rostock.de/oekologie/literature/RMB/RMB%2020/RMB(20)%2025-44.pdf)

**Uncertainty**

**3.13 Consideration of uncertainty:** Yes

By means of their correlation coefficient to the synthetic degradation index

**3.14 Comments:**

none

ID: 72

ZKI

## 1. General information

- 1.01 GIG:** Baltic  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Estonia
- 1.05 Specification:** none
- 1.06 Method name:** *Macrozoobenthos community index*
- 1.07 Original name:** *Makrozoobentose koosluse indeks*
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** Eutrophication, General degradation
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
ZKI was tested against tot-N and tot-P loads; correlation coefficient R-squared ranging from 0.32 to 0.62
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Jonne Kotta<br>jonne.kotta@sea.ee<br>Estonian Marine Institute, University of Tartu | <b>1.14 Method reported by</b><br>Kristjan Herkül<br>kristjan.herkyl@sea.ee<br>Estonian Marine Institute, University of Tartu |
|--|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
HELCOM, 2006. Manual for Marine Monitoring in the COMBINE Programme of HELCOM. [http://www.helcom.fi/groups/monas/CombineManual/en\\_GB/Contents/](http://www.helcom.fi/groups/monas/CombineManual/en_GB/Contents/)
- 2.02 Short description**  
Three stations are visited in every waterbody once per year. Three replicate samples are taken from each station using Ekman type bottom grab sampler. The samples are sieved on a 0.25 mm mesh and the residuals are held in deep-freezer (-18°C) until analyzing in laboratory.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Grab
- 2.05 Specification:** Ekman grab
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** July to September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3 replicates
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
0.02 square-metres \* three replicates \* three stations = 0.18 square-metres

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 0.25 mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Other, Species/species groups  
Juvenile Gammarus spp identified to genus level; insect larvae identified to family or order level; oligochaetes identified to class level.
- 2.15 Record of abundance:** Individual counts

in relation to Area

Unit Number of individuals per one square-metre

2.16 Quantification of biomass: n.a.

Dry weight (60°C, 48 h) of each taxon measured to the nearest 0.0001 g

2.17 Other biological data: none

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

3.01 List of biological metrics

Dry biomass of each benthic invertebrate taxon.

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: Average metric scores

3.04 From which biological data are the metrics calculated?

Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

#### Reference conditions

3.05 Scope of reference conditions: Site-specific

3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Historical data, Least Disturbed Conditions

3.07 Reference site characterisation

Number of sites: Multiple

Geographical coverage: Estonian coastal sea

Location of sites: Estonian coastal sea

Data time period: Historical data from 1950s-1960s

Criteria:

n.a.

3.08 Reference community description

n.a.

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: High-good boundary derived from metric variability at near-natural reference sites

3.11 Boundary setting procedure

n.a.

3.12 "Good status" community: n.a.

#### Uncertainty

3.13 Consideration of uncertainty: No (to be done)

3.14 Comments:

none

ID: 66

BBI

## 1. General information

- 1.01 GIG:** Baltic  
CW\_B0, CW\_B2 and CW\_B3
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Finland
- 1.05 Specification:** none
- 1.06 Method name:** *Brackish water benthic index*
- 1.07 Original name:** *Brackish water benthic index*
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Tested against traditional physicochemical monitoring data in the complex Archipelago Sea.
- 1.10 Internet reference:** <http://ambio.allenpress.com/archive/0044-7447/36/2/pdf/i0044-7447-36-2-250.pdf>
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Perus, J., E. Bonsdorff, S. Bäck, H.-G. Lax, A. Villnäs & V. Westberg, 2007. Zoobenthos as Indicators of Ecological Status in Coastal Brackish Waters: A Comparative Study from the Baltic Sea. *Ambio* 36: 250–256.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Jens Perus<br>jens.perus@environment.fi<br>West Finland Regional Environment Centre | <b>1.14 Method reported by</b><br>Jouko Rissanen<br>firstname.lastname@environment.fi (jouko rissanen)<br>Finnish Environment institute |
|--|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Lax, H.-G., 2008. Pehmeiden pohjien pohjaeläinten ja sedimentin näytteenotto rannikkovesien VPD-seurannassa. Vaasa.
- 2.02 Short description**  
Water basin is divided to sub areas according depth (<10m and >10m) and salinity (0-2, 2-4 and >4 psu). In each sub areas five random stations are sampled within 3 or 6 years intervals. One replicate per station.
- 2.03 Method to select the sampling/survey site or area:** Stratified sampling/surveying
- 2.04 Sampling/survey device:** Dredge
- 2.05 Specification:** VanVeen and Ekman-Birger
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
profundal soft bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** May to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
3 or 6 years
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Five stations per subarea
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Sum of five spatial replicates á

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 0,5 mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Family, Genus, Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to Area  
Unit individuals/square meter
- 2.16 Quantification of biomass:** n.a.

wet weight

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments:**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Please look: Perus, J., Bonsdorff, E., Bäck, S., Lax, H.-G., Villnäs, A. ja V. Westberg. 2007. Zoobenthos as Indicators of Ecological Status in Coastal Brackish Waters: A Comparative Study from the Baltic Sea. *Ambio* 36: 250–256.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Weighted average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple spatial replicates

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** CW\_B3 only (for the meantime)

**Location of sites:** Arhipelago Sea (Finland)

**Data time period:** 1990-2000

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 187

MarBIT

## 1. General information

- 1.01 GIG:** Baltic  
CW B12
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Germany
- 1.05 Specification:** currently, only Baltic Sea
- 1.06 Method name:** **Marine Biotic Index Tool**
- 1.07 Original name:** *Marine Biotic Index Tool*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

General degradation

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

General degradation was tested against a range of sites from cleaner and more degraded sites (determined by expert judgement). 10 samples were used at each site to assess the status. No further statistics has been made.

- 1.10 Internet reference:** <http://www.marilim.de/marbit>

- 1.11 Pertinent literature of mandatory character:**  
n.a.

**1.12 Scientific literature:**

Meyer, T., T. Berg & K. Fühaupter, 2009. Ostsee- Makrozoobenthos- Klassifizierungssystem für die Wasserrahmenrichtlinie. Referenz-Artenlisten, Bewertungsmodell und Monitoring. (3. überarbeitete Fassung vom 20. Januar 2009). Bericht für das BMBF (Förderkennzeichen 0330678).

**1.13 Method developed by**

Torsten Berg  
berg@marilim.de  
MariLim Gesellschaft für Gewässeruntersuchung mbH

**1.14 Method reported by**

Torsten Berg  
berg@marilim.de  
MariLim Gesellschaft für Gewässeruntersuchung mbH, Heinrich-  
Wöhlk-Straße 14, 24232 Schönkirchen

**1.15 Comments**

Peer-reviewed paper in preparation.

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Rumohr, H., 1990. Soft bottom macrofauna: Collection and treatment of samples. ICES Techniques in Environmental Sciences 8, 1–19. Revised version 2001 Prüfverfahren-SOP: Makrozoobenthos-Untersuchungen in marinen Sedimenten (Weichboden). Qualitätssicherungsstelle des Bund/Länder-Messprogramms Nord- und Ostsee am Umweltbundesamt.

**2.02 Short description**

Sites known to have the targeted habitat are selected, then random samples over the complete depth gradient of the water body/type are taken within the habitat. At each location, one single sample is taken, so there are single samples covering the targeted water body.

- 2.03 Method to select the sampling/survey site or area:** Expert knowledge

- 2.04 Sampling/survey device:** Grab  
Kautsky sampling frame

- 2.05 Specification:** Kautsky frame, 0.1 square metres ; van Veen grab, warp rigged, 0.1 square metres

- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom, hard bottom, phytal fauna

- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

- 2.08 Sampling/survey month(s):** spring from March to April

- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one per year

- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
20 single samples per water body and habitat

- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
sum of 20 samples = 2 square metres

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 1000µm (size of mesh in the field)

- 2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
All groups are determined to the most specific level possible, which is species level in general. The following groups are not determined further: Platyhelminthes, Nemertina, Chironomidae, Hemichordata, Oligochaeta. To genus level: Bathyporeia smaller 4mm, Gammarus smaller 4mm, Marenzelleria smaller 2mm (breadth at 7. segment), Nereididae smaller 2mm, Nephtys smaller 2mm.

**2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** number of individuals

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** Since the samples are taken by divers when using a sampling frame, animals are qualitatively recorded, that cannot quantitatively be caught: Asterias, siphons of Mya, and similar large low-density species.

**2.19 Comments**  
to B-14, B-15: sub-sampling is only done when a taxon occurs in masses. The exact definition of this and the procedure applied for subsampling under these conditions can be found in the SOP.

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**  
1. Taxonomic spread index (TSI) 2. Abundance distribution vs. log-normal distribution 3. Fraction of sensitive taxa 4. Fraction of tolerant taxa

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

Median

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple spatial replicates

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Modelling (extrapolating model results)  
Autecological data from existing scientific literature

**3.07 Reference site characterisation**

**Number of sites:** - not applicable -

**Geographical coverage:** - not applicable -

**Location of sites:** n.a.

**Data time period:** - not applicable -

**Criteria:**

For this BQE, there are no actual reference sites available.

**3.08 Reference community description**

The reference community comprises all species, that by all known scientific knowledge are able to live under the given abiotic regime (e.g. salinity, depth, substrate, exposure). This community is derived from autecological data and verified via historical sample material. However, only steady species are accounted for, not guest species from e.g. the North Sea. Also, very rare species not typical of the community, are disregarded, since it is unlikely to find them with the given sampling effort.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
High-good boundary derived from metric variability at near-natural reference sites  
The boundaries for the final EQR are always transformed boundaries at 0.2, 0.4, 0.6, and 0.8

**3.11 Boundary setting procedure**

The GM boundary was set where a statistical significance occurs with respect to the change of the community (measured individually and separately on each of the 4 metrics). The HG boundary was in general set half-way from there and up to the maximum index value. The MP and PB boundaries were derived from the normative definitions and translated into ecologically sensible values for each of the 4 indices. (All details on this procedure can be found in the report given for question A-22).

- 3.12 "Good status" community:** 1. Only an insignificant reduction of species composition has taken place (in practice, every major taxonomical group should be present).<sup>2</sup>2. The deviation of the abundance distribution from the ideal log-normal model is not significant.<sup>3</sup>3. The fraction of sensitive species in the community matches the fraction found under reference conditions with only a minor reduction.<sup>4</sup>4. The fraction of tolerant species in the community matches the fraction found under reference conditions with only a minor increase.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

Strictly, the scope of the reference conditions is several water bodies, but not all in one water type. Per water type, there are more than one references.

ID: 67

FI-AL-CO

## 1. General information

- 1.01 GIG:** Baltic  
CW\_B0, CW\_B2 and CW\_B3
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Macroalgae
- 1.04 Country:** Finland
- 1.05 Specification:** Not in Bothnian Bay
- 1.06 Method name:** *Fucus index*
- 1.07 Original name:** *Fucus index*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Ruuskanen, A., 2009. Rannikon makrofyttiseurannan menetelmäpäivitys. Helsinki. 32 p. Manuscript.
- 1.12 Scientific literature:**  
n.a.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Ari Ruuskanen<br>ari.ruuskanen@monivesi.fi<br>Monivesi Oy | <b>1.14 Method reported by</b><br>Jouko Rissanen<br>firstname.lastname@environment.fi (jouko rissanen)<br>Finnish Environment institute |
|--|---|
- 1.15 Comments**  
Method was not accepted for intercalibration by other countries around Baltic Sea.

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Ruuskanen, A., 2009. Rannikon makrofyttiseurannan menetelmäpäivitys. Helsinki. 32 p. Manuscript.
- 2.02 Short description**  
The lower limit of growing zone of bladder-wrack (*Fucus vesiculosus*) is measured by diving in 10 to 30 places at each sites in every 3 years. Sites are located in average at 50 km intervals in the outer archipelago of Finnish coast
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.  
SCUBA diving
- 2.05 Specification:** Scuba diving
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Fucus vesiculosus zone
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** August to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
3 years interval
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
several sites per water body
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** n.a.
- 2.15 Record of abundance:** Individual counts  
**in relation to** n.a.  
No abundance  
**Unit** depth of the lower limit of Fucus zone
- 2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

Only lower depth of Fucus zone is measured.

### 3. Data evaluation

#### **Evaluation**

**3.01 List of biological metrics**

n.a.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### **Reference conditions**

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** Gulf of Finland, Finland

**Location of sites:** Gulf of Finland, Finland

**Data time period:** 1993-2008

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 4

EE-PP-CO

## 1. General information

- 1.01 GIG:** Baltic  
B12 and B13
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Estonia
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment of ecological status of coastal waters using phytoplankton indicators*
- 1.07 Original name:** *Rannikuvete ökoloogilise seisundi hindamine fütoplanktoni indikaatorite alusel*
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** Eutrophication, Impact of alien species
- Has the pressure-impact-relationship been tested?**  
Yes, with qualitative data (e.g. response at reference against impacted sites).  
440 samples from 22 coastal water stations were examined to establish pressure-impact relationship between phytoplankton metrics (chlorophyll a) and TN measured from June to September. Linear regression analysis showed significant correlation ( $r^2=0.59$ ).
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Pinnaveekogumite moodustamise kord ja nende pinnaveekogumite nimestik, mille seisundiklass tuleb määrata, Pinnaveekogumite seisundiklassid ja seisundiklassidele vastavad kvaliteedinäitajate väärtused ning seisundiklasside määramise kord. <http://www.rigiteataja.ee/ert/act.jsp?id=13210253&replstring=33>
- 1.12 Scientific literature:**  
n.a.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Andres Jaanus<br>andres@sea.ee<br>Estonian Marine Institute, University of Tartu | <b>1.14 Method reported by</b><br>Andres Jaanus<br>andres@sea.ee<br>Estonian Marine Institute, University of Tartu |
|---|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
[http://www.helcom.fi/groups/monas/CombineManual/AnnexesC/en\\_GB/annex4](http://www.helcom.fi/groups/monas/CombineManual/AnnexesC/en_GB/annex4)  
(chlorophyll) [http://www.helcom.fi/groups/monas/CombineManual/AnnexesC/en\\_GB/annex6](http://www.helcom.fi/groups/monas/CombineManual/AnnexesC/en_GB/annex6) (phytoplankton biomass)
- 2.02 Short description**  
Chlorophyll: Plankton: Water samples from standard depths (1, 5 and 10 m) are pooled and 100-200 ml of water is poured into a glass bottle containing fixative (acid Lugol's solution). Samples are stored in dark.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** Niskin PWS, 1.5 L
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** June to September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
6-7 samples per assessment season and station
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3 replicates
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Integrated sample from 3 (2) horizons (upper 10 m layer); 0.2 liters for phytoplankton biomass

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 2  $\mu\text{m}$  (resolution of inverted microscope using 400x magnification)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups  
Mainly to species level; depends on the facilities of inverted microscope techniques; flagellates are often identified to

genus or order level

**2.15 Record of abundance:** Individual counts

**in relation to** Volume

**Unit** Number of individuals per liter; for chlorophyll a

**2.16 Quantification of biomass:** Chlorophyll-a concentration, Utermöhl technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** Flow-through samples onboard passenger ferries are sampled from 5 metre depth

**2.19 Comments**

none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Total median wet weight autotrophic biomass (including autotrophic ciliate *Mesodinium rubrum*) mg/l, chlorophyll a median concentration µg/l

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Worst metric score

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

Aggregated data from multiple spatial replicates

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** 4 coastal water bodies out of 15

**Geographical coverage:** 100-400 km<sup>2</sup>

**Location of sites:** Narva Bay (southeastern Gulf of Finland); Tallinn Bay (southern Gulf of Finland); Haapsalu Bay (West-Estonian coast); Pärnu Bay (northwestern Gulf of Riga, Estonia)

**Data time period:** Monitoring data from 1993 to 2004

**Criteria:**

Spatio-temporal variability has to be taken into account; abundance and biomass are affected by seasonal cycle of light period and intensity, by limiting nutrients and by hydrodynamics (upwelling, riverine outflow).

**3.08 Reference community description**

Reference communities are dominated by nanoplanktonic flagellates and cyanobacteria in small amounts.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

Reference conditions (RC) were set at 10th or 20th percentile of all monitoring values. The HG boundary was set RC\*1.2.

☐

The GM boundary was set RC\*1.5. The MP boundary was set RC\*3. The PB boundary was set RC\*4.5.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 93

CHL-FI

## 1. General information

**1.01 GIG:** Baltic  
CW\_B0, CW\_B2, CW\_B3

**1.02 Category:** Coastal Waters

**1.03 BQE:** Phytoplankton

**1.04 Country:** Finland

**1.05 Specification:** none

**1.06 Method name:** *Assessment system for coastal waters using phytoplankton chlorophyll-a*

**1.07 Original name:** *Kasviplanktonin a-klorofyllin luokitus rannikkovesissä*

**1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)

**1.09 Detected pressure(s):** Eutrophication

***Has the pressure-impact-relationship been tested?***

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Finnish coastal water monitoring data (about 700 sites) were examined to establish pressure-impact relationship between nutrients (TN and TP) and chlorophyll a measured in mid and late summers showed significant R-squares ( $p < 0.0001$ ).

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Vuori et al., 2006. The basis for typology and ecological classification of water bodies in Finland. The Finnish Environment 807. Suomen ympäristökeskus & Riista- ja kalatalouden tutkimuslaitos 2008. Pintavesien ekologisen luokittelun vertailuolot ja luokan määrittäminen. Luokitteluopas (in press).

**1.12 Scientific literature:**

Kauppila, P., 2007. Phytoplankton quantity as an indicator of eutrophication in Finnish coastal waters. Applications within the Water Framework Directive. Monographs of the Boreal Environment Research 31: 58 pp.

**1.13 Method developed by**

0

**1.14 Method reported by**

Pirkko Kauppila

pirkko.kauppila@ymparisto.fi

SYKE

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Chlorophyll a is measured from a composite sample and analysed according to Lorenzen, 1967. The chl samples are extracted with ethanol (ethyl alcohol). Monitoring is described briefly in @Niemi J. (ed.) 2009. Environmental monitoring in Finland. Finnish Environment Institute, The Finnish Environment 12 / 2009.

**2.02 Short description**

Chlorophyll a measured from composite sample (two times Secchi depth).

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** none

**2.06 Sampled/surveyed habitat:** n.a.

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** Summer period from July to September used for ecological classification for chlorophyll a

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Two to five samplings per sampling season (mid to late summer)

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

One to five sites exist within a coastal water body.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Finnish coastal water area, defined according to the WFD is 34 000 km<sup>2</sup>. Altogether 760 sites comprise the coastal monitoring network.

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** n.a.

**2.13 Sample treatment:** n.a.

**2.14 Level of taxonomical identification:** n.a.

- 2.15 Record of abundance:** n.a.  
**in relation to** n.a.  
**Unit**
- 2.16 Quantification of biomass:** Chlorophyll-a concentration
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
n.a.
- 3.02 Does the metric selection differ between types of water bodies?** n.a.
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
n.a.

#### Reference conditions

- 3.05 Scope of reference conditions:** n.a.
- 3.06 Key source(s) to derive reference conditions:**  
Modelling (extrapolating model results)
- 3.07 Reference site characterisation**
- Number of sites:** 29 sites in coastal marine areas around Finland
  - Geographical coverage:** From the Bothnian Bay to the eastern Gulf of Finland
  - Location of sites:** in outer archipelagos
  - Data time period:** mid summers 1925-1934
  - Criteria:**  
n.a.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise
- 3.11 Boundary setting procedure**  
The procedure described in the IC technical report and applied in the national classification system. The procedure also described in Vuori et al. (2006). The basis for typology and ecological classification of water bodies in Finland. The Finnish Environment 807 (in Finnish with a English summary). Suomen ympäristökeskus & Riista- ja kalatalouden tutkimuslaitos 2008. Pintavesien ekologisen luokittelun vertailuolot ja luokan määrittäminen. Luokitteluopas (in press).
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
Accuracy of reference values have been estimated (Kauppila 2007).

ID: 21

DK-AN-CO

## 1. General information

- 1.01 GIG:** Baltic, North-East-Atlantic  
coastal waters, type OW3, Hjelm Bugt
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Angiosperms  
Zostera marina
- 1.04 Country:** Denmark
- 1.05 Specification:** all areas with soft/sandy bottoms
- 1.06 Method name:** *Depth limit of eelgrass*
- 1.07 Original name:** *ålegræssets dybdegrænse*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Eelgrass depth limits have been found to respond to changes in underwater light climate - tested based on nation-wide Danish data sets (e.g. Nielsen et al. 2002). To the extent that the light climate reflects nutrient concentrations and nutrient load, depth limits also respond to changes in nutrient levels (Nielsen et al. 2002). Reductions in nutrient load to Danish coastal waters have led to reductions in nutrient concentrations but have, however, not led to improvements in light climate and likely therefore not led to improved depth limits (Hjort et al 2009 - the national Danish monitoring report for marine waters 2009).

- 1.10 Internet reference:** [http://www2.dmu.dk/1\\_om\\_dmu/2\\_tvaer-funk/3\\_fdc\\_mar/programgrundlag/TekAnv2004\\_2009/Del3/TA04\\_3\\_1\\_Bundvegetation.pdf](http://www2.dmu.dk/1_om_dmu/2_tvaer-funk/3_fdc_mar/programgrundlag/TekAnv2004_2009/Del3/TA04_3_1_Bundvegetation.pdf)
- 1.11 Pertinent literature of mandatory character:**  
Sampling method: Technical guidelines for marine vegetation surveys. [http://www2.dmu.dk/1\\_om\\_dmu/2\\_tvaer-funk/3\\_fdc\\_mar/programgrundlag/TekAnv2004\\_2009/Del3/TA04\\_3\\_1\\_Bundvegetation.pdf](http://www2.dmu.dk/1_om_dmu/2_tvaer-funk/3_fdc_mar/programgrundlag/TekAnv2004_2009/Del3/TA04_3_1_Bundvegetation.pdf) Krause-Jensen, D. & M.B. Rasmussen, 2009. Historisk udbredelse af ålegræs i danske kystområder. Danmarks Miljøundersøgelser, Århus Universitet. 38 pp. Technical report from NERI no 755. <http://www.dmu.dk/Pub/FR755.pdf> (will appear ultimo December 2009) In Danish with English summary. Assessment method: - not yet published
- 1.12 Scientific literature:**  
Boström, C., S.P. Baden & D. Krause-Jensen, 2003. The seagrasses of Scandinavia and the Baltic Sea. In Green, E.P. & F.T. Short (eds), World Atlas of Seagrasses. University of California Press, Berkeley: 27-37. Duarte, C.M., N. Marbà, D. Krause-Jensen & M. Sánchez-Camacho, 2007. Publications using data on eelgrass depth limits from Danish coastal waters: Testing the Predictive Power of Seagrass Depth Limit Models. Estuaries and Coasts 30 (4): 652-656. Greve, T.M. & D. Krause-Jensen, 2005. Predictive modelling of eelgrass (*Zostera marina*) depth limits. Marine Biology 146: 849-858. Greve, T.M. & D. Krause-Jensen, 2005. Stability of eelgrass (*Zostera marina* L.) depth limits: influence of habitat type. Marine Biology 147: 803-812. Krause-Jensen, D., M.F. Pedersen & C. Jensen, 2003. Regulation of Eelgrass (*Zostera marina*). Cover along Depth Gradients in Danish Coastal Water. Estuaries 26 (4A): 866-877. Krause-Jensen, D., P. Henriksen, T. Rielsing & H. Schubert, 2005. Integrated ecological assessment of Danish Baltic Sea coastal areas by means of phytoplankton and macrophytobenthos. Estuarine, Coastal and Shelf Science 63: 109-118. Krause-Jensen, D., T.M. Greve & K. Nielsen, 2005. Eelgrass as a Bioindicator under the European Water Framework Directive. Water Resources Management 19: 63-75.
- 1.13 Method developed by**  
various  
  
The Danish Environmental Authorities and National Environmental Research Institute (NERI) in collaboration
- 1.14 Method reported by**  
Dorte Krause-Jensen  
dkj@dmu.dk  
National Environmental Research Institute, University of Aarhus (NERI-AU)
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Technical guidelines for marine vegetation surveys. [http://www2.dmu.dk/1\\_om\\_dmu/2\\_tvaer-funk/3\\_fdc\\_mar/programgrundlag/TekAnv2004\\_2009/Del3/TA04\\_3\\_1\\_Bundvegetation.pdf](http://www2.dmu.dk/1_om_dmu/2_tvaer-funk/3_fdc_mar/programgrundlag/TekAnv2004_2009/Del3/TA04_3_1_Bundvegetation.pdf)
- 2.02 Short description**  
A diver swims along a depth gradient (transect) extending from the shore to the deepest eelgrass shoot and makes point observations of eelgrass cover (%) connected with information on water depth and position. For each meter change in water depth there are at least 7-10 observations of cover. The depth limit of eelgrass is defined as the maximum depth of 10% eelgrass cover. An average is calculated based on all the transects of the area. The diver also assesses the maximum depth limit, i.e. the depth of the deepest shoots. - The divers swims along the edge of the vegetation (transversal to the depth gradient) and gives about 7 observations of the maximum depth limit.
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** n.a.  
diver observations
- 2.05 Specification:** none

- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom habitats -seagrass beds
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** n.a.
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
we use one annual sampling
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
We use about 5 sites per area
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Percent coverage  
in relation to n.a.
- Unit**
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**

I have only specified methods for the indicator 'Depth limit of eelgrass'. We do measure other vegetation variables, but these are not yet fully developed for use in the Water Framework Directive. Comment to B-02: In my terminology Phytobenthos includes microphytobenthos and macrophytobenthos. The macrophytobenthos equals 'macrophytes and includes macroalgae and seagrasses and other angiosperms.

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
So far we only use the depth limit of eelgrass as vegetation indicator. We are developing other indicators such as Total macroalgal cover (i.e. the change in macroalgal cover with depth)
- 3.02 Does the metric selection differ between types of water bodies?** n.a.
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
n.a.

### **Reference conditions**

- 3.05 Scope of reference conditions:** n.a.
- 3.06 Key source(s) to derive reference conditions:**  
Historical data
- 3.07 Reference site characterisation**
- Number of sites:** A large net of historical data amounting to several hundreds in total
- Geographical coverage:** The historical data are distributed across most of the Danish coastal waters with the majority along the open coasts
- Location of sites:** Various water body types along open coasts and fjords of Denmark
- Data time period:** 1880s-1920s
- Criteria:**  
We assumed that the period 1880s-1920s represented a period with low nutrient loads characteristic of reference conditions. Moreover, as the majority of the Danish eelgrass populations were killed by the wide-spread 'wasting disease' in the early 1930s we used data before 1930 to describe the reference.
- 3.08 Reference community description**  
Around year 1900 eelgrass meadows covered most of the Danish coastal waters.
- 3.09 Results expressed as EQR?** n.a.

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

Eelgrass depth limits have been shown to respond to changes in water clarity which again relates to nutrient levels. Historical data on depth limits from a period with high water clarity and low nutrient levels were used to characterise the reference situation. The boundaries were set as a specific deviation (25-30%) from the reference.

**3.12 "Good status" community:** At good status the eelgrass meadows grow deep, deviating only by 25-30% from reference depth limits.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 176

RO-AL-CO

## 1. General information

- 1.01 GIG:** Black Sea  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Angiosperms, Macroalgae  
Zostera nana
- 1.04 Country:** Romania
- 1.05 Specification:** Romanian Black Sea shore
- 1.06 Method name:** *Assessment system for coastal waters using macroalgae*
- 1.07 Original name:** n.a.
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction  
*Has the pressure-impact-relationship been tested?*  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
n.a.
- n.a.
- 1.14 Method reported by**  
Oana Dumitrescu  
oana8900@yahoo.com  
Department of Environmental Protection, National Institute for  
Environmental Protection Research and Development
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Minicheva, G.G. Macrophytobenthos - Methods of sampling, treatment and estimation of parameters.
- 2.02 Short description**  
Macroalgae are collected using a frame of 10x10 cm (for *Cystoseira barbata*) and 20x20 cm (for other species) and a rake. The samples are taken from different depths, then placed into a plastic bag and supplied with a detailed label with information about the sampling place and conditions. An underwater camera is used to take pictures.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Rake
- 2.05 Specification:** rake, scalpels, frames, plastic bags, labels.
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
rocky bottom, hard artificial bottom, sandy bottom (for *Zostera nana*).
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** all year
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3 replicates
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Sum of 3 spatial replicates = 0,12 square-metres

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 0
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Sometimes species of *Enteromorpha* and *Cladophora* to genus level; others to species level.
- 2.15 Record of abundance:** n.a.  
The biota's abundance was not calculated until present, but in future it will be based on individual

- counts.
- in relation to** Area
- Unit** Number of individuals per one square-metre.
- 2.16 Quantification of biomass:** n.a.  
Determination of fresh weight by weighting at the electronic balance, after the identification and separation of the species.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
- Fresh weight:  $B(g/m^2) = a \times 25$ , a= wet biomass weighted from a surface of 20/20  
cm.<sup>2</sup> 25= coefficient for reporting on one square-metre.<sup>2</sup>- Presence/Absence
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time  
Data from single spatial replicate
- Reference conditions**
- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** 5 sites in the Romanian Black Sea waters  
**Geographical coverage:** Only reference zones between Mangalia and Vama Veche considered representative for Romanian Black Sea waters.  
**Location of sites:** Constanta, Eforie, Mangalia, 2 Mai, Vama Veche.  
**Data time period:** Historical data before 1981 covering 11 years.  
**Criteria:**  
The anthropogenic impact is more reduced within the sites considered representative for Romanian Black Sea waters.
- 3.08 Reference community description**  
1. Macroalgal communities of high diversity should be dominated quantitatively by brown algae (*Cystoseira barbata*, *Cystoseira crinita*) and red algae (*Phyllophora* sp.)<sup>2</sup>. A high number of brown and red algae.
- 3.09 Results expressed as EQR?** No Presence/Absence of brown algae and angiosperms considered as indicators.

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Using discontinuities in the relationship of anthropogenic pressure and the biological response.  
Not established yet.
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** - A high number of sensitive taxa (brown algae - *Cystoseira* sp., red algae - *Phyllophora* sp., angiosperms - *Zostera nana*).<sup>2</sup>- A high specific diversity.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 92

DK-PP-CO

## 1. General information

- 1.01 GIG:** Black Sea, North-East-Atlantic  
Baltic GIG: B12, B13, B14; NEA GIG: NEA1/26c, NEA8
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Denmark
- 1.05 Specification:** Until politically approved, the method is used only at intercalibration sites
- 1.06 Method name:** *Assessment system for coastal waters using chlorophyll-a as indicator of phytoplankton biomass*
- 1.07 Original name:** n.a.
- 1.08 Status: Method is/will be used in** n.a.
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Relationships between nitrogen input and site-specific total nitrogen (TN) as well as between TN and Chl a are established using recent monitoring data from 35 sites. From modelled time series of nutrient inputs to the Danish straits boundary values for nutrient input for different periods of eutrophication have been selected and used for predicting site-specific boundaries for TN and Chl a.
- 1.10 Internet reference:** <http://www2.dmu.dk/Pub/FR683.pdf>
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Carstensen, J. & P. Henriksen, 2009. Phytoplankton biomass response to nitrogen inputs: a method for WFD boundary setting applied to Danish coastal waters. *Hydrobiologia* 633 (1): 137-149.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Jacob Carstensen and Peter Henriksen<br>jac@dmu.dk<br>National Environmental Research Institute, Aarhus University | <b>1.14 Method reported by</b><br>Peter Henriksen<br>pet@dmu.dk<br>National Environmental Research Institute, Aarhus University,<br>Denmark |
|---|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Danish Technical guidelines [http://www2.dmu.dk/1\\_om\\_dmu/2\\_tvaer-funk/3\\_fdc\\_mar/programgrundlag/TekAnv2004\\_2009/Del2/TA04\\_2\\_3\\_klorofyl.pdf](http://www2.dmu.dk/1_om_dmu/2_tvaer-funk/3_fdc_mar/programgrundlag/TekAnv2004_2009/Del2/TA04_2_3_klorofyl.pdf) Based on the HELCOM COMBINE manual <http://www.helcom.fi/groups/monas/CombineManual/>
- 2.02 Short description**  
For the open sea, the standard sampling depths for chlorophyll-a are in the upper water at the following depths: 1 m, 5 m, 10 m, 15 m and 20 m. The sample from 1 m or an integrated sample (1-10 m) is analysed.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** n.a.
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** Baltic GIG: May-September, NEA GIG: March-September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Monthly sampling as a minimum
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
One
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Samples of 1-2 litres

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** Any organism retained on GF/F filters
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** n.a.

- 2.15 Record of abundance:** n.a.  
**in relation to** n.a.  
**Unit**
- 2.16 Quantification of biomass:** Chlorophyll-a concentration
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Summer (May-September) mean Chl a concentration or 90th percentile of Chl a concentration in samples collected from March through September
- 3.02 Does the metric selection differ between types of water bodies?** n.a.
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
n.a.  
Metric calculated from data collected during a whole year or based on data from a 6 year period

#### Reference conditions

- 3.05 Scope of reference conditions:** Site-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**
- Number of sites:** 35 sites in Danish waters used in calculation of reference conditions. No reference sites available.
- Geographical coverage:** n.a.
- Location of sites:** n.a.
- Data time period:** Estimated historical nutrient loadings combined with monitoring data covering 1989-2005
- Criteria:**  
n.a.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** No Chl a concentrations

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites
- 3.11 Boundary setting procedure**  
Nutrient inputs to the Danish straits were hincasted to 1900 based on estimates of the nitrogen surplus from Danish agriculture and estimated changes in point sources. Discussions with the Danish EPA have led to characterise different ecological status classes by different periods in time. The period up to 1950 is considered having a high ecological status, corresponding to a nitrogen input of about 22,000 tonnes N per year. In the 1950s and early 1960s the ecological status was considered to be good, corresponding to a nitrogen input of about 32,000 tonnes N per year. In the late 1960s and 1970s the situation started worsening and the ecological status was considered to be moderate, corresponding to an average nitrogen input of about 73,000 tonnes N per year. In the 1980s the situation got really poor (average of 91,000 tonnes N per year) and in certain years the status may even have been considered bad (average of 110,000 tonnes N per year for the 3 worst years). Nitrogen inputs in the 1990s were highly variable with an average of 66,000 tonnes N per year, an input level similar to the 1970s and the status could be characterised as moderate. In the most recent years the nitrogen input has been about 50,000 tonnes N per year, a status that may be characterised as between good and moderate status. Given these N-input boundaries the corresponding site specific TN concentrations were calculated and from TN-Chl a relationships the boundary Chl a concentration defined.
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** Yes  
Described in: Carstensen, J., Henriksen, P. (2009) Phytoplankton biomass response to nitrogen inputs: a method for WFD

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boundary setting applied to Danish coastal waters, *Hydrobiologia*, vol. 633 no. 1, pp. 137-149.

**3.14 Comments:**

none

ID: 231

CymoSkew

## 1. General information

**1.01 GIG:** Mediterranean**1.02 Category:** Coastal Waters**1.03 BQE:** Angiosperms**1.04 Country:** Greece**1.05 Specification:****1.06 Method name:** *CymoSkew***1.07 Original name:** *CymoSkew***1.08 Status: Method is/will be used in** Second RBMP (2015)**1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Pollution by organic matter*Has the pressure-impact-relationship been tested?***1.10 Internet reference:****1.11 Pertinent literature of mandatory character:**

n.a.

**1.12 Scientific literature:**

n.a.

**1.13 Method developed by**

sorfanid@inale.gr

**1.14 Method reported by**

Sotiris Orfanidis

sorfanid@inale.gr

National Agricultural Research Foundation (NAGREF)-Fisheries  
Research Institute (FRI)**1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Quantitative sampling of seagrasses.

**2.02 Short description**

Since the spatial variation of the *Cymodocea nodosa*'s leaf length is not known a random nested sampling design on a hierarchy of spatial scales, ranging from 10s of m (area) to 100s of m (site) to km (meadow), is suggested. In each meadow two sites are randomly chosen along the same isobath (1.2 to 4 m) that are ca. 500-800 m apart. In each site two areas are randomly selected that are ca. 50-80 m apart. In each area five metallic quadrats (25 x 25 cm) were randomly placed by divers on the bottom and subsequently all the shoots within a quadrat were very carefully uprooted with the help of a knife. Samples were labelled and placed individually in plastic bags. During sampling and transportation to the laboratory samples were kept in a cool box.

**2.03 Method to select the sampling/survey site or area:** Random sampling/surveying**2.04 Sampling/survey device:** n.a.**2.05 Specification:** metallic frame (25 x 25 cm)**2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom**2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone**2.08 Sampling/survey month(s):** June to July**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

10-20 replicates per meadow

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

0.625-1.25sqm

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** all down to light microscope scale**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

60 random intermediate or adult leaves are measured

**2.14 Level of taxonomical identification:** Species/species groups**2.15 Record of abundance:** n.a.

leaf length (mm)

in relation to n.a.

Unit

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** leaf length (mm)

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

CymoSkew index was estimated following the formula: 
$$\text{Skewness index} = \frac{n \cdot M_3}{[(n-1) \cdot (n-2) \cdot \sigma^3]}$$
 where  $M_3 = \sum (x_i - \text{Mean})^3 \cdot x_i$  = ln-transformed relative frequencies of adult and intermediate photosynthetic leaf lengths distinct values produced in frequency tables  $\sigma$  = standard deviation  $n$  = ln-transformed relative frequencies of 60 adult and intermediate photosynthetic leaf lengths distinct values

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

##### 3.04 From which biological data are the metrics calculated?

Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Habitat-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites

##### 3.07 Reference site characterisation

**Number of sites:** 2 meadows (4 sites, 8 areas) in North Aegean

**Geographical coverage:** Less impacted areas

**Location of sites:** Thasos Island, Vrasidas cape (Kavala Gulf, North Aegean)

**Data time period:** Last five years (2004-2009)

##### Criteria:

Dense (mean shoot density=msd=1936 shoots m<sup>-2</sup>\*) meadow of Cymodocea. In deeper waters coexistence with Posidonia. CymoSkew < 1,5.

##### 3.08 Reference community description

Dense seagrass communities without opportunistic epiphytes

**3.09 Results expressed as EQR?** No

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites

##### 3.11 Boundary setting procedure

Statistically (nested Anova, post hoc comparisons, linear relationships)

**3.12 "Good status" community:** Dense seagrass meadow of (ca. 1099 shoots m<sup>-2</sup>). CymoSkew >1,5-2,5

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

##### 3.14 Comments:

General comment: CymoSkew has also been successfully applied to Slovenia coasts (paper under preparation)

ID: 175

POSWARE

## 1. General information

- 1.01 GIG:** Mediterranean  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Angiosperms  
Posidonia oceanica
- 1.04 Country:** Italy
- 1.05 Specification:** none
- 1.06 Method name:** **POSWARE**
- 1.07 Original name:** POSWARE
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** General degradation
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione\\_tecnica.html](http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione_tecnica.html)
- 1.11 Pertinent literature of mandatory character:**  
[http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione\\_tecnica.html](http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione_tecnica.html) ISPR, 2009. Sviluppo di una metodologia adeguata per il campionamento, analisi ed elaborazione dei dati sulle praterie di Posidonia oceanica delle zone costiere italiane, ai fini dell'applicazione della Direttiva 2000/60/CE, in accordo con quanto discusso ed elaborato all'interno del Gruppo geografico di Intercalibrazione MED-GIG. Relazione di fine contratto di ricerca: 2007-2008 - Stazione Zoologica "A. Dohrn" di Napoli, Laboratorio di Ecologia del Benthos. Responsabile: Dott.ssa M.Cristina Buia.
- 1.12 Scientific literature:**  
n.a.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>M.C. Buia<br>mcbuia@szn.it<br>Stazione Zoologica "A. Dohrn" di Napoli, Laboratorio di Ecologia del Benthos | <b>1.14 Method reported by</b><br>Tiziano Bacci<br>tiziano.bacci@isprambiente.it<br>ISPR (ex ICRAM) - Advanced Institute for Environmental Protection and Research |
|---|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
ISPR, 2008. Protocolli per il campionamento e la determinazione degli elementi di qualità biologica e fisico-chimica nell'ambito dei programmi di monitoraggio ex 2000/60/CE delle acque costiere.
- 2.02 Short description**  
Station 15 m: sampling stations randomly positioned in 3 areas of 400 square meters distant one from each other about 10 meters
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.  
Scuba diver
- 2.05 Specification:** bed density (quadrats 40\*40 cm); lower limit (balise, camera); sediment (PVC corer)
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Seagrass
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** August to September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
e. g. station 15 m: 18 replicates (shoots), 9 replicates (density), 3 replicates (visual census) - station lower limit: 6 replicates (shoots), 6 replicates (density), 1 replicates (visual census)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Station 15 m: estimate about 1600 square meters - station lower limit: along transept of 50-60 m

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 0
- 2.13 Sample treatment:** Organisms of the complete sample are identified.

- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts, Percent coverage, Relative abundance  
morphometry, lepidochronology, biomass, granulometry, other measures  
**in relation to** Area  
**Unit** e.g density (number of shoots per square-metre); morphometry (mm); epiphytes biomass (mg / shoot) ; etc.
- 2.16 Quantification of biomass:** n.a.  
epiphytes biomass (mg / shoot); biomass of leaves per shoot (g dry wt); leaf production per shoot (g /year)
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Depth (m), density (nr shoots/m<sup>2</sup>), rhizome production (mg/year), rhizome elongation (mm/year), leaf production (nr leaves/year)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Habitat-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites
- 3.07 Reference site characterisation**  
**Number of sites:** 215 Tyrrhenian sites in the Mediterranean Sea  
**Geographical coverage:** Tyrrhenian Sea  
**Location of sites:** Maratea, Feraxi, Mortola, Monte Russu (1), Monte Russu (2), Talomone, Punta Licosa, Monterosso  
**Data time period:** n.a.  
**Criteria:**  
Reference conditions have been set in all classification systems on the basis of the assumption that these occur in unimpacted areas with unpolluted water quality and no hydromorphological alterations to the shore or seabed. For each national classification systems, reference values were determined from reference sites on the basis of the following criteria: 1: No significant pressures in waterbodies according to IMPRESS (article 5) 2: additional quantitative criteria: i) Mooring density (max 2 mooring.ha<sup>-1</sup>), ii) harbour or mooring facility distance (min 3km), iii) Population density (no settlements within 3km), iv) no beach regeneration (within 15km), v) no trawling, vi) no conspicuous invasive species, vii) no hydromorphological alterations, viii) no evidence of meadow deterioration.
- 3.08 Reference community description**  
Maximum value of density (nr shoots/m<sup>2</sup>), rhizome production (mg/year), rhizome elongation (mm/year), leaf production (nr leaves/year) of the meadow at 0-10; 11-20; 21-30; 31-40 meters depths.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** Best value of density (nr shoots/m<sup>2</sup>), rhizome production (mg/year), rhizome elongation (mm/year), leaf production (nr leaves/year) of the meadow at 0-10; 11-20; 21-30; 31-40 meters

depth.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 186

POMI

## 1. General information

- 1.01 GIG:** Mediterranean  
all types within coastal waters
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Angiosperms  
Posidonia oceanica
- 1.04 Country:** Spain
- 1.05 Specification:** Autonomous regions of Catalunya and Balears Islands
- 1.06 Method name:** *Posidonia oceanica Multivariate Index*
- 1.07 Original name:** *Posidonia oceanica Multivariate Index*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, General degradation, Habitat destruction, Heavy metals, Hydromorphological degradation, Impact of alien species, Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

- Qualitative test on the metrics used (reported in Martínez-Crego et al. 2008 MEPS 361: 93-109). Quantitative: test using the EQR generated by POMI on 14 sites; metrics for pressures were urban sewage discharge, urban soil surface, tourism pressure, harbours pressure (as defined in the IMPRESS document of our water authority), and a combination of all four. We used linear regression,  $p < 0.05$  in all cases.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

ORDEN ARM/2656/2008, de 10 de septiembre, por la que se aprueba la instrucción de planificación hidrológica. BOE 229, 22 de septiembre de 2008.

**1.12 Scientific literature:**

Martínez-Crego, B., A. Vergés, J. Romero & T. Alcoverro, 2008. Selection of multiple seagrass indicators for environmental biomonitoring. Marine Ecology Progress Series 361: 93-109. Romero, J., B. Martínez-Crego, T. Alcoverro & M. Pérez, 2007. A multivariate index based on the seagrass Posidonia oceanica (POMI) to assess ecological status of coastal waters under the Water Framework Directive (WFD). Marine Pollution Bulletin 55: 196-204.

**1.13 Method developed by**

Javier Romero  
jromero@ub.edu  
University of Barcelona

**1.14 Method reported by**

Javier Romero  
jromero@ub.edu  
University of Barcelona

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Martínez-Crego, B., A. Vergés, J. Romero & T. Alcoverro, 2008. Selection of multiple seagrass indicators for environmental biomonitoring. Marine Ecology Progress Series 361: 93-109.

**2.02 Short description**

At each site, a transect is prepared following the 15 m isobath. Three sites, one at the origin (0 m), other intermediate (25 m) and the third terminal (50 m) are chosen and marked with pegs and buoys (for future samplings). Close to each bar, over an area of 25 m<sup>2</sup>, samples or measures are performed randomly (n=2-8, depending on the metric) resulting in a final sampling size per site of 3xn.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** n.a.  
Diving

**2.05 Specification:** Diving

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** september

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

between 6 and 24, depending on the metrics

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Area surveyed: based on a transect of 50 m at constant depth, i.e. ca 250 m<sup>2</sup>

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Individual seagrass shoots, variable size, 2-7 leaves, ca. 1 cm width, 5-80 cm long
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** n.a.  
No taxonomical identification
- 2.15 Record of abundance:** Individual counts, Percent coverage  
**in relation to** Area  
**Unit** shoots per square meter and percent cover
- 2.16 Quantification of biomass:** n.a.  
leaf surface
- 2.17 Other biological data:** in addition to abundance, other metrics are used (growth form, N content...)
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Shoot Density, Shoot Cover, Plagiotropic rhizomes, Shoot Foliar Surface, Leaf Necrosis, N content in rhizome, P content in rhizomes, Sucrose in rhizomes,  $\delta^{15}N$  ratio in rhizomes,  $\delta^{34}S$  ratio in rhizomes, Epiphyte N content, [Cu] in rhizomes, [Pb] in rhizomes, [Zn] in rhizomes
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.  
Multivariate PCA
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Least Disturbed Conditions, Modelling (extrapolating model results)
- 3.07 Reference site characterisation**  
**Number of sites:** 3  
**Geographical coverage:** Catalan coast  
**Location of sites:** Northern part of the Catalan coast  
**Data time period:** same time of sampling  
**Criteria:**  
n.a.
- 3.08 Reference community description**  
Meadows displaying the higher (or lower, depending on the metrics) values for each parameter
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
with the exception that the bad class was restricted to cases of meadow die-off, see below
- 3.11 Boundary setting procedure**  
According to the behavior of other BQEs, it was assumed that the "bad status" corresponds to the absence (due to anthropic impacts) of the targeted seagrass (*P. oceanica*), and the boundary between bad and poor arbitrarily set at 0.1. The response of EQR (based on POMI) to pressures was progressive (linear) and no discontinuities were apparent, so the rest of the EQR scale (0.1 to 1) was divided into four equal classes.
- 3.12 "Good status" community:** n.a.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

a) by choosing three sampling sites, distant 100 m one from each other, and performing the entire protocol at each site, in eight of the meadows of the monitoring network, in two different sampling periods and in two depths; b) by performing a sensitivity test of the POMI (adding random variation to the descriptors)

**3.14 Comments:**

none

ID: 171

BENTIX

## 1. General information

- 1.01 GIG:** Mediterranean  
CW-M2, CW-M3
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Greece
- 1.05 Specification:** none
- 1.06 Method name:** **BENTIX**
- 1.07 Original name:** BENTIX
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

- 1.10 Internet reference:** <http://www.hcmr.gr/listview3.php?id=1195>

**1.11 Pertinent literature of mandatory character:**

EC intercalibration results decision, annex 6, Greek translation.

**1.12 Scientific literature:**

Simboura, N., 2004. Benthic Index vs Biotic Index in monitoring: an answer to Borja et al., 2003. *Marine Pollution Bulletin* 48 (3-4): 403-404. [Simboura, N. & A. Zenetos, 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new Biotic index. \*Mediterranean Marine Science\* 3 \(2\): 77-111.](#) [Simboura, N., E. Papatthanassiou & D. Sakellariou, 2007. The use of a biotic index \(Bentix\) in assessing long term effects of dumping coarse metalliferous waste on soft bottom benthic communities. \*Ecological Indicators\* 7 \(1\): 164-180.](#) [Simboura, N. & M. Argyrou, 2006. Implementation of the Water Framework Directive in Cyprus: application of the Bentix index in Limassol Bay. \*Proceed. 8th Hell. Symp. Oceanogr. and Fisheries, Thessaloniki.\*](#) [Simboura, N., P. Panayotidis, E. Papatthanassiou, 2005. A synthesis of the Biological Quality Elements for the implementation of the European Water Framework Directive in the Mediterranean Ecoregion: the case of Saronikos Gulf. \*Ecological Indicators\* 5: 253-266.](#) [Simboura, N. & S. Reizopoulou, 2007. A comparative approach of assessing ecological status in two coastal areas of Eastern Mediterranean. \*Ecological Indicators\* 7: 455-468.](#) [Simboura, N. & S. Reizopoulou, 2008. An intercalibration of classification metrics of benthic macroinvertebrates in coastal and transitional ecosystems of the Eastern Mediterranean ecoregion \(Greece\). \*Marine Pollution Bulletin\* 56: 116-126.](#)

**1.13 Method developed by**

Nomiki Simboura  
msim@ath.hcmr.gr  
Hellenic Centre for Marine Research

**1.14 Method reported by**

Nomiki Simboura  
msim@ath.hcmr.gr  
Hellenic Centre for Marine Research

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

n.a.

**2.02 Short description**

Two replicate samples are collected at each station for the analysis of zoobenthos. Samples for fauna analysis are sieved on board through a 1 mm sieve and stored in 4 % formalin solution, stained with Rose Bengal. Samples are sorted in the lab and are grouped into the main benthic groups. Subsequently most of the specimens are identified to the species level and only when this was not possible (broken material) to a higher taxonomic level (genus or family).

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Grab

**2.05 Specification:** Van Veen Grab, Ponar Grab

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones

**2.08 Sampling/survey month(s):** May to August

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Once a year is sufficient - preferable warm season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Two replicates are sufficient.

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

For example 5-10 stations (2 replicates each) for a coastal gulf (ex. Athens gulf) is sufficient.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 0.5 mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to Area  
Unit Number of individuals per one square-metre
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Complete list of species with abundance data in a station
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** n.a.
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites  
The setting of the RC was based on the autoecology of species; a species list was compiled to identify the species characterizing each type of community, habitat and water body type thus establishing RC on an ecological basis (Simboura et al., 2005). Reference conditions of the method are not water body type specific neither habitat type specific. Around 10 sites corresponding to these reference conditions under pristine conditions were used to validate the numerical value of the method under high status.
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** Aegean and Ionian seas  
**Location of sites:** Cyclades islands, Ionian coasts (western Greece)  
**Data time period:** Data from 1985-1992  
**Criteria:**  
The sites are from undisturbed areas, the fauna is composed of mostly sensitive species (over 75%), diversity is among the highest values expected for the specific habitat, a list of characteristic species and typical abundances is compiled for every community and habitat type under reference conditions. These species are expected to be found in expected abundance in reference sites.
- 3.08 Reference community description**  
General reference conditions are based on the normative definition, which states, "All the disturbance-sensitive taxa associated with undisturbed conditions should be present". Reference sites from Greek data were defined, presenting reference conditions for biological element macroinvertebrates. The fauna is composed of mostly sensitive species (GI) and corresponding mean Benthic values are amongst the highest: Benthic>5. In these cases the composition of the fauna corresponds to sensitive species over 75%. In special cases where muddy bottoms are encountered within a reference site Benthic values are expected to reach values over 4 and sensitive species percentage over 50%. Species ecology Another aspect biological reference conditions setting is based on the autoecology of species. Each species is designated with an ecological identity as extracted from scientific literature, so it is possible to identify the species belonging to, or characterizing each type of community > habitat > water body, and thus to establish reference conditions on an ecological basis. Species reference lists are established for each kind of habitats-communities (Simboura & Zenetos, 2002) and the link among community-habitat-water body type is given (Simboura et al., 2005, Site presentation, Simboura) following the EUNIS habitat classification scheme for European coasts. Diversity indices Other indices as the Shannon Diversity index

and species Richness are expected to be among the highest according to the given type of habitat, in the sites under reference conditions. For example over a large set of data from Greek coastal areas and for a sample size of 0.1m<sup>2</sup>, H<sub>max</sub>=6.3 and S<sub>max</sub>=110 for mixed sediments, while for muds the respective values were H<sub>max</sub>=4,6 and S<sub>max</sub>=39. Generally for the above standard reference sample area and for mixed sediments H values in reference sites are expected to be over 5 and S over 80 and for muddy bottoms H over 4-4.5 and S over 30. However, discrepancies in the values of these indices may arise from sampling methodology differences and habitat particularities. Another point to be considered is that in transitional zones of disturbance (ecotone) Shannon diversity and species richness maybe significantly high leading to misinterpretation of reference conditions; so diversity values should be cross-checked with biotic indices.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Using paired metrics that respond in different ways to the influence of the pressure  
The centre of good class derived from paired metrics. An equidistant subdivision of good and moderate classes was applied. The highest and lowest values of the method were set by the mathematical formula of the method.

### **3.11 Boundary setting procedure**

Class boundary values were set by plotting the percentage of sensitive taxa and of tolerant taxa against the decreasing values of the Benthic index on the x-axis. The point where the two curves cross corresponds to the central value of the Good class. Here the two groups of sensitive and tolerant species are each 50% of the fauna. This point corresponds with the ecotone point of the transitional zone, middle of good class. The points at equal distances (0.5) in each side of the crossline represent the high-good boundary limit with value 4.5, and at the other side of the center the boundary between good/moderate with value 3.5. The HG and GM boundaries and the center of good class are indicated by vertical lines. At the border of good to high status (Benthic=4.5) the sensitive group accounts roughly for more than 60% or more than 2/3 of the fauna, while the tolerant group as a whole (tolerant plus opportunists) accounts for less than 40% or less than 1/3 of the fauna. It is important to stress here that for purely muddy habitats (with a percentage of fines over 80%) where the benthic fauna is normally dominated by some tolerant species, and only in this class border among high and good, a possible refinement of the boundary limit would change 4,5 to 4. The condition of the communities under Good status is to some extent in accordance with the normative conditions, which states that "The level of diversity and abundance of invertebrate taxa is slightly outside the range associated with undisturbed conditions. Most of the sensitive taxa of the type specific communities are present". At the border of good to moderate status (Benthic=3.5) the sensitive group accounts roughly for less than 40% or less than 1/3 of the fauna, while the tolerant group as a whole (tolerant plus opportunists) accounts for more than 60% or more than 2/3 of the fauna. Also for purely muddy habitats where the benthic fauna is normally dominated by some tolerant species, a refinement of the boundary limit changes 3,5 to 3. The condition of the fauna under Moderate status is in accordance with the normative conditions, which states that "The level of diversity and abundance of invertebrate taxa is moderately outside the range associated with undisturbed conditions. Most of the sensitive taxa of the type specific communities are absent".

**3.12 "Good status" community:** n.a.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

The software for the calculation of the index sets the limits of parameters under which the results are not within the confidence limits. These parameters are based on the lowest number of scoring species and the lowest number of the species in the matrix that is needed to calculate the index.

**3.14 Comments:**

none

ID: 177

M-AMBI

## 1. General information

- 1.01 GIG:** Mediterranean  
Mediterranean GIG - Sedimentary shallow (CW-M3)
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Italy
- 1.05 Specification:** none

**1.06 Method name:** *Multivariate-AZTI Marine Biotic Index*

**1.07 Original name:** *Multivariate-AZTI Marine Biotic Index*

**1.08 Status: Method is/will be used in** Second RBMP (2015)

**1.09 Detected pressure(s):** General degradation, Pollution by organic matter

*Has the pressure-impact-relationship been tested?*

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:** <http://www.azti.es>

**1.11 Pertinent literature of mandatory character:**

ISPRA, 2009. Sviluppo di una metodologia adeguata per il campionamento, analisi ed elaborazione dei dati del macrozoobenthos costiero italiano, al fine dell'applicazione della direttiva 2000/60/CE, in accordo con quanto discusso ed elaborato (Eventuali risultati). All'interno dell'gruppo geografico di intercalibrazione med-gig. Relazione di fine contratto di ricerca: 2007-2008 - Università degli Studi di Pavia, Dipartimento di Ecologia del Territorio e degli Ambienti Terrestri, Responsabile: Prof.ssa. Anna Occhipinti [http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione\\_tecnica.html](http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione_tecnica.html) DECRETO 14 aprile 2009, n. 56. Regolamento recante «Criteri tecnici per il monitoraggio dei corpi idrici e l'identificazione delle condizioni di riferimento per la modifica delle norme tecniche del decreto legislativo 3 aprile 2006, n. 152, recante Norme in materia ambientale, predisposto ai sensi dell'articolo 75, comma 3, del decreto legislativo medesimo». (GU n. 124 del 30-5-2009 - Suppl. Ordinario n.83) - Testo in vigore dal: 14-6-2009.

**1.12 Scientific literature:**

Muxika, I., A. Borja & J. Bald, 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. *Marine Pollution Bulletin* 55: 16-29.

**1.13 Method developed by**

Angel Borja

[aborja@pas.azti.es](mailto:aborja@pas.azti.es)

AZTI-Tecnalia, Marine Research Division, Herrera Kaia,  
Portualdea s/n, 20110 Pasaia, Spain

**1.14 Method reported by**

Benedetta Trabucco

[benedetta.trabucco@isprambiente.it](mailto:benedetta.trabucco@isprambiente.it)

ISPRA (ex ICRAM) - Advanced Institute for Environmental Protection and Research

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

ISPRA, 2008. Protocolli per il campionamento e la determinazione degli elementi di qualità biologica e fisico-chimica nell'ambito dei programmi di monitoraggio ex 2000/60/CE delle acque marine costiere. [http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione\\_tecnica.html](http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione_tecnica.html)

**2.02 Short description**

Two stations, along a transect off-coast. The first one on sandy sediment (% of sand equal or more than 75%). The second one on silty sediment (% of sand equal or less than 25%).

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Grab

**2.05 Specification:** Van Veen Grab (0,1 m<sup>2</sup>, 18/20l)

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom

**2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone

**2.08 Sampling/survey month(s):** six-monthly (Spring/Fall)

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

Twice a year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Three replicates

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Average of three spatial replicates (0,1m<sup>2</sup>).

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** 1 mm (mesh-size sieve)

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Species/species groups

**2.15 Record of abundance:** Individual counts  
in relation to Area

**Unit** Number of individuals per 0,1 square-metre

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

AMBI Index ( $AMBI = [(0 \times \%GI) + (1.5 \times \%GII) + (3 \times \%GIII) + (4.5 \times \%GIV) + (6 \times \%GV)]/100$ )  
GI = species belonging to the I class (sensitive species);  
GII = species belonging to the II class (sensitive-tolerant species);  
GIII = species belonging to the III class (tolerant species);  
GIV = species belonging to the IV class (second order opportunistic species);  
GV = species belonging to the V class (first order opportunistic species).  
Shannon-Wiener Index 'H'; species richness value S. It elaborates these three components through multivariate statistic analysis, allowing to obtain an index value from 0 to 1

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

multivariate statistic analysis

##### 3.04 From which biological data are the metrics calculated?

Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Expert knowledge, Historical data, Least Disturbed Conditions

##### 3.07 Reference site characterisation

**Number of sites:** 19 (222 samples) and 24 western Mediterranean sites

**Geographical coverage:** Northern Adriatic sea and western Mediterranean

**Location of sites:** Northern Adriatic sea and western Mediterranean

**Data time period:** 1993-2004

##### Criteria:

As defined in the WFD.

##### 3.08 Reference community description

As defined in the WFD.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient

##### 3.11 Boundary setting procedure

Expert judgment.

**3.12 "Good status" community:** As defined in the WFD.

#### Uncertainty

**3.13 Consideration of uncertainty:** Yes

Implementation of the species list. Implementation of the human-pressures list.

##### 3.14 Comments:

none



ID: 8

SI-BI-CO

## 1. General information

- 1.01 GIG:** Mediterranean  
coastal Med GIG; types aren't relevant, method used wherever there is soft-bottom
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Slovenia
- 1.05 Specification:** for soft-bottom
- 1.06 Method name:** *Methodology for assessment of ecological status of coastal waters using benthic invertebrates*
- 1.07 Original name:** *Metodologija vrednotenja ekološkega stanja obalnih voda z bentoškimi nevretenčarji*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** General degradation
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [http://www.mop.gov.si/fileadmin/mop.gov.si/pageuploads/podrocja/okolje/pdf/vode/ekolosko\\_stanje/metod\\_vredn\\_ekoloskega\\_st\\_obalnih\\_voda\\_bentoskimi\\_nevretenčarji.pdf](http://www.mop.gov.si/fileadmin/mop.gov.si/pageuploads/podrocja/okolje/pdf/vode/ekolosko_stanje/metod_vredn_ekoloskega_st_obalnih_voda_bentoskimi_nevretenčarji.pdf)
- 1.11 Pertinent literature of mandatory character:**  
Lipej, I., P. Mozetič, M. Orlando-Bonaca, B. Mavric, M. Sisko & N. Bettoso, 2007. Opredelitev ekološkega stanja obalnega morja v skladu z Vodno Direktivo (Water Framework Directive, 2000/60 EC). Dopolnjeno zaključno poročilo, poročila MBP 96: 180 pp.
- 1.12 Scientific literature:**  
n.a.
- |  |  |
|--|--|
| <b>1.13 Method developed by</b><br>prof. dr. Lovrenc Lipej (MBP NIB, Slovenia), but M-AMBI index used was developed by Borja et al.<br>lipej@mbss.org<br>Marine Biology Station Piran, NIB | <b>1.14 Method reported by</b><br>Borut Mavrič<br>mavric@mbss.org<br>Marine Biology Station Piran, National Institute of Biology |
|--|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
Samples taken on soft bottom, in the depth 7-9m (below deeper boundary of sea grass meadows). Replicates taken randomly. Replicates are treated separately. They are washed through 1 mm mesh size net with salt water and conserved with ethanol.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Grab
- 2.05 Specification:** Van Veen Grab
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** may and august/septembr
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
twice per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3 replicates per sampling station
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
0,3 m2 per season = 0,6 m2 per year

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 1 mm (mesh-size of sewing net)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to Area

Unit 0,1 m<sup>2</sup>

- 2.16 Quantification of biomass:** n.a.  
**2.17 Other biological data:** none  
**2.18 Special cases, exceptions, additions:** none  
**2.19 Comments:**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
M-AMBI, which includes Species richness, Shannon-Wiener diversity index and AMBI
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates

#### Reference conditions

- 3.05 Scope of reference conditions:** Habitat-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge
- 3.07 Reference site characterisation**  
**Number of sites:** 2 sites  
**Geographical coverage:** stations from one water body with minimal anthropogenic influences  
**Location of sites:** Uvala svetega Jerneja, SI5-WB2  
**Data time period:** one year  
**Criteria:**  
the lowest anthropogenic influences
- 3.08 Reference community description**  
High diversity and species richness, high abundance of sensitive species (EG1 and EG2), lack or low abundance of EG3 and EG4 species and lack of EG5 species.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
High-good boundary derived from mean EQR at near-natural reference sites, taking in consideration also natural variability (20%; upper anchor lies 15% from EQR from near-natural RS), other boundaries are derived using equidistant division
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** High diversity and species richness, high abundance of sensitive species (EG1 and EG2), low abundance of EG3 and EG4 species and lack of EG5 species.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 174

CARLIT

## 1. General information

- 1.01 GIG:** Mediterranean  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Macroalgae
- 1.04 Country:** Italy
- 1.05 Specification:** Only the water bodies which have the 80% of coast line as rocky shore
- 1.06 Method name:** *Cartography of littoral and upper-sublittoral rocky-shore communities*
- 1.07 Original name:** *Cartography of littoral and upper-sublittoral rocky-shore communities*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** General degradation

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Ballesteros E., Torras X., Pinedo S, Garcí'a M., Mangialajo L., Torres de M., 2007. A new methodology based on littoral community cartography for the implementation of the European Water Framework Directive. *Marine Pollution Bulletin*, 55: 172-180. Mangialajo L., Ruggieri N., Asnaghi V., Chiantore M. C., Povero P., Cattaneo-Vietti R., 2007. Ecological status in the Ligurian Sea: The effect of coastline urbanisation and the importance of proper reference sites. *Marine Pollution Bulletin*, 55: 30-41.

- 1.10 Internet reference:** <http://www.icram.org/nav2/dipartimento1.htm>

**1.11 Pertinent literature of mandatory character:**

MCW - Sistema di classificazione ecologica. [http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione\\_tecnica.html](http://www.apat.gov.it/site/it-IT/APAT/Pubblicazioni/Documentazione_tecnica.html)

**1.12 Scientific literature:**

Ballesteros, E., X. Torras, S. Pinedo, M. García, L. Mangialajo & M. Torres, 2007. A new methodology based on littoral community cartography for the implementation of the European Water Framework Directive. *Marine Pollution Bulletin* 55: 172-180. Mangialajo, L., N. Ruggieri, V. Asnaghi, M.C. Chiantore, P. Povero & R. Cattaneo-Vietti, 2007. Ecological status in the Ligurian Sea: The effect of coastline urbanisation and the importance of proper reference sites. *Marine Pollution Bulletin* 55: 30-41.

**1.13 Method developed by**

E. Ballesteros, L. Mangialajo  
luisa.mangialajo@unice.fr  
CEAB, CSIC, Spain ; Université de Nice-sophia antipolis - EA4228, ECOMERS

**1.14 Method reported by**

Paola Gennaro and Luisa Mangialajo  
paola.gennaro@isprambiente.it  
High Institute for Environmental Protection and Research (ISPRA)

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Mangialajo, L., G. Sartoni & F. Giovanardi, 2008. Quaderno Metodologico sull'elemento biologico MACROALGHE e sul calcolo dello stato ecologico secondo la metodologia CARLIT.

**2.02 Short description**

Rocky-shore coasts are sampled by visual census of the dominant macroalgal community carried out by small boats so as to sail along the coast as close as possible (3-4 m) to the rock face. The observed linear development of each macroalgal community is noted down on a cartographic support (aerial photography at 1:5000 scale) with the geomorphological features of the studied coast line; the minimum length of the sampling unit is 50m. For some species (*Cystoseira* spp.) the cover of macroalgal belt are recorded and some macroalgae talli samples are collected for the taxonomic identification.

- 2.03 Method to select the sampling/survey site or area:** n.a.

- 2.04 Sampling/survey device:** n.a.  
visual census

- 2.05 Specification:** little boat for visual census and cartographic supports to report the length of the observed macroalgal communities

- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
littoral and upper-sublittoral rocky-shore

- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones

- 2.08 Sampling/survey month(s):** April to June

- 2.09 Number of sampling/survey occasions (in time) to classify site or area**

One sampling per year

- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

If the whole coastline is censused, no replicates are needed. If the rocky coastline is too vast for reasonably do a complete cartography, number of sites (replicates) will depend on the amplitude of the studied water mass.

- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

All the available rocky shore covered by macroalgal community

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** No minimum size requested because it is a no destructive sampling
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Other  
The method is based on a simplification of more widespread macroalgal communities study in the upper infralittoral fringe in the Mediterranean Sea. On the base of existing literature and expert judgement, 19 categories have been created (table updated in 2008 for the Italian application) with associated values ranging from 1 to 20. Such categories follow mostly a taxonomic schema, grouping species, genus and orders.
- 2.15 Record of abundance:** n.a.  
Geographic coordinates of beginning and end of each stretch of coast dominated by one of the 19 categories mentioned above
- in relation to** n.a.  
Abundance of each category is related to the length of coastline (property of zones non influenced by tides)
- Unit** Meters
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Meters of coastline covered by a specific categorized community (togliere specie, il carlit prende in considerazione talune specie, ma solo quelle habitat forming, a livello di paesaggio. Non ha nulla a che vedere con il conteggio di specie che fanno, per esempio, nei fondi molli)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** n.a.
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Habitat-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Least Disturbed Conditions
- 3.07 Reference site characterisation**
- Number of sites:** 48 (WFD intercalibration technical report - Part 3 – Coastal and Transitional Waters - Section 1 – General part, and Section 4 – Macroalgae.)
- Geographical coverage:** Ligurian region
- Location of sites:** Ligurian region
- Data time period:** spring/summer 2000
- Criteria:**  
The absence of pressures had to be illustrated. The communities at the sites had to correspond with the description of the reference community description. Spatio-temporal variability had to be taken into account of the community's composition and abundance affected by geomorphological features, irradiance exposure, intense and frequency of natural disturbances, e.g. hydrodynamism and by seasonal cycle of light period and intensity.
- 3.08 Reference community description**  
1. Littoral and upper-sublittoral rocky-shore macroalgal communities of high diversity should be dominated quantitatively by structuring brown algae mainly of the order Fucales (Cystoseira spp.) which develop in high irradiance sites by long and continuous belt. In shadow zones (exposed steep vertical cliffs) Lithophyllum byssoides develops, forming important organogenic structures (trottoir).
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites  
Equidistant division of the EQR gradient

#### **3.11 Boundary setting procedure**

In Italy, Carlit method has been tested at Regional scale, in the Ligurian Sea, applying it in a moderate urban gradient (figure 10) and in four Marine Protected Areas (MPAs), proposed as hypothetical reference sites at a regional scale (Mangialajo et al., 2007). This study shows that Carlit index is suitable to detect different kinds of anthropogenic pressures obtaining a good correlation with different water column variables. (WFD intercalibration technical report - Part 3 – Coastal and Transitional Waters - Section 1 – General part, and Section 4 – Macroalgae.)

**3.12 "Good status" community:** As defined in the WFD.

### **Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

#### **3.14 Comments:**

none

ID: 79

SI-AL-CO

## 1. General information

- 1.01 GIG:** Mediterranean  
coastal MED-GIG
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Macroalgae
- 1.04 Country:** Slovenia
- 1.05 Specification:** in coastal WBs (rocky or sedimentary with at least 20% of rocky coast)
- 1.06 Method name:** **Methodology for assessment of ecological status of coastal waters using macroalgae**
- 1.07 Original name:** Metodologija za vrednotenje ekološkega stanja obalnih voda z makrofitskimi algami
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** n.a.  
organic pollution - mainly nutrients
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [http://www.mop.gov.si/fileadmin/mop.gov.si/pageuploads/podrocja/okolje/pdf/vode/ekolosko\\_stanje/metod\\_vredn\\_ekoloskega\\_st\\_obalnih\\_voda\\_fitobentosom\\_makrofiti.pdf](http://www.mop.gov.si/fileadmin/mop.gov.si/pageuploads/podrocja/okolje/pdf/vode/ekolosko_stanje/metod_vredn_ekoloskega_st_obalnih_voda_fitobentosom_makrofiti.pdf)
- 1.11 Pertinent literature of mandatory character:**  
Lipej, L., P. Mozetic, M. Orlando-Bonaca, B. Mavric, M. Sisko & N. Bettoso, 2007. Opredelitev ekološkega stanja morja v skladu z Vodno Direktivo (Water Framework Directive, 2000/60/EC). Dopolnjeno zaključno poročilo, poročila MBP 96: 180 pp.
- 1.12 Scientific literature:**  
Orlando-Bonaca, M., L. Lipej & S. Orfanidis, 2008. Benthic macrophytes as a tool for delineating, monitoring and assessing ecological status: the case of Slovenian coastal waters. Marine Pollution Bulletin 56 (4): 666-676.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Martina Orlando Bonaca (for Slovenia), but Sotiris Orfanidis developed the EEI, which we use<br>orlando@mbss.org<br>Marine biology station, National Institute of Biology | <b>1.14 Method reported by</b><br>Martina Orlando Bonaca<br>orlando@mbss.org<br>Marine biology station, National Institute of Biology |
|--|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Orfanidis, S., P. Panayotidis & N. Stamatis, 2001. Ecological evaluation of transitional and coastal waters: A marine benthic macrophytes-based model. Mediterr. Mar. Sci. 2 (2): 45-65.
- 2.02 Short description**  
At each sampling site, in a depth range of 2 to 4 m, three samples are randomly scraped from the bottom (20 x 20 cm). All samples are collected between 8 and 12 a.m.
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying
- 2.04 Sampling/survey device:** Scraper
- 2.05 Specification:** quadrat 20x20 cm
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
hard bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** May-June and August-September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
3 replicates (3 quadrates) per each sampling station
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
sum of 3 spatial replicates = 3 x 400 cm<sup>2</sup> per station per season

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 2 mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** n.a.

in relation to Area

Unit percentage coverage in the sampled area

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

Only species covering at least 1% of the sampling area are assessed. In cases where the coverage of morphologically similar species could not be measured precisely, these species are grouped together (as spp.).

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

The Greek EEI (Ecological Evaluation Index) proposed by Orfanidis et al. (2001, 2003) is used. The macroalgae species are divided into two Ecological State Groups (ESG I and II). The EEI is a number ranging from 2 to 10. To determine the EEI of water bodies, the procedure from Orfanidis et al. (2001, 2003) is used.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites

**3.07 Reference site characterisation**

**Number of sites:** one MPA in Slovenian coastal waters

**Geographical coverage:** One reference zone in a MPA is considered representative for Slovenian coastal waters

**Location of sites:** Strunjan Nature Reserve (Slovenia)

**Data time period:** spring-summer 2006

**Criteria:**

High ecological status of macroalgae; low pressures and impacts - natural coastal environment well preserved; no sources of anthropogenic pollution; no non-indigenous species that can affect autochthonous species and habitats.

**3.08 Reference community description**

Macroalgae community of high diversity, dominated by brown algae, mainly *C. barbata*, with also abundant *P. pavonica*, *Halimeda tuna* and *H. incurva*.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise

**3.11 Boundary setting procedure**

Boundaries are set according to biotic index (EEI). No statistical analysis exclusively to set boundaries. No discontinuities. Continuum of possibilities with gradual disappearance/appearance of different indicator species.

**3.12 "Good status" community:** The dominance of the late-successional species of the genera *Cystoseira* form communities is indicative of High/Good ES, which is characterized, for example, by low nutrient and clear water conditions, whilst the dominance of opportunistic seaweeds (as *Ulva* and *Gracilaria*) and Cyanobacteria form communities is indicative of degraded state, which is characterized by high nutrients, heavy metals and turbid conditions. The coexistence of the late-successional species like *Cystoseira*, *Sargassum*, *Corallina* with opportunistic species like *Ulva*, *Cladophora*, *Gracilaria*, Cyanobacteria species form communities is indicative of intermediate (moderate) ES.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 253

CARLIT/BENTHOS

## 1. General information

- 1.01 GIG:** Mediterranean  
All types (MedGIG group did not differ between types for macroalgae)
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Macroalgae
- 1.04 Country:** Spain
- 1.05 Specification:** Catalonia, Comunitat Valenciana and Balearic Islands
- 1.06 Method name:** **CARLIT/BENTHOS**
- 1.07 Original name:** CARLIT/BENTHOS
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, Heavy metals, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

See it in ARÉVALO, R., S. PINEDO & E. BALLESTEROS (2007). Changes in the composition and structure of Mediterranean rocky-shore communities following a gradient of nutrient enrichment: descriptive study and test of proposed methods to assess water quality regarding macroalgae. *Marine Pollution Bulletin*, 55: 104-113.

- 1.10 Internet reference:** none (soon at: [www.gencat.cat/aca](http://www.gencat.cat/aca))
- 1.11 Pertinent literature of mandatory character:**  
ORDEN ARM/2656/2008, de 10 de septiembre, por la que se aprueba la instrucción de planificación hidrológica. BOE 229, 22 de septiembre de 2008. European Commission, 2008. Commission Decision of 30 October 2008 establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring System classifications as a result of the intercalibration exercise. Official Journal of the European Union, L332/20-L332/44.
- 1.12 Scientific literature:**  
Enric Ballesteros, Xavier Torras, Susana Pinedo, María García, Luisa Mangialajo, Mariona de Torres (2007). A new methodology based on littoral community cartography dominated by macroalgae for the implementation of the European Water Framework Directive. *Marine Pollution Bulletin* 55:172-180. Alessandro Carletti & Anna-Stiina Heiskanen, 2009. Water Framework Directive intercalibration technical report. Part 3: Coastal and Transitional waters. JRC Scientific and Technical Reports. EUR 23838 EN/3 - 2009.
- |  |  |
|--|--|
| <b>1.13 Method developed by</b><br>Enric Ballesteros and Xavier Torras<br>kike@ceab.csic.es; xtorras@ceab.csic.es<br>Centre d'Estudis Avançats de Blanes, CSIC | <b>1.14 Method reported by</b><br>Xavier Torras<br>xtorras@ceab.csic.es<br>Centre d'Estudis Avançats de Blanes, CSIC |
|--|--|

**1.15 Comments**

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
European Commission (EC). 2003. Common implementation strategy for the Water Framework Directive (2000/60/EC). Working Group COAST. Guidance document nº 5. Transitional and Coastal Waters - Tipology, reference conditions and classification systems. European Commission (EC). 2003. Common implementation strategy for the Water Framework Directive (2000/60/EC). Working Group COAST. Guidance document nº 6. Towards a guidance on establishment of the intercalibration network and the process on the intercalibration exercise.
- 2.02 Short description**  
A sampling unit is a sector of coast, at least 50 meters, with an homogeneous community category (corresponding to a single community or combination of communities). The sectors are translated on a graphical display.
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** n.a.  
Visual sampling
- 2.05 Specification:**
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Upper-sublittoral communities on rocky coasts
- 2.07 Sampled/surveyed zones in areas with tidal influence:**
- 2.08 Sampling/survey month(s):** April-June
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
Once a year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
None if all the rocky coast is surveyed. Expert knowledge advice otherwise
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Around 400 Km on study coast and 250km on Reference Zone coasts

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Macroalgae (>1cm)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
Algal communities (or combination of communities), the main algal species and the mussel *Mytilus galloprovincialis* or other dominant macroinvertebrates
- 2.15 Record of abundance:** Abundance classes  
**in relation to** n.a.  
Length of coast  
**Unit** meters of coast length covered by a community
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:**
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Communities or categories of community occupation
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Habitat-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** 250 km of coast  
**Geographical coverage:** Only reference zones in natural parks from Corsica and Balearic Islands considered representative for the entire Mediterranean Sea  
**Location of sites:** Façade maritime du Parc Naturel Régional de Corse (France), Parc Natural de Ses Salines (Balearic Islands, Spain) and Reserva Marina del Nord de Menorca (Balearic Islands, Spain).  
**Data time period:** Springtime 2002  
**Criteria:**  
Undisturbed (or with only very minor disturbances) sites that cover a wide range of coastal geomorphologies, from different geological origins (volcanic, granite, calcareous, metamorphic) to different wave exposures and coastal morphologies
- 3.08 Reference community description**  
Rocky shores places exposed to high irradiance levels and characterized by dense communities of several *Cystoseira* species: *C. mediterranea/amentacea* var. *stricta*, *C. crinita*, *C. brachyparpa* var. *balearica*, *C. foeniculacea/barbata/spinosa* var. *tenuior/compressa* var. *pustulata*. Alternatively, in shadow zones (steep vertical cliffs, high hydrodynamic conditions) *Lithophyllum byssoides* develops, forming important organogenic structures (trottoir).
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.
- 3.11 Boundary setting procedure**  
Boundaries are set according to biotic index and/or combined with the results of or multivariate analysis. No statistical analysis exclusively to set boundaries. No discontinuities. Continuum of possibilities with gradual disappearance/appearance of different indicator species.
- 3.12 "Good status" community:** At good status sites (in hydrodynamic environments and non-polluted waters) highly structured

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and productive *Cystoseira mediterranea/stricta/crinita* communities are well developed.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

ID: 31

IT-PP-CO

## 1. General information

- 1.01 GIG:** Mediterranean  
The parameter that has been intercalibrated during MED GIG Phase I, is Chlorophyll "a", that was
- 1.02 Category:** referred to three intercalibration types based on salinity (density) Cfr. Decision 2008/915/EC
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Italy
- 1.05 Specification:** Until 2006, the trophic classification of the coastal areas of Italy was, by law (Dlgs 152-1999), based on TRIX index calculation.
- 1.06 Method name:** **Assessment System for Coastal Waters Based on BQE "Phytoplankton"**
- 1.07 Original name:** *Sistema di valutazione per le acque costiere basato sull'EQB "Fitoplancton"*
- 1.08 Status: Method is/will be used in** Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Impact of alien species, Pollution by organic matter
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
More than 30 years of experience in the Eutrophication control, in relation to the eutrophied areas of the Northern Adriatic Sea. In particular, nutrients loads coming from the Po river basin have been related to the trophic levels of the coastal areas of the Emilia Romagna Region, using Trophic Index (TRIX) and the related trophic scale as a management tool. At present, it is under way the adoption of a classification system based on Chlorophyll concentration, as reported in the Commission Decision 2008/915/EC, as a result of the MED GIG Phase I.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
-
- 1.14 Method reported by**  
Franco Giovanardi  
franco.giovanardi@isprambiente.it; f.giovanardi@icram.org  
ISPRA (High Institute for Protection and Environmental Research)
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
n.a.
- 2.02 Short description**  
n.a.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** every two months
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
six time per year
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
n.a.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n.a.
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Individual counts  
in relation to Volume

Unit Chlorophyll concentration as mg/m<sup>3</sup> and n. of cells/L

**2.16 Quantification of biomass:** Chlorophyll-a concentration

Determination of chlorophyll-a concentration by fluorimetry

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

In our national monitoring programme (period 2000-2007 and 2008-August 2009), in general vertical profiles of chlorophyll measurements are available (as fluorimetric units) with a frequency of 15 days. Together with these measures, surface nutrients concentrations and quantitative determinations of Phytoplankton species are also available. From 2009 the new monitoring programme provides the same determinations, but with different frequencies (six times per year).

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

Chlorophyll concentrations, nutrients concentration and species determination

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

In the assessment system based on Chlorophyll concentrations, different metrics are taken into consideration, depending on three typologies of a hydrological kind, based on stability of the watercolumn (high, mean and low). The metrics are the parameters

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data

**3.07 Reference site characterisation**

**Number of sites:** Among the same sites already used for defining typologies (Tyrrhenian and Adriatic sites)

**Geographical coverage:** The reference conditions cover the entire coastal development of the Italian peninsula

**Location of sites:** n.a.

**Data time period:** n.a.

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** n.a.

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** n.a.

**3.14 Comments:**

At present, the classification criterion for BQE Phytoplankton is based on chlorophyll concentration and our Country is temporarily adopting this criterion with opportune legislative tools. The boundaries G/H and M/G were chosen on the basis of the experts judgement and the procedure of classification is well described in the cited Commission Decision. Who is completing this questionnaire is also the coordinator for TW and CW MED GIG phase II. We are now making the effort to develop a new tool of assessment for the BQE Phytoplankton, taking into account both Phyto Biodiversity and the related sensitivity to the pressures (i.e. Diversity Indexes/nutrients loads relationships). In this period, we are preparing a common database of quantitative phytoplankton data and probably, from the early months of 2010 (MED GIG meeting of February 2010), will be made available a common criterion in order to start with the IC exercise for the Mediterranean Eco-region,

following of course the updated IC guidelines provided by the JRC. In other words, it is not possible now to refill adequately this questionnaire, especially the topic C, but we are ready to produce all the information you need, as recommended by Mrs Wendy Bonne, the JRC GIG coordinator for TW and CW.

ID: 56

SK-PP-RI

## 1. General information

- 1.01 GIG:** Mediterranean coastal Med-GIG
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Slovenia
- 1.05 Specification:** none
- 1.06 Method name:** *Methodology for assessment of ecological status of coastal waters using phytoplankton*
- 1.07 Original name:** *Metodologija vrednotenja ekološkega stanja obalnih voda s fitoplanktonom*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).
- 1.10 Internet reference:** [http://www.mop.gov.si/si/delovna\\_podrocja/direktorat\\_za\\_okolje/sektor\\_za\\_vode/ekolosko\\_stanje\\_povrsinskih\\_vod\\_a/](http://www.mop.gov.si/si/delovna_podrocja/direktorat_za_okolje/sektor_za_vode/ekolosko_stanje_povrsinskih_vod_a/)
- 1.11 Pertinent literature of mandatory character:**  
Lipej, L., P. Mozetič, M. Orlando-Bonaca, B. Mavrič, M. Šiško & N. Bettoso, 2007. Opredelitev ekološkega stanja morja v skladu z Vodno direktivo (Water Framework Directive, 2000/60/EC). Dopolnjeno zaključno poročilo (Poročila MBP, 96), 180 pp.
- 1.12 Scientific literature:**  
n.a.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Patricija Mozetič<br>mozetic@mbss.org<br>Marine Biology Station, National Institute of Biology | <b>1.14 Method reported by</b><br>Janja France<br>france@mbss.org<br>Marine Biology Station, National Institute of Biology |
|---|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Grasshoff, K., M. Ehrhardt & K. Kremling, 1983. Methods of Seawater Analysis. 2nd, Revised and Extended Edition. Verlag Chemie, Weinheim, 419 pp. Holm-Hansen, O., C.J. Lorenzen, R.W. Holmes & J.G.H. Strickland, 1965. Fluorometric determination of chlorophyll. J. Cons. perm. int. Explor. Mer. 30: 3-15.
- 2.02 Short description**  
At each sampling site, samples are collected at 4 standard depths (surface, 5m, 10 or 15 m and bottom) using 5 l Niskin bottles, samples are then kept in cold and dark place before further processing (filtering).
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** Niskin bottles
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
pelagic
- 2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant
- 2.08 Sampling/survey month(s):** all
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
minimum once per month
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
no replicates
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
integrated water column chl-a concentrations based on 4 discrete sampling depths

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 0.7 µm
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
A sub-sample of 400 ml of seawater is filtered through a GF/F filter.
- 2.14 Level of taxonomical identification:** n.a.
- 2.15 Record of abundance:** n.a.

in relation to n.a.

Unit

2.16 Quantification of biomass: n.a.

Fluorometric determination of chlorophyll-a concentrations

2.17 Other biological data: none

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

##### 3.01 List of biological metrics

Yearly geomeans of integrated Chlorophyll-a concentrations

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: Not relevant

##### 3.04 From which biological data are the metrics calculated?

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

3.05 Scope of reference conditions: Surface water type-specific

##### 3.06 Key source(s) to derive reference conditions:

Existing near-natural reference sites, Historical data

##### 3.07 Reference site characterisation

Number of sites: 1

Geographical coverage: Northern Adriatic, Gulf of Trieste

Location of sites: 1 NM off coast, 21 m deep, southern part of the Gulf of Trieste

Data time period: period 1989-2002 (sampling once per month)

##### Criteria:

The reference site has the lowest average chl-a concentrations. There are no runoffs from land that could introduce an increased amount of nutrients and suspended matter that could alter water column transparency. Indeed, the site is characterized by the highest water transparency of the area and low nutrient concentrations.

##### 3.08 Reference community description

Chlorophyll biomass shows a normal seasonal cycle with two peaks (first extended from February to April and second in November). Average chl-a biomass is about 0.9 µg/l.

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites

##### 3.11 Boundary setting procedure

No direct response of chl-a biomass to environmental pressures (e.g. nutrient concentrations) was found. Boundaries were therefore set using existing data according to expert judgement. HG boundary was set as 90-percentile of yearly geomeans of chl-a concentrations at the reference site GM boundary was set as 90-percentile of yearly geomeans of chl-a concentrations at a site under the influence of river and wastewater discharges MP and PB boundaries were set according to equidistant principle

3.12 "Good status" community: Average chl-a concentration reaches around 1.4 µg/l. Mean phytoplankton abundance is higher compared to that at the reference site, while the diversity shows no differences.

#### Uncertainty

3.13 Consideration of uncertainty: No (to be done)

##### 3.14 Comments:

none

ID: 188

ES-PP-CO

## 1. General information

**1.01 GIG:** Mediterranean  
Type IIA, Type III western

**1.02 Category:** Coastal Waters

**1.03 BQE:** Phytoplankton

**1.04 Country:** Spain

**1.05 Specification:**

**1.06 Method name:** *Water quality based on chlorophyll-a*

**1.07 Original name:** *n.a.*

**1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)

**1.09 Detected pressure(s):** Catchment land use, Eutrophication, Flow modification, General degradation

*Has the pressure-impact-relationship been tested?*

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

No clear relationship was found between chlorophyll-a concentration and pressures. Not used for boundary setting.

**1.10 Internet reference:** It will be soon at: [www.gencat.cat/aca](http://www.gencat.cat/aca)

**1.11 Pertinent literature of mandatory character:**

ORDEN ARM/2656/2008, de 10 de septiembre, por la que se aprueba la instrucción de planificación hidrológica. BOE 229, 22 de septiembre de 2008.  European Commission, 2008. Commission Decision of 30 October 2008 establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring System classifications as a result of the intercalibration exercise. Official Journal of the European Union, L332/20-L332/44.

**1.12 Scientific literature:**

Carletti, A. & A.-S. Heiskanen, 2009. Water Framework Directive intercalibration technical report. Part 3: Coastal and Transitional waters. JRC Scientific and Technical Reports. EUR 23838 EN/3 - 2009.

**1.13 Method developed by**

Jordi Camp Sancho  
evafl@icm.csic.es  
Institut de Ciències del Mar, CSIC

**1.14 Method reported by**

Jordi Camp Sancho  
evafl@icm.csic.es  
Institut de Ciències del Mar, CSIC

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

European Commission (EC), 2003. Common implementation strategy for the Water Framework Directive (2000/60/EC). Working Group COAST. Guidance document nº 5. Transitional and Coastal Waters - Typology, reference conditions and classification systems.  European Commission (EC), 2003. Common implementation strategy for the Water Framework Directive (2000/60/EC). Working Group COAST. Guidance document nº 6. Towards a guidance on establishment of the intercalibration network and the process on the intercalibration exercise.

**2.02 Short description**

107 inshore stations (beaches and rocky areas) are sampled along the Catalan coast, distributed into the 34 water bodies (WB) defined for the internal hydrologic basins. They are located between 0 and 25 m from the coastal line where the water column is around 1 m depth and are sampled at surface monthly along the whole year. Salinity, Chl-a and dissolved inorganic nutrients data are obtained, among other environmental parameters.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Bucked samples

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
Surface waters

**2.07 Sampled/surveyed zones in areas with tidal influence:** not relevant

**2.08 Sampling/survey month(s):** All year

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

1 sample / month, in some cases the frequency could be 4 samples (seasonally) /year (see Technical report. Part 3: Coastal

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

1 sample per site, between 2 and 21 samples per water body

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

60 ml per sample ( x water body site number x 4 years)

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** Aprox. 0,7 µm (GF/F filters)
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** n.a.
- 2.15 Record of abundance:** n.a.  
in relation to n.a.  
Unit
- 2.16 Quantification of biomass:** n.a.  
Determination of chlorophyll-a concentration by fluorometric analysis
- 2.17 Other biological data:** Harmful species, total diatoms, total dinoflagellates, total coccolithophorids, total nanoflagellates in selected samples (not used)
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Chlorophyll-a (90th percentile)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
n.a.  
Data from multiple spatial replicates and sampling during 4 years for each water body

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** 4 sites for type III  
**Geographical coverage:** Type III: 1 rocky deep sites, 2 sedimentary shallow and 1 sedimentary deep  
**Location of sites:** Towns: Cambrils, Roses, Salou and Tarragona  
**Data time period:** From 5 to 15 years, monthly sampled depending on the site  
**Criteria:**  
From the potential reference areas (Agencia Catalana de l'Aigua, 2005. IMPRESS document) the two lowest chlorophyll-a sites were selected as references for each substrate typology in type III (rocky deep, sedimentary shallow and sedimentary deep)
- 3.08 Reference community description**  
Low anthropogenic impact (low inorganic dissolved nutrient concentration and chlorophyll-a values)
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
High-good boundary derived from metric variability at near-natural reference sites
- 3.11 Boundary setting procedure**  
See Alessandro Carletti & Anna-Stiina Heiskanen, 2009. Water Framework Directive intercalibration technical report. Part 3: Coastal and Transitional waters. Section 3. JRC Scientific and Technical Reports. EUR 23838 EN/3 - 2009.
- 3.12 "Good status" community:** n.a.

### **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none



ID: 121

DE-AN-CO

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Angiosperms, Macroalgae  
Zostera marina; Zostera noltii
- 1.04 Country:** Germany
- 1.05 Specification:** none
- 1.06 Method name:** *Assessment method of macrophytes in the Wadden Sea*
- 1.07 Original name:** *Bewertungsverfahren für Makrophyten im Wattenmeer*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication, Habitat destruction, Hydromorphological degradation  
*Has the pressure-impact-relationship been tested?*  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
Dolch, T., C. Buschbaum & K. Reise, 2009. Seegrass Monitoring im Schleswig-Holsteinischen Wattenmeer 2008. Forschungsbericht zur Bodenkartierung ausgewählter Seegrassbestände.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Karsten Reise; Tobias Dolch; Christian Buschbaum  
Karsten.Reise@awi.de  
Alfred Wegener Institute; Wadden Sea Station Sylt
- 1.14 Method reported by**  
Tobias Dolch; Christian Buschbaum  
Tobias.Dolch@awi.de; Christian.Buschbaum@awi.de  
Alfred Wegener Institute; Wadden Sea Station Sylt
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Dolch, T. & K. Reise, 2008. Seegrass Monitoring im Schleswig-Holsteinischen Wattenmeer 2007. Forschungsbericht zur Bodenkartierung ausgewählter Seegrassbestände.
- 2.02 Short description**  
the spatial occurrence of Seagrass beds is detected by recording the outer boundaries with a differential GPS
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.  
airborne mapping
- 2.05 Specification:** differential GPS
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Seagrass bed
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Intertidal zone
- 2.08 Sampling/survey month(s):** mid July to mid September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
the whole Wadden Sea area of Schleswig-Holstein is surveyed within six years
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
due to habitat mapping no replicates are necessary
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** bottom cover more than 5%
- 2.13 Sample treatment:** n.a.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Percent coverage  
in relation to Area  
Unit percent coverage
- 2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** macroalgae cover; epiphyte cover; species composition; substrates

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

n.a.

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** Not relevant

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

**3.05 Scope of reference conditions:** Site-specific

**3.06 Key source(s) to derive reference conditions:**

Expert knowledge, Historical data

**3.07 Reference site characterisation**

**Number of sites:** two

**Geographical coverage:** North Frisian Wadden Sea

**Location of sites:** North Frisian Wadden Sea

**Data time period:** Historical data from the 1930s

**Criteria:**

availability

**3.08 Reference community description**

Well developed Seagrass community in the intertidal zone in the Northern Wadden Sea

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites

**3.11 Boundary setting procedure**

Due to the complexity of the procedure please see in Dolch et al. (2009) in which it is described in detail.

**3.12 "Good status" community:** Due to the complexity of the procedure please see in Dolch et al. (2009) in which it is described in detail.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 35

BEQI

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Belgium
- 1.05 Specification:** none
- 1.06 Method name:** *Benthic Ecosystem Quality Index*
- 1.07 Original name:** *Benthic Ecosystem Quality Index*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation, Impact of alien species, Pollution by organic matter, Riparian habitat alteration
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Cf Publication Van Hoey et al., 2007 for cases.
- 1.10 Internet reference:** <http://www.beqi.eu>
- 1.11 Pertinent literature of mandatory character:**  
Van Hoey, G., E. Pecceu, J. Derweduwen, A. De Backer, J. Wittoeck, H. Hillewaert, S. Vandendriessche & K. Hostens, 2009. Macrobenthos monitoring at the Belgian coast in 2008, in accordance with the Water Framework Directive. ILVO report BM2009-2, 101pp.  Van Hoey, G., J. Drent, T. Ysebaert & P. Herman, 2007. The Benthic Ecosystem Quality Index (BEQI), intercalibration and assessment of Dutch coastal and transitional waters for the Water Framework Directive: Final report. NIOO Rapporten, 2007-02. NIOO. 244 pp.  Van Hoey, G., J. Wittoeck, H. Hillewaert, K. Van Ginderdeuren & K. Hostens, 2008. Macrobenthos monitoring at the Belgian coast and the evaluation of the availability of reference data for the Water Framework Directive. ILVO report.
- 1.12 Scientific literature:**  
n.a.
- |  |   |
|--|---|
| <p><b>1.13 Method developed by</b><br/>Gert Van Hoey<br/>gert.vanhoey@ilvo.vlaanderen.be<br/>Institute for Agriculture and Fisheries Research, Department<br/>Fishery, Section Biological Environmental Research</p> | <p><b>1.14 Method reported by</b><br/>Gert Van Hoey<br/>gert.vanhoey@ilvo.vlaanderen.be<br/>Institute for Agriculture and Fisheries Research, Department<br/>Fishery, Section Biological Environmental Research</p> |
|--|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
ISO standard (ISO 16665:2005(E)) "Water quality – Guidelines for quantitative sampling and sample processing of marine soft-bottom macrofauna".
- 2.02 Short description**  
Habitat approach, the main habitat types within a water body were sampled in such way to get a confident ecological quality classification (enough samples, spatially and eventually temporal distributed within a habitat). The samples were taken randomly within the habitat area.
- 2.03 Method to select the sampling/survey site or area:** Random sampling/surveying
- 2.04 Sampling/survey device:** Grab
- 2.05 Specification:** Van Veen Grab (0.1m<sup>2</sup>)
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)  
soft bottom sediments (muddy sediments [Macoma balthica habitat], fine muddy sand)
- 2.07 Sampled/surveyed zones in area with tidal influence can subdivide in (Nephtys cirrosa habitat)**
- 2.08 Sampling/survey month(s):** Oktober
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per year (preferential autumn)
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Depends on habitat type samples (18 for Macoma balthica habitat, 20 for Abra alba habitat and 18 for Nephtys cirrosa habitat)
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Depends on habitat type samples (1.8m<sup>2</sup> for Macoma balthica habitat, 2.0m<sup>2</sup> for Abra alba habitat and 1.8m<sup>2</sup> for Nephtys cirrosa habitat)

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 1 mm
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
Determination to the lowest level possible. Oligochaeta to level of order. Some polychaeta to the level of family (Cirratulidae). Taxonomy between assessment and reference data were set consistent.
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** Number of individuals per one square-metre
- 2.16 Quantification of biomass:** n.a.  
Wet weights of macrofauna, and converted to Ash Free Dry Weight by conversion factors
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Level 1 – At the ecosystem level, the BEQI uses the relationship between macrobenthic biomass (B) and system productivity (P) Level 2 - Habitat surface area changes (not used for the Belgian Coastal Waters) Level 3 – At the third level the BEQI analyses and evaluates the benthic macrofauna community per habitat. The BEQI level 3 uses four biological parameters: number of species, total density (ind.m<sup>-2</sup>), total biomass (g AFDW.m<sup>-2</sup>), and similarity (Bray-Curtis similarity based on 4th root transformed density data).
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates

### **Reference conditions**

- 3.05 Scope of reference conditions:** Habitat-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** No reference sites; the reference data is selected out of a dataset of 631 samples  
**Geographical coverage:** Belgian Part of the North Sea (within 6 nautical mile)  
**Location of sites:** No reference sites  
**Data time period:** 1994-2004  
**Criteria:**  
No reference sites in the Belgian Coastal waters.
- 3.08 Reference community description**  
The reference benthic characteristics of each habitat were defined on the randomisation of a reference dataset, reflecting the spatial and temporal variability expected in that habitat, based on existing data and knowledge.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
The references values were determined for each boundary setting based on the randomised reference dataset and the used sampling effort.
- 3.11 Boundary setting procedure**  
The boundary setting procedure is based on the output of the randomisation procedure of the reference dataset. The reference value of the good/moderate boundary is determined based on the 5th percentile (number of species, similarity) or on the 2.5th and 97.5th percentile (density, biomass) out of the permutation distribution of each parameter of the reference dataset. The moderate/poor and poor/bad reference value were determined by equal scaling (respectively 2/3 and 1/3 of the

good/moderate reference value), whereas the median value (number of species, similarity) or the 25th and 75th percentile (density, biomass) out of the permutation distribution was used as the reference value of the high/good boundary.

**3.12 "Good status" community:** Is not defined textual.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

A power analysis is used to determine the level of confidence of the analysis. The possibility to detect with a power of 75% a certain deviation from the median value for each evaluated parameter with the used sampling effort was evaluated. Four classes were defined: (1) when it is possible to detect changes with a factor < 2, the power is evaluated as GOOD; (2) changes with a factor 2-5, the power is evaluated as MODERATE; (3) changes with a factor 5-10, the power is evaluated as LOW; (4) changes with a factor > 10, the power is evaluated as VERY POOR.

**3.14 Comments:**

none

ID: 3

DKI

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
NEA 1/26, NEA 8
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Denmark
- 1.05 Specification:** Polysaline open sea areas
- 1.06 Method name:** *Danish Quality Index*
- 1.07 Original name:** *Danskt Kvalitets Indeks*
- 1.08 Status: Method is/will be used in** neither first nor second RBMP
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction, Heavy metals, Pollution by organic compounds (e.g. DDT, PCB), Pollution by organic matter

**Has the pressure-impact-relationship been tested?**

Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).

Three multi-metric benthic macrofauna indices, including DKI, were used to assess marine benthic ecological status (EcoQS) in seven different pollution gradients mainly in western Scandinavia. The impacts included organic load, hypoxia, sediment metals, urban effluents and physical disturbance. The indices responded in a similar threshold response fashion, to the impacts irrespective of factor identity. Index values from Good and High status were significantly ( $p < 0.01$ ) higher than values from Moderate and worse values.

- 1.10 Internet reference:** [http://www.sciencedirect.com/science?\\_ob=MIimg&\\_imagekey=B6V6N-4M0BHVB-3-9&\\_cdi=5819&\\_user=642076&\\_orig=browse&\\_coverDate=12%2F31%2F2007&\\_sk=999449998&view=c&wchp=dGLzV-zzSkzS&md5=2374f051d81ae05acd6afb08a16aeea2&ie=/sdarticle.pdf](http://www.sciencedirect.com/science?_ob=MIimg&_imagekey=B6V6N-4M0BHVB-3-9&_cdi=5819&_user=642076&_orig=browse&_coverDate=12%2F31%2F2007&_sk=999449998&view=c&wchp=dGLzV-zzSkzS&md5=2374f051d81ae05acd6afb08a16aeea2&ie=/sdarticle.pdf)

**1.11 Pertinent literature of mandatory character:**

Carletti, A. & A.-S. Heiskanen (eds), 2009. Water Framework Directive Intercalibration technical report Part 3. Coastal and transitional waters, JRC Scientific and Technical Reports. EUR 23838 EN/3.

**1.12 Scientific literature:**

Borja, A., A.B. Josefson, A. Miles, I. Muxika, F. Olsgaard, G. Phillips, J.G. Rodriguez & B. Rygg, 2007. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic eco-region, according to the European Water Framework Directive. *Marine Pollution Bulletin* 55: 42-52. Josefson, A.B., J.L.S. Hansen, G. Asmund & P. Johansen, 2008. Threshold response of benthic macro fauna integrity to metal contamination in West Greenland. *Marine Pollution Bulletin* 56: 1265-1274. Josefson, A.B., M. Blomkvist, J.L.S. Hansen, R. Rosenberg & B. Rygg, 2009. Assessment of marine benthic quality change in gradients of disturbance: comparison of multi-metric indices. *Marine Pollution Bulletin* 58: 1263- 1277.

**1.13 Method developed by**

Alf B Josefson  
aj@dmu.dk  
National Environmental Research Institute, Aarhus University

**1.14 Method reported by**

Alf B Josefson  
aj@dmu.dk  
National Environmental Research Institute, Aarhus University

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Holme, N.A. & A.D. McIntyre, 1984. *Methods for the study of marine benthos*. IBP Handbook 16, Blackwell, Oxford.

**2.02 Short description**

Three to six Van Veen are taken (blindly) at a site or area using ships. Alternatively 40 Haps are taken, one at each geographical position, mostly regularly spaced within an area. For the case of point sites, 5-10 Haps are taken blindly at each site and sampling occasion.

- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying

- 2.04 Sampling/survey device:** Corer, Grab

- 2.05 Specification:** 0.1 m<sup>2</sup> Van Veen Grab, 0.0143 m<sup>2</sup> Haps-corer

- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom (sand - mud)

- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone

- 2.08 Sampling/survey month(s):** April to June

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One per year

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Six 0.1 m<sup>2</sup> Van Veen, or 40 Haps samples

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

0.6 m<sup>2</sup>

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** 1 mm (mesh-size of sieve)
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Other, Species/species groups  
Species level (or if not possible to determine, genus or family level): Echinodermata, Polychaeta, Crustacea, Mollusca  
Higher Group level: Nemertea, Nematoda, Turbellaria
- 2.15 Record of abundance:** Individual counts  
**in relation to** Area  
**Unit** individuals per m<sup>2</sup>
- 2.16 Quantification of biomass:** n.a.  
wet weight or dry weight are recorded of each taxon
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
The DK1 is applied on 0.1 m<sup>2</sup> samples and therefore Haps samples are pooled to this sample size (6-7 Haps)

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Shannon' s H, AMBI (Borja et al. 2000), Number of species, Number of individuals
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Least Disturbed Conditions
- 3.07 Reference site characterisation**  
**Number of sites:** Depends on type, but typically 5-50 sites  
**Geographical coverage:** Sites the least impacted - farthest from impact source  
**Location of sites:** n.a.  
**Data time period:** Recent data from least impacted sites  
**Criteria:**  
Fauna as in C-11 and impact factor close to background.
- 3.08 Reference community description**  
High diversity (H and richness). Dominance of sensitive species sensu Borja et al. 2000.
- 3.09 Results expressed as EQR?** Yes

### **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient  
Using discontinuities in the relationship of anthropogenic pressure and the biological response.
- 3.11 Boundary setting procedure**  
Usually, the border between Good and Moderate EcoQS (G/M) is determined as some deviation from a reference situation. Reference data, however, are difficult to find. An alternative procedure is described to estimate the G/M border, not requiring reference data. Threshold values, where faunal structure deterioration commences, were identified from non-linear regressions between indices and impact factors. Index values from the less impacted side of the thresholds were assumed to come from environments of Good and High EcoQS, and the 5th percentile of these data, was defined as the G/M border.
- 3.12 "Good status" community:** High diversity (H and richness). Dominance of sensitive species sensu Borja et al. (2000).

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

Status (DKI values) based on individual Van Veen samples are compared to the Good-Moderate border value (obtained for instance as in C-15) using Wilcoxon signed ranks test. If DKI values from a site or area area (water body) are significantly ( $p < 0.05$ ) above the border value, status is acceptable.

**3.14 Comments:**

none

ID: 128

M-AMBI

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Benthic Invertebrates
- 1.04 Country:** Germany
- 1.05 Specification:** NEA, North Sea
- 1.06 Method name:** **Multivariate AZTI Marine Benthos Index**
- 1.07 Original name:** *Multivariate AZTI Marine Benthos Index*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Catchment land use, Eutrophication, General degradation, Habitat destruction, Hydromorphological degradation
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Muxika et al., 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. *Marine Pollution Bulletin* 55 (1-6): 16-29.
- |   |   |
|---|---|
| <b>1.13 Method developed by</b><br>Muxika et al. 2007, Angel Borja [aborja@azti.es], German adaptations by Heyer 2007<br>heyerkarin@t-online.de<br>consultant | <b>1.14 Method reported by</b><br>Dr. Karin Heyer<br>heyerkarin@t-online.de<br>consultant |
|---|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Muster- Standardarbeitsanweisung für Laboratorien des Bund/Länder-Messprogramms Prüfverfahren-SOP:  
  
Makrozoobenthos-Untersuchungen in marinen Sedimenten (Weichboden)
- 2.02 Short description**  
5 to 20 sediment samples are taken from 1 ecotope. Each sample is sieved separately (1mm, 0,5mm mud) and residue is stored and transferred to the laboratory. Benthic species are separated and identified to the lowest taxonomic level.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge, Random sampling/surveying
- 2.04 Sampling/survey device:** Corer, Grab
- 2.05 Specification:** Van Veen-grab (0.1m<sup>2</sup>), corers with 9-15cm diameter
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
soft bottom
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** May or September / October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
6-10 replicates per ecotope
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
1m<sup>2</sup> per ecotope, 2-4 ecotopes per waterbody, average of several years

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** 1000µm, 500µm in mud sediments
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Genus, Species/species groups  
all to species level except some Oligochaeta, (Keine Vorschläge), Diptera, priapulida,...
- 2.15 Record of abundance:** Individual counts

in relation to Area

Unit Number of individuals per one square-meter

2.16 Quantification of biomass: n.a.

determination of fresh weight, ash free dry weight- but not used for assessment

2.17 Other biological data: none

2.18 Special cases, exceptions, additions: none

2.19 Comments  
none

### 3. Data evaluation

#### Evaluation

3.01 List of biological metrics

1) species richness, 2) Shannon diversity(log2), 3) AMBI  $((0*\%EGI)+(1,5*\%EGII)+(3*\%EGIII)+(4.5*\%EGIV)+(6*\%EGV))/100$ , EG= ecological group of the benthic species

3.02 Does the metric selection differ between types of water bodies? No

3.03 Combination rule for multi-metrics: n.a.

factorial analysis

3.04 From which biological data are the metrics calculated?

Data from single sampling/survey occasion in time

Data from single spatial replicate

#### Reference conditions

3.05 Scope of reference conditions: Habitat-specific

3.06 Key source(s) to derive reference conditions:

Expert knowledge, Historical data, Least Disturbed Conditions

3.07 Reference site characterisation

Number of sites: subtidal coast: 17

Geographical coverage: Lower Saxony only

Location of sites: different sites Wadden Sea of Lower Saxony

Data time period: reference time: 1959 up to now

Criteria:

The communities at the sites had to correspond with description of the reference community description referring to a certain habitat.

3.08 Reference community description

Benthic communities, species numbers, diversity typically for the habitat (sediment, salinity, exposure)- low number of opportunistic species.

3.09 Results expressed as EQR? Yes

#### Boundary setting

3.10 Setting of ecological status boundaries: Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites

3.11 Boundary setting procedure

The boundary setting procedure is orientated at the normative descriptions of the WFD (Annex V; 1.2.3). The boundaries were additionally adjusted by the assessment of expert judgement (Heyer 2007). The M-Ambi relates to pressures of sediment enrichment, eutrophication and hazardous substances (Muxika et al. 2007).

3.12 "Good status" community: High portion of sensitive taxa, complex communities, low number of opportunists, high species number and high diversity assemblages.

#### Uncertainty

3.13 Consideration of uncertainty: No (to be done)

3.14 Comments:

The M-Ambi method is similar used by Spain and France. The British and Denish Indices are closely related. Germany uses the AMBI software from AZTI tecnalia in Spain ([www.azti.es](http://www.azti.es)) including local german reference values for species richness, AMBI index and Shannon diversity and german boundaries (Heyer 2007, 2009).

ID: 132

DE-AL-CO

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
Note: Introduced to 1/26, 3/4; not yet intercalibrated
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Macroalgae
- 1.04 Country:** Germany
- 1.05 Specification:** none
- 1.06 Method name:** *Opportunistic Macroalgae-cover/acreage on soft sediment intertidal in coastal waters*
- 1.07 Original name:** *Flächenausdehnung opportunistischer eulitoraler Makroalgen (Grünalgen) in Küstengewässern*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:** [http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt\\_Makrophyten.html](http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt_Makrophyten.html)
- 1.11 Pertinent literature of mandatory character:**  
Dolch, T., C. Buschbaum & Reise, K., 2008. Seegrass-Monitoring im Schleswig-Holsteinischen Wattenmeer. AWI, Sylt. Jaklin, S., B. Petersen, W. Adolph, G. Petri & W. Heiber, 2007. Aufbau einer Matrix für die Gewässertypen nach EG-WRRL im Küstengebiet der Nordsee, Schwerpunkt Flussgebietseinheiten Weser und Elbe. Abschlussbericht Teil A: Nährstoffe, Fische, Phytoplankton, Makrophyten (Makroalgen und Seegrass). Berichte des NLWKN 2007. Kolbe, K., 2007. Intercalibration Report (NEA GIG). Assessment of German Coastal Waters (NEA1/26, NEA3/4) and Transitional Waters (NEA11) by Macroalgae and Angiosperms. NLWKN Wilhelmshaven.
- 1.12 Scientific literature:**  
n.a.
- 1.13 Method developed by**  
Karsten Reise, Kerstin Kolbe, Sandra Jaklin, Winny Adolph  
Kerstin.Kolbe@nlwkn-ny.niedersachsen.de,  
Karsten.Reise@awi.de  
Lower Saxony Water Management, Coastal Defense and Nature Conservation Agency (NLWKN - Lower Saxony); State Agency for Agriculture, Environment and Rural Areas (LLUR-Schleswig-Holstein)
- 1.14 Method reported by**  
Wilfried Heiber  
Wilfried.Heiber@nlwkn-bra.niedersachsen.de  
Lower Saxony Water Management, Coastal Defense and Nature Conservation Agency (NLWKN)
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Monitoring-Handbuch des Bund-Länder-Messprogramms Meeresumwelt (MHB). [http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt\\_Makrophyten.html](http://www.blmp-online.de/Monitoringhandbuch/Kennblaetter/Kennblatt_Makrophyten.html)
- 2.02 Short description**  
Throughout the flight the areas covered by algae are recorded on topographic maps
- 2.03 Method to select the sampling/survey site or area:** n.a.
- 2.04 Sampling/survey device:** n.a.  
Aerial mapping
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)  
Only habitats within the NEA types CW 2 (26) and 4; habitats within the types CW 1 and 3
- 2.07 Sampled/surveyed zones in areas with tidal influence, but have not been assessed**
- 2.08 Sampling/survey month(s):** May to September
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
3 - 5 occasions per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
n.a.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
Total area surveyed
- Sample processing**
- 2.12 Minimum size of organisms sampled and processed:** -----
- 2.13 Sample treatment:** n.a.

- 2.14 Level of taxonomical identification:** Family
- 2.15 Record of abundance:** Percent coverage  
**in relation to** Area  
**Unit** Percent coverage of intertidal
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
The sub-component macroalgae (with it's metric 'opportunistic macroalgae-cover) is part of the assessment tool for the quality component Macrophytes ; other sub-components are 'saltmarsh vegetation' and 'seagrass' (specific metrics look there)
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Weighted average metric scores  
Sub-components (s. C-01): Weighted average metric score per sub-component (macroalgae at present with only one metric). Biological Quality element 'Macrophytes': Weighted average metric score (score of the the sub-components - see C-01.):'
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Note: Classification is based on the 6-year-median of the annual maximal for each water body.

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** n.a.  
**Location of sites:** n.a.  
**Data time period:** Historical descriptive literature before the 1960s  
**Criteria:**  
Absence of eutrophication. According to this pressure at the present no reference site situation in the Wadden Sea.
- 3.08 Reference community description**  
Intertidal soft substrates are not covered or almost not covered by opportunistic macroalgae.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** n.a.  
Good-moderate boundary derived from normative descriptions in the WFD (deviation from the reference situation not more than 50 % (L.S.) respect. 100 % (S.-H.) - (boundary high-good))
- 3.11 Boundary setting procedure**  
n.a.
- 3.12 "Good status" community:** n.a.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
It will be checked if the sub-component 'Macroalgae' might be supplemented by a metric which focuses the species diversity of brown, red and green algae. The differences in the boundary setting between Schleswig-Holstein and Niedersachsen which are existing actually will be checked.



ID: 102

RSL

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Macroalgae
- 1.04 Country:** Ireland
- 1.05 Specification:** none
- 1.06 Method name:** *Rocky Intertidal Macroalgae - Reduced Species List*
- 1.07 Original name:** *Rocky Intertidal Macroalgae - Reduced Species List*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, Hydromorphological degradation, Impact of alien species
- Has the pressure-impact-relationship been tested?**  
No, pressure-impact relationship has not been tested.
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
<http://www.environ.ie/en/Legislation/Environment/Water/FileDownload,20824,en.pdf>
- 1.12 Scientific literature:**  
Wilkinson, M., P. Wood, E. Wells & C. Scanlan, 2007. Using attached macroalgae to assess ecological status of British estuaries for the European Water Framework Directive. Marine Pollution Bulletin 55: 136 -150.
- |  |   |
|--|---|
| <b>1.13 Method developed by</b><br>Robert Wilkes<br>r.wilkes@epa.ie<br>Environmental Protection Agency | <b>1.14 Method reported by</b><br>Robert Wilkes<br>r.wilkes@epa.ie<br>Environmental Protection Agency |
|--|---|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
ISO 19493 Water quality. Guidance on marine biological surveys of hard-substrate communities.
- 2.02 Short description**  
Full search of section of rocky intertidal shore is undertaken. Depending on length of shore site is divided into 4-5 shore heights and 5 quadrats are placed at each shore height. All seaweed species present in quadrats are noted and %cover recorded. Other subhabitats such as rockpools and overhangs also searched and species present recorded. 'Shore description' as described in published method is recorded also.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.
- 2.05 Specification:** In situ shore survey
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
rocky shore intertidal
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Intertidal zone
- 2.08 Sampling/survey month(s):** May to October
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
minimum three sites per WB at least twice in 6-year cycle
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
min 3 sites per WB
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
n.a.

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** n/a
- 2.13 Sample treatment:** Organisms of the complete sample are identified.
- 2.14 Level of taxonomical identification:** Species/species groups
- 2.15 Record of abundance:** Percent coverage  
in relation to Area  
Unit % cover

- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** none
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
%cover not currently used in classification

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Number of green species☐Number of brown species☐Number of red species☐Number of opportunists☐Number of species in Ecological Status Group 1 ☐Number of species in Ecological Status Group 2 ☐Shore Description
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Existing near-natural reference sites, Expert knowledge, Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** n.a.  
**Geographical coverage:** Many sites from UK and Ireland  
**Location of sites:** n.a.  
**Data time period:** n.a.  
**Criteria:**  
No specific reference sites have been identified. There are a number of rocky intertidal sites around the coast of the UK and Ireland that can be considered to be reference conditions for which data is available within the Marine Benthic Algal Database.
- 3.08 Reference community description**  
Diverse community of red, green and brown seaweeds with high levels of species richness. Cover variable depending on local physical conditions but species richness relatively constant temporally. Red species present as richest group along with a high proportion of long-lived spp. Opportunist and green species should constitute a lower proportion of the algal present
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Equidistant division of the EQR gradient
- 3.11 Boundary setting procedure**  
Degradation through coastal morphological change or increased pressure, specifically dredging activity causing increased sedimentation and excess deposition with restriction of light and limiting growth to opportunist or tolerant species only.  
☐  
Increased nutrients inputs from both direct and indirect sources such as sewage outfalls and land run-off contribute to eutrophication problems. These may exacerbate the growth of opportunist species which in turn may smother the underlying algal community resulting in a decrease in species richness and general diversity.☐Freshwater run-off or outflows reducing salinity can also lead to a dominance of more tolerant species such as the opportunist macroalgae whereby less tolerant species may be restricted in both richness and abundance.
- 3.12 "Good status" community:** Good status☐Most disturbance-sensitive macroalgae associated with undisturbed conditions are present. The level of macroalgal cover shows slight signs of disturbance. There is a slight deviation from the reference conditions. There is a slightly less diverse community of red, green and brown seaweeds with a corresponding decrease in species richness. Cover variable depending on local physical conditions. Greatest reduction in red spp. and greater proportion of short-lived spp. present.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**

---

none

ID: 185

CFR

## 1. General information

- 1.01 GIG:** North-East-Atlantic
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Macroalgae
- 1.04 Country:** Spain
- 1.05 Specification:** Spanish North East Atlantic regions
- 1.06 Method name:** *Quality of Rocky Bottoms*
- 1.07 Original name:** *Calidad de Fondos Rocosos*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation, Habitat destruction

**Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

The pressure-impact relationship was analysed in a qualitative way in 11 stations, comparing differences between impaired and reference sites under different types of pollution sources, assuming that there are contaminant concentration gradients associated to the distance from the discharge points. However, no physicochemical data were taken and the quality category of each station was estimated "a priori" based on expert judgement, according to their situation along the pollution gradient and depending on the apparent quality of the macroalgae communities. The correlation between the CFR values and the expected quality values was  $R^2=0.87$ ,  $p<0.0001$ .

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

MMA, 2008. Instrucción de Planificación Hidrológica. ORDEN ARM/2656/2008. Ministerio de Medio Ambiente, y Medio Rural y Marino. European Commission, 2008. Commission Decision of 30 October 2008 establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring System classifications as a result of the intercalibration exercise. Official Journal of the European Union, L332/20-L332/44.

**1.12 Scientific literature:**

Juanes, J.A., X. Guinda, A. Puente & J.A. Revilla, 2008. Macroalgae, a suitable indicator of the ecological status of coastal rocky communities in the NE Atlantic. *Ecological Indicators* 8 (4): 351-359. Guinda, X., J.A. Juanes, A. Puente & J.A. Revilla, 2008. Comparison of two methods for quality assessment of macroalgae assemblages, under different pollution types. *Ecological Indicators* 8 (5): 743-753.

**1.13 Method developed by**

José A. Juanes de la Peña  
juanesj@unican.es  
IH Cantabria, University of Cantabria

**1.14 Method reported by**

José A. Juanes de la Peña  
juanesj@unican.es  
IH Cantabria, University of Cantabria

**1.15 Comments**

Although the original metric is described in Juanes et al. (2008) (see A-22), a slightly modified version for the CFR application was published in the last WFD intercalibration technical report (JRC, 2008).

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

JRC, 2008. Water Framework Directive intercalibration technical report. Part 3: Coastal and Transitional waters. Joint Research Centre. European Commission.  
Luxembourg. [http://circa.europa.eu/Public/irc/jrc/jrc\\_eewai/library?l=/intercalibration\\_2/jrc51341-volumecoastpdf/\\_EN\\_1.0\\_&a=d](http://circa.europa.eu/Public/irc/jrc/jrc_eewai/library?l=/intercalibration_2/jrc51341-volumecoastpdf/_EN_1.0_&a=d)

**2.02 Short description**

For each replicate, a qualitative assessment is carried out along a specific transect surface (intertidal) or area (subtidal) in order to obtain information about the three indicators: % coverage of characteristic populations of macroalgae, fraction of opportunistic species and richness of conspicuous characteristic macroalgae (for details see Juanes et al., 2008 and JRC, 2008, referred above).

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** n.a.  
Visual

**2.05 Specification:** Qualitative assessment (non-destructive)

**2.06 Sampled/surveyed habitat:** Single habitat(s)  
Hard bottom

**2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones

**2.08 Sampling/survey month(s):** June to September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

One occasion per sampling season

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

3 replicates

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Variable area (10-100 m<sup>2</sup> aprox.)

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:** Macroalgae populations

**2.13 Sample treatment:** n.a.

**2.14 Level of taxonomical identification:** Other

The taxonomical identification for the coverage estimation is based on a predefined, well-established list of "Characteristic" and "Opportunistic" macroalgae, identified as the dominant species (e.g. *Gelidium corneum*) or higher taxonomic level (e.g. Ceramiales)

**2.15 Record of abundance:** Individual counts, Percent coverage, Relative abundance

**in relation to** Area

**Unit** % Coverage (for Characteristic Macroalgae), % (for Fraction of Opportunistics) and Number (for Richness)

**2.16 Quantification of biomass:** n.a.

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

**3. Data evaluation**

**Evaluation**

**3.01 List of biological metrics**

CFR=C+F+R; C: Coverage of Characteristic Macroalgae; F: Fraction of Opportunistics; R: Richness of Characteristic Macroalgae

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Weighted average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple spatial replicates  
Data from single sampling/survey occasion in time

**Reference conditions**

**3.05 Scope of reference conditions:** Habitat-specific

**3.06 Key source(s) to derive reference conditions:**

Existing near-natural reference sites, Expert knowledge

**3.07 Reference site characterisation**

**Number of sites:** 31 transects along the Cantabrian coast (N Spain)

**Geographical coverage:** Whole Cantabrian coast

**Location of sites:** Not polluted zones of the Cantabrian coast

**Data time period:** 1 year

**Criteria:**

Absence of pressures. Communities at the sites correspond with the description of the reference community. Spatio-temporal variability had to be taken into account.

**3.08 Reference community description**

The intertidal zonation pattern of the macroalgae communities in the Cantabrian coast can be divided in two main fringes (in the range of application of the CFR index); the mid-littoral (dominated by *Corallina* spp. and accompanied by calcareous encrusters, *Caulacanthus ustulatus*, etc...) and the infralittoral (dominated by *Bifurcaria bifurcata* and accompanied by species belonging to the genera *Gelidium*, *Cystoseira*, *Chondracanthus*, *Chondrus*, *Stypocalon*, *Codium*, etc...). The subtidal zones are generally dominated by *Gelidium corneum* or *Cystoseira baccata*, which are accompanied by species belonging to the genera *Laminaria*, *Saccorhiza*, *Corallina*, *Codium*, *Halidrys*, *Spatoglossum*, etc... In reference conditions these communities present high coverage values and the presence of opportunistic macroalgae (e.g. species belonging to the genera *Ulva*, *Enteromorpha*, *Ectocarpus*, etc...) is very reduced.

**3.09 Results expressed as EQR?** Yes

**Boundary setting**

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites

**3.11 Boundary setting procedure**

Pressures evaluated in qualitative terms (see A-13) and boundaries initially set according to expert judgement and finally taken over from intercalibration exercise.

**3.12 "Good status" community:** Good coverage of characteristic macroalgae populations with a reduced presence of opportunistic macroalgae.

**Uncertainty**

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

none

ID: 25

RSL

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Macroalgae
- 1.04 Country:** United Kingdom
- 1.05 Specification:** none
- 1.06 Method name:** *Macroalgae - Rocky Shore Reduced Species List*
- 1.07 Original name:** *Macroalgae - Rocky Shore Reduced Species List*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication, General degradation  
Toxic substances, disturbance

**Has the pressure-impact-relationship been tested?**

Yes, with qualitative data (e.g. response at reference against impacted sites).

The tool was not tested against known gradients relating to specific pressures. However, a large number of sites were assessed using a combination of multivariate analysis and expert opinion. These sites spanned the range of quality from High down to Poor/Bad. Analysis outputs were tested against expert opinion, particularly against the sites with most data.

- 1.10 Internet reference:** [http://www.wfduk.org/bio\\_assessment/bio\\_assessment/macro\\_rockyshore](http://www.wfduk.org/bio_assessment/bio_assessment/macro_rockyshore)
- 1.11 Pertinent literature of mandatory character:**  
UKTAG Coastal water assessment methods: Macroalgae- Rocky shore reduced species list by Water Framework Directive. United Kingdom Technical Advisory Group (WFD-UKTAG).
- 1.12 Scientific literature:**  
Wells, E., 2006. Water Framework Directive Marine Plants Task Team Tools paper, Intertidal Coastal Waters macroalgae. Rocky Shore Tool. Draft version 3. Paper No. MPTT/MAT01. Wells, E., M. Wilkinson, P. Wood & C. Scanlan, 2007. The use of macroalgal species richness and composition on intertidal rocky seashores in the assessment of ecological quality under the WFD. Marine Pollution Bulletin 55 (1-6): 151-161. Wells, E., NN. Field Guide to British Seaweeds as required for assistance in the classification of waterbodies under the WFD. Environment Agency.
- |   |  |
|---|--|
| <b>1.13 Method developed by</b><br>Dr. Emma Wells, Prof. Martin Wilkinson, Paul Wood, Clare Scanlan<br>emma@wellsmarine.org, M.Wilkinson@hw.ac.uk,<br>clare.scanlan@sepa.org.uk<br>Wells Marine (Consultancy); Heriot-Watt University, Edinburgh;<br>SEPA | <b>1.14 Method reported by</b><br>Dr. Clare Scanlan<br>clare.scanlan@sepa.org.uk<br>Scottish Environment Protection Agency |
|---|--|
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Agency-specific standard operating procedures, as derived from the MPTT tool paper and field guide.
- 2.02 Short description**  
Sites are selected based on previous knowledge/records, where is considered typical or where there is suitable, safely accessible substratum. Mobile substratum or boulder shores are not considered suitable, only bedrock. All sub-habitats on the shore are sampled between high water and low water; sampling should take place on a good spring tide and the lower shore should be surveyed around the time of low water. Particular physical features of the shore are recorded. All possible sub-habitats should be examined and identified. Attention should be paid to e.g. turfs, crevices, overhangs and rock pools. Larger and commoner species may be identified in situ; other taxa should be examined microscopically and identified using appropriate keys.
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** n.a.  
Not applicable - macroalgae
- 2.05 Specification:** Not applicable - macroalgae collected by hand
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Rocky shore may be considered single habitat, but all sub-habitats within this are sampled
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Intertidal zone
- 2.08 Sampling/survey month(s):** May to September (inclusive)
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Generally a minimum of three sites per WB, but may vary

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

Minimum sampling time is 30 minutes, maximum 120 minutes; recommended minimum of 100m shore length to max of ca. 300m, but is shore specific

**Sample processing**

**2.12 Minimum size of organisms sampled and processed:** Not applicable - all visible macroalgae collected, but epiphytes also examined

**2.13 Sample treatment:** Organisms of the complete sample are identified.

**2.14 Level of taxonomical identification:** Genus, Species/species groups  
Identification is to a mixed taxonomic level, with many taxa identified to genus only (e.g. *Ectocarpus* sp, *Blidingia* sp), others to species (e.g. *Leathesia difformis*, *Odonthalia dentata*) and red encrusting algae to type (calcareous encrusters) only. Once within the WFD reporting cycle, a full species list should be carried out to check that the RSL is still representative. On this occasion, all taxa should be identified to species level.

**2.15 Record of abundance:** Individual counts  
Presence of taxon recorded, i.e. count total number of species present

**in relation to** n.a.

The number of species present is related to shore type, and "de-shoring factor" is used to normalise species number.

**Unit** Number of taxa per site

**2.16 Quantification of biomass:** n.a.  
Not measured

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** Boulder shores and shores of mobile substrata are considered unsuitable. Estuarine rocky shores are considered unsuitable.

**2.19 Comments**  
none

**3. Data evaluation**

**Evaluation**

**3.01 List of biological metrics**  
Normalised number of macroalgal taxa (normalised to shore diversity); Proportion of Chlorophyta taxa; Proportion of Rhodophyta taxa; Proportion of opportunistic taxa; Ecological status group ratio.

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** Average metric scores

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time

Metrics may be calculated for single site, waterbody single occasion or waterbody multiple occasions.

**Reference conditions**

**3.05 Scope of reference conditions:** Habitat-specific

**3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions

**3.07 Reference site characterisation**

**Number of sites:** Ca. 350 (to derive reference conditions and boundary conditions)

**Geographical coverage:** From all parts of the United Kingdom

**Location of sites:** Too numerous to list

**Data time period:** Current data, recent data and historical data back to and including the 1960s.

**Criteria:**

Sites had to be considered as free from obvious pressures at the time of sampling. Communities at the sites and species richness totals had to reflect those of shores regarded as high quality according to expert opinion. Sites had to be of suitable substratum type. Species lists for individual sites had to have been compiled as single occasion sampling lists only (not aggregated lists from separate surveys, as these artificially inflate expected species numbers), and to have been surveyed by reputable phycologists.

**3.08 Reference community description**

This varies to some extent geographically within the UK and this is reflected in the slight different RSL lists for different parts of the UK and the Republic of Ireland. A general description is: There should be a diverse community of red, green and

brown seaweeds. Cover is variable depending on local physical conditions but species richness should be relatively constant temporally. Red species are present as the richest group along with a high proportion of long-lived species.

**3.09 Results expressed as EQR?** Yes

### **Boundary setting**

**3.10 Setting of ecological status boundaries:** Calibrated against pre-classified sampling sites

#### **3.11 Boundary setting procedure**

The tool can potentially respond to more than one pressure. Insufficient data were available to be able to set boundaries according to individual pressures. Boundaries were set for all potential pressures by analysing a large data set and using a combination of multivariate analysis and expert judgement. The boundary levels are different for each of the geographically-based RSLs, using the metrics identified in C01 (see the references cited).

**3.12 "Good status" community:** There would be a slightly less diverse community of red, green and brown species than at reference conditions. Cover would be variable according to local physical conditions. The greatest reduction in species would be seen in the numbers of sensitive red algae and there would be a greater proportion of short-lived species present.

### **Uncertainty**

**3.13 Consideration of uncertainty:** Yes

The uncertainty of the classification obtained has been quantified. Confidence in class may be calculated for a single survey's data or for data from aggregated temporal surveys. A spreadsheet with embedded calculations is used to calculate the class, as per the standard equations, and the final EQR (face value class), but it also calculates the probability of the water body being in each of the five WFD status classes. The face value class may not be the same as the most probable class given by the CofC assessment, because the EQR is constrained to be between 0 and 1. This typically occurs where the EQR is close to a boundary - the face value may be Good, but the CofC assessment may say there is a 40% chance of High, 50% of Good and 10% of Moderate. There is therefore 90% confidence of Good or better. Full details of the statistical methodology used are provided in the report "Confidence of Class for Marine Plant Tools".

#### **3.14 Comments:**

Full details of the statistical methodology used are provided in the report "Confidence of Class for Marine Plant Tools".

ID: 129

DE-PP-CO

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
NEA 3/4; NEA 1/26
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Germany
- 1.05 Specification:** Phaeocystis ist only used in the waterbodies of the Ems
- 1.06 Method name:** *Assessment of phytoplankton in coastal waters*
- 1.07 Original name:** n.a.
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication
- Has the pressure-impact-relationship been tested?**  
Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure).  
Eutrophication as total-N, data for the German Coast; chlorophyll a and total-N are defined within the salinity ranges (18-30, 30-32) Eutrophication as blooms of Phaeocystis; data from Norderney; frequency of Phaeocystis cell counts above 1.000.000 cells/L over a period of 6 years (percentage);
- 1.10 Internet reference:**
- 1.11 Pertinent literature of mandatory character:**  
n.a.
- 1.12 Scientific literature:**  
Topcu, D., U. Brockmann & U. Claussen, 2006. Assessments of the eutrophication status in the German Wadden Sea, based on background concentrations of nutrients and chlorophyll. In Laursen, K. (eds), Monitoring and Assessment in the Wadden Sea. Proceedings from the 11. Scientific Wadden Sea Symposium, Esbjerg, Denmark, 4.-8. April 2005. NERI Technical Report No. 573: 53-71.
- 1.13 Method developed by**  
0
- 1.14 Method reported by**  
Annika Grage  
annika.grage@nlwkn-ol.niedersachsen.de  
Lower Saxony Water Management, Coastal Defence and Nature Conservation Agency
- 1.15 Comments**  
none

## 2. Data acquisition

### Field sampling/surveying

- 2.01 Sampling/Survey guidelines**  
Lorenzen, C.J., 1967. Determination of Chlorophyll and Phaeo-pigments: spectrophotometric equations. Limnol. Oceanogr. 12: 343-346. Strickland, J.D. & T.R. Parsons, 1968. A practical handbook of seawater analysis. Fish. Res. Bd. Can. Bull. 167: 1-311.
- 2.02 Short description**  
water sampling (water surface)
- 2.03 Method to select the sampling/survey site or area:** Expert knowledge
- 2.04 Sampling/survey device:** Water sampler
- 2.05 Specification:** none
- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
water surface
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones
- 2.08 Sampling/survey month(s):** growth period
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
one per month
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
n.a.
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
1-4 samples per month and waterbody

### Sample processing

- 2.12 Minimum size of organisms sampled and processed:** original water sample, as long as they are detectable
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
an adequate/specified sub-sample ist taken from the mixed water sample and taken for chlorophyll a analysis or

Phaeocystis cell counts

**2.14 Level of taxonomical identification:** Genus, Other, Species/species groups

not relevant because in the assessment only Phaeocystis is needed right now

**2.15 Record of abundance:** Individual counts

Phaeocystis is counted as colonies (~ 1000 cells) and single cells

**in relation to** Volume

**Unit** number of Phaeocystis cells per liter

**2.16 Quantification of biomass:** Chlorophyll-a concentration

**2.17 Other biological data:** Biovolume, species

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

none

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

concentration of chlorophyll a in µg/L, number of Phaeocystis cells per liter (expressed as frequency (%))

**3.02 Does the metric selection differ between types of water bodies?** Yes

**3.03 Combination rule for multi-metrics:** n.a.

Phaeocystis can not enhance the assessment results derived from chlorophyll a concentrations

**3.04 From which biological data are the metrics calculated?**

Data from single sampling/survey occasion in time

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Historical data, Modelling (extrapolating model results)

**3.07 Reference site characterisation**

**Number of sites:** multiple data from the German Bight

**Geographical coverage:** German Bight

**Location of sites:** coastal region of German Bight

**Data time period:** modelling of reference conditions verified by data before eutrophication

**Criteria:**

n.a.

**3.08 Reference community description**

n.a.

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise

**3.11 Boundary setting procedure**

n.a.

**3.12 "Good status" community:** n.a.

#### Uncertainty

**3.13 Consideration of uncertainty:** n.a.

**3.14 Comments:**

none

ID: 183

IE-PP-CO2

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Ireland
- 1.05 Specification:** none
- 1.06 Method name:** *The Elevated Phytoplankton (Single Taxa) Counts Tool*
- 1.07 Original name:** *The Elevated Phytoplankton (Single Taxa) Counts Tool*
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Surface Water Regulations SI 272. <http://www.environ.ie/en/Legislation/Environment/Water/FileDownload,20824,en.pdf>

**1.12 Scientific literature:**

Cusack, C. & F. O'Biern. Marine Ecological Tools for Reference, Intercalibration and Classification. Associated datasets and digital information objects connected to this resource are available at: Secure Archive For Environmental Research Data (SAFER) managed by Environmental Protection Agency Ireland. <http://erc.epa.ie/safer/resource?id=c67ae163-8911-102b-aa08-55a7497570d3>. Jowett, D. 2006. NEA GIG Milestone 6 Report. [http://circa.europa.eu/Public/irc/jrc/jrc\\_eewai/library](http://circa.europa.eu/Public/irc/jrc/jrc_eewai/library)

**1.13 Method developed by**

Caroline Cusack

Metric Project, Marine Institute, Ireland

**1.14 Method reported by**

Georgina McDermott

[g.mcdermott@epa.ie](mailto:g.mcdermott@epa.ie)

Environmental Protection Agency, Ireland

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

CEN TC230 N601 NWIP Marine Phytoplankton Still at a draft stage.

**2.02 Short description**

A bucket is used for surface samples and Ruttner water sampler used for depth samples. Equal volumes from 3-5 stations are then added in to a container. This sample is well mixed and a subsample is taken, usually 30mls and preserved with Lugol's iodine. A salinity reading and fluorescence reading are also taken.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Bucket or a Standard Ruttner Water Sampler (depending on depth of sample)

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones

**2.08 Sampling/survey month(s):** Jan-Feb; End of May to beginning of September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

4 times a year, once in winter and 3 times during the summer.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Varies, depending on size of waterbody. Usually > 5

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

The waterbody

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** >10 um

**2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.

25ml or 10ml of the sample is left to settle overnight in a sedimentation chamber and this is then analysed. Either the full base plate, half base plate, middle transect or fields of view are counted depending on the biomass of the sample. Samples taken during a bloom are analysed using a sedgewick rafter cell, 1 ml is placed in cell and left for 15 minutes to settle and then counted.

- 2.14 Level of taxonomical identification:** Other, Species/species groups  
Some cells are identified to centric, pennate diatom or armoured and unarmoured dinoflagellates. These are classified to size ranges from 0-20 µm, 20-50 µm and > 50 µm. Some cell can't be identified to species level using light microscopy and are then left at genus level e.g.. Alexandrium spp, Coscinodiscus spp.
- 2.15 Record of abundance:** Individual counts  
in relation to Volume  
Unit Cells/L
- 2.16 Quantification of biomass:** n.a.
- 2.17 Other biological data:** length and width of individual indetermined cells
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
none

### 3. Data evaluation

#### Evaluation

- 3.01 List of biological metrics**  
Single elevated taxa counts
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Not relevant
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Historical data
- 3.07 Reference site characterisation**  
**Number of sites:** 6 waterbodies  
**Geographical coverage:** n.a.  
**Location of sites:** Atlantic Waters off the west and south west of Ireland  
**Data time period:** Data usually ran from 1991-2005  
**Criteria:**  
The reference sites used were in areas where there is minimal disturbances. Sites were chosen that were pristine or near to based on expert judgement.
- 3.08 Reference community description**  
n.a.
- 3.09 Results expressed as EQR?** Yes

#### Boundary setting

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites
- 3.11 Boundary setting procedure**  
Boundaries of 100,000, 250,000, 500,000 and 1 million cells/L as well as different percentage frequency occurrences were examined. This was examined in waterbodies considered to be at high ecological status or near reference conditions.
- 3.12 "Good status" community:** Where an individual taxa blooms is greater than 250,000 cells/L between 20%-39% of the sampling occasions during a 6 year cycle.

#### Uncertainty

- 3.13 Consideration of uncertainty:** No (to be done)
- 3.14 Comments:**  
none

ID: 182

IE-PP-CO1

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
n.a.
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Ireland
- 1.05 Specification:** none
- 1.06 Method name:** **90th percentile and median chlorophyll**
- 1.07 Original name:** 90th percentile and median chlorophyll
- 1.08 Status: Method is/will be used in** First RBMP (2009), Second RBMP (2015)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

Surface Water Regulations SI 272. <http://www.environ.ie/en/Legislation/Environment/Water/FileDownload,20824,en.pdf>

**1.12 Scientific literature:**

Cusack, C. & F. O'Biern, NN. Marine Ecological Tools for Reference, Intercalibration and Classification. Associated datasets and digital information objects connected to this resource are available at: Secure Archive For Environmental Research Data (SAFER) managed by Environmental Protection Agency Ireland. <http://erc.epa.ie/safer/resource?id=c67ae163-8911-102b-aa08-55a7497570d3>. Jowett, D. 2006. NEA GIG Milestone 6 Report. [http://circa.europa.eu/Public/irc/jrc/jrc\\_eewai/library](http://circa.europa.eu/Public/irc/jrc/jrc_eewai/library)

**1.13 Method developed by**

Shane O'Boyle  
s.oboyle@epa.ie  
Environmental Protection Agency, Ireland

**1.14 Method reported by**

Georgina McDermott  
g.mcdermott@epa.ie  
Environmental Protection Agency, Ireland

**1.15 Comments**

none

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

n.a.

**2.02 Short description**

A bucket is used for surface samples and Ruttner water sampler used for depth samples. 500mls of a sample is filtered through a Whatman glass microfibre filters (GF/C) 47 mm in diameter. The filters are then stored in labelled centrifuge tubes (labelled with station no, date, depth, amount filtered) and place in a cool box (keeping them out of sunlight). Stored in fridge overnight, analysed the next day.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler

**2.05 Specification:** Bucket or a Standard Ruttner Water Sampler (depending on depth of sample)

**2.06 Sampled/surveyed habitat:** All available habitats per site (Multi-habitat)

**2.07 Sampled/surveyed zones in areas with tidal influence:** Both tidal zones

**2.08 Sampling/survey month(s):** Jan-Feb; End of May to beginning of September

**2.09 Number of sampling/survey occasions (in time) to classify site or area**

4 times a year, once in winter and 3 times during the summer.

**2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**

Varies, depending on size of waterbody. Usually > 5

**2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**

The waterbody

### Sample processing

**2.12 Minimum size of organisms sampled and processed:** n.a.

**2.13 Sample treatment:** n.a.

**2.14 Level of taxonomical identification:** n.a.

**2.15 Record of abundance:** n.a.

in relation to n.a.

**Unit**

**2.16 Quantification of biomass:** Chlorophyll-a concentration  
Using the hot methanol extraction technique

**2.17 Other biological data:** none

**2.18 Special cases, exceptions, additions:** none

**2.19 Comments**

Two different extraction methods, each with different extraction efficiencies are used in the Republic of Ireland. Separate class boundary criteria for both extraction methods, therefore had to be developed (EPA: Chl total pigments: hot methanol extraction, Marine Institute: Chl a acetone extraction). In this questionnaire, I am referring only to the hot methanol extraction.

### 3. Data evaluation

#### Evaluation

**3.01 List of biological metrics**

90th percentile and median of Chlorophyll in the waterbody

**3.02 Does the metric selection differ between types of water bodies?** No

**3.03 Combination rule for multi-metrics:** n.a.

At the moment we are using the worst quality class but we hope to develop a weighted average scores in the future.

**3.04 From which biological data are the metrics calculated?**

Aggregated data from multiple sampling/survey occasions in time  
Aggregated data from multiple spatial replicates

#### Reference conditions

**3.05 Scope of reference conditions:** Surface water type-specific

**3.06 Key source(s) to derive reference conditions:**

Historical data

**3.07 Reference site characterisation**

**Number of sites:** n.a.

**Geographical coverage:** Reference sites in Atlantic waters

**Location of sites:** Atlantic Waters off the west and south west of Ireland

**Data time period:** n.a.

**Criteria:**

The analyses used to set these boundaries were based on samples where the contemporaneous dissolved oxygen saturation values were within the limits regarded as undisturbed for the particular waterbody from which each sample was collected. To counter the effect of oxygen depletion due to organic pollution being masked by the supersaturating effect of phytoplankton photosynthesis it was necessary to use supporting environmental data such as biological oxygen demand (BOD) to assess the potential for this. In absence of BOD measurements, ammonia levels were used as a proxy for organic pollution.

**3.08 Reference community description**

Dissolved oxygen conditions are 80%-120%; 90th percentile chlorophyll: 6.667 ug/L (hot methanol); 3.333 ug/L (cold acetone) median chlorophyll: 3.333 ug/L (hot methanol); 1.667 ug/L (cold acetone)

**3.09 Results expressed as EQR?** Yes

#### Boundary setting

**3.10 Setting of ecological status boundaries:** High-good boundary derived from metric variability at near-natural reference sites

**3.11 Boundary setting procedure**

For coastal waters the High/Good boundary for chlorophyll was set by applying a factor of 1.5 to the background (ref) concentrations to account for natural variability. Good/Moderate boundary was set at twice the High/Good boundary in the absence of sufficient empirical observations.

**3.12 "Good status" community:** At good status the 90th percentile chlorophyll range is between 10.0-20.0 ug/L and the median chlorophyll range is 5.0-10.0 ug/L. Again, these values are for the hot methanol extraction technique.

#### Uncertainty

**3.13 Consideration of uncertainty:** No (to be done)

**3.14 Comments:**

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none

ID: 189

Spanish Phytoplankton Tool (NEA CW)

## 1. General information

- 1.01 GIG:** North-East-Atlantic  
Coastal Waters 1/26a and 1/26e
- 1.02 Category:** Coastal Waters
- 1.03 BQE:** Phytoplankton
- 1.04 Country:** Spain
- 1.05 Specification:** Atlantic regions (Basque Country, Cantabria, Asturias, Galicia and Andalucía)
- 1.06 Method name:** *Spanish Phytoplankton Tool for North East Atlantic Coastal Waters*
- 1.07 Original name:** *Spanish Phytoplankton Tool for North East Atlantic Coastal Waters*
- 1.08 Status: Method is/will be used in** First RBMP (2009)
- 1.09 Detected pressure(s):** Eutrophication

**Has the pressure-impact-relationship been tested?**

No, pressure-impact relationship has not been tested.

**1.10 Internet reference:**

**1.11 Pertinent literature of mandatory character:**

ORDEN ARM/2656/2008, de 10 de septiembre, por la que se aprueba la instrucción de planificación hidrológica. BOE 229, 22 de septiembre de 2008. European Commission, 2008. Commission Decision of 30 October 2008 establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring System classifications as a result of the intercalibration exercise. Official Journal of the European Union, L332/20-L332/44.

**1.12 Scientific literature:**

Revilla, M., A. Borja, J. Bald, J. Franco & V. Valencia, 2008. A method based on chlorophyll-a concentration for the assessment of phytoplankton status in coastal and transitional waters. XI International Symposium on Oceanography of the Bay of Biscay. Revista de Investigación Marina 3: 219–220. [www.azti.es](http://www.azti.es). Revilla, M., J. Franco, J. Bald, A. Borja, A. Laza, S. Seoane & V. Valencia, 2009. Assessment of the phytoplankton ecological status in the Basque coast (northern Spain) according to the European Water Framework Directive. Journal of Sea Research 61: 60–67. Revilla, M., M. Garmendia, J. Franco & A. Borja (submitted). Comparison of methods for phytoplankton quality assessment in the Basque estuaries (North Spain). Revista de Investigación Marina. <http://www.azti.es>. Revilla, M., O. Briz-Miquel, P. Carrillo de Albornoz, M. Escalona, P. García, X. Guinda, P. Pérez, V. Pérez, N. Rodríguez & P. Serret, 2008. Description of national methods included in the intercalibration. Spain Member State Report for the Phytoplankton Element: Coastal Waters NEA 1/26 type. January 2, 2008. Technical Report.

**1.13 Method developed by**

Several: M. Revilla (coordinator), O. Briz-Miquel, P. Carrillo de Albornoz, M. Escalona, P. García, X. Guinda, P. Pérez, V. Pérez, N. Rodríguez, and P. Serret  
[mrevilla@pas.azti.es](mailto:mrevilla@pas.azti.es)  
Several institutions covering all regions: AZTI-Tecnalia (coordination), Instituto Canario de Ciencias Marinas, INDUROT-Universidad de Oviedo, EGMASA-Junta de Andalucía, IH Cantabria-Universidad de Cantabria, Centro de Investigación e Información Ambiental

**1.14 Method reported by**

Marta Revilla  
[mrevilla@pas.azti.es](mailto:mrevilla@pas.azti.es)  
AZTI-Tecnalia; Marine Research Division

**1.15 Comments**

The tool was agreed among all the regional governments within the Spanish State for the purpose of the European intercalibration (Revilla et al., 2008a; 2009). However, it includes only the reference conditions and the class boundaries between high, good and moderate status. A completed tool that includes also the poor and bad status categories has been applied in the CW and TW of the Basque Country (north Spain) (Revilla et al., 2008b; submitted).

## 2. Data acquisition

### Field sampling/surveying

**2.01 Sampling/Survey guidelines**

Several regional governments and their corresponding laboratories are involved in the water monitoring of the Spanish coast and, therefore, sampling strategy and analytical techniques can present some regional variation. In the Basque Country, standard protocols are used for sampling and laboratory analysis. Lorenzen, C.J. & S.W. Jeffrey, 1980. Determination of chlorophyll in seawater. UNESCO Technical Papers in Marine Science: 35. Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitteilungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 9: 1-38.

**2.02 Short description**

CTD vertical profiles (fluorescence, salinity, oxygen, temperature and PAR) are conducted along the whole water column. Simultaneously, water samples are collected in surface (0-1 m) for phytoplankton counts, chlorophyll-a and additional physico-chemical variables (such as, nutrients, suspended solids and turbidity). Physico-chemical variables only to be used as complementary information; they are not involved in the classification of the phytoplankton element.

**2.03 Method to select the sampling/survey site or area:** Expert knowledge

**2.04 Sampling/survey device:** Water sampler  
Also, CTD fluorescence is used

**2.05 Specification:** Niskin bottle or clean bucket

- 2.06 Sampled/surveyed habitat:** Single habitat(s)  
Surface waters (0-1 m depth)
- 2.07 Sampled/surveyed zones in areas with tidal influence:** Subtidal zone
- 2.08 Sampling/survey month(s):** For chlorophyll-a, a minimum of four months that represent all seasons (winter, spring, summer and fall). For phytoplankton counts, a minimum of two months (once in spring and once in summer)...
- 2.09 Number of sampling/survey occasions (in time) to classify site or area**  
One occasion per sampling season, and several seasons per year, over a 6-year period
- 2.10 Number of spatial replicates per sampling/survey occasion to classify site or area**  
Usually, 1 replicate per sampling station and several stations per water body
- 2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area**  
For chlorophyll-a: about 1-4 L per sampling station; for phytoplankton counts: about 50-100 mL per sampling station.

### **Sample processing**

- 2.12 Minimum size of organisms sampled and processed:** For chlorophyll-a, a single measurement (CTD/filter) is allowed. For phytoplankton (Utermöhl), at least 2 cm are counted with 400 x. This usually implies 50-100 units from the dominant taxa.
- 2.13 Sample treatment:** Sample is divided (sub-sampling) and organisms of a sub-sample are identified.  
When chlorophyll-a is determined by spectrophotometry, a water sample of about 10 L is collected in the field and, subsequently, a subsample of about 1-4 L is filtered in the laboratory (the exact volumen depends on the particulate matter); the remaining water is used for several physico-chemical analysis. For phytoplankton counts, samples of 125-250 ml are collected and fixed with Lugol or glutaraldehyde. Then, the volumen of water used in sedimentation chambers for phytoplankton counting by the Utermöhl technique is about 50 or 100 mL.
- 2.14 Level of taxonomical identification:** Family, Genus, Other, Species/species groups  
Most diatoms and armoured dinoflagellated are identified at the genus or species level. However, broader groups are also used when it is not possible to identify at the higher levels. The taxa usually grouped are the naked dinoflagellates, euglenophytes, small flagellates, small coccoids, chlorophytes and cryptophytes.
- 2.15 Record of abundance:** Individual counts  
in relation to Volume  
Unit Cells/L
- 2.16 Quantification of biomass:** Chlorophyll-a concentration  
Also, determination of chlorophyll-a concentration by CTD fluorescence, regularly calibrated by spectrophotometry
- 2.17 Other biological data:** No
- 2.18 Special cases, exceptions, additions:** none
- 2.19 Comments**  
Chl-a is extracted in cold acetone.

## **3. Data evaluation**

### **Evaluation**

- 3.01 List of biological metrics**  
Sub-metric1 (biomass indicator): 90th percentile of chlorophyll-a with all data recorded at a sampling area during a 6-year period. Sub-metric2 (bloom indicator): percentage of samples, at a sampling area during a 6-year period, where any single taxa exceeds a threshold. The threshold varies from 500,000 to 1,000,000 cells/L along the Spanish coast to account for regional variability in upwelling influence and also in the level of taxonomical identification.
- 3.02 Does the metric selection differ between types of water bodies?** No
- 3.03 Combination rule for multi-metrics:** Average metric scores
- 3.04 From which biological data are the metrics calculated?**  
Aggregated data from multiple sampling/survey occasions in time  
In some Spanish regions the metrics are calculated also with aggregated data from multiple spatial replicates (several sampling stations within a water body).

### **Reference conditions**

- 3.05 Scope of reference conditions:** Surface water type-specific
- 3.06 Key source(s) to derive reference conditions:**  
Expert knowledge, Historical data, Least Disturbed Conditions  
Offshore station not influenced by anthropogenic nutrient sources due to its distance from land

### 3.07 Reference site characterisation

- Number of sites:** 1-2 sites per water body type and coastal sub-area (i. e., Eastern Cantabrian Coast, Western Cantabrian Coast, Iberian Upwelling Coast, Gulf of Cadiz and Canary Islands).
- Geographical coverage:** All the Atlantic coastal sub-areas in Spain: Eastern Cantabrian Coast, Western Cantabrian Coast, Iberian Upwelling Coast, Gulf of Cadiz and Canary Islands).
- Location of sites:** All the Atlantic regions in Spain (Basque Country, Cantabria, Asturias, Galicia and Andalucía)
- Data time period:** 5-15 years
- Criteria:**

The absence or almost negligible pressure in reference sites was illustrated by low values in chl-a concentration and bloom frequency. Other complementary information, such as nutrients, upwelling intensity, river flow and coastal geomorphology (coast exposure and shelf width) was considered (Revilla et al., 2008a; 2009).

### 3.08 Reference community description

Composition-metrics were not developed for phytoplankton quality assessment. Chlorophyll-metric reference for Type 1a: 2.33 ug/L (Eastern Cantabrian Coast), 3.33 ug/L (Gulf of Cadiz) and 0,67 ug/L (Canary Islands). Chlorophyll-metric reference for Type 1e: 4.00 ug/L (Western Cantabrian Coast) and 5.33 ug/L (Iberian Upwelling Coast). Bloom-metric reference: 16.7% (Type 1a) and 25% (Type 1e).

- 3.09 Results expressed as EQR?** Yes

## **Boundary setting**

- 3.10 Setting of ecological status boundaries:** Boundaries taken over from the intercalibration exercise  
Calibrated against pre-classified sampling sites

### 3.11 Boundary setting procedure

The boundary values were established by data analysis and expert judgement. For the Basque CW some of these parameters were established during the first phase of the European intercalibration exercises in the Northeast Atlantic (European Commission, 2008). The reference condition (2.33 µg/L) was set using historical data from coastal and offshore stations in the eastern Cantabrian Sea considered under no risk of eutrophication; the High/Good and Good/Moderate boundaries were established assuming some degree of deviation (50% from the reference to the first class boundary and 100% from the first to the second class boundary). The boundaries among the worse status classes (Moderate/Poor and Poor/Bad) do not require to be intercalibrated at the European level. Those boundaries were established for the Basque CW by assuming a constant increment of 3.5 µg L<sup>-1</sup>, which is similar to the increment from the first (High/Good) to the second (Good/Moderate) class boundary.

- 3.12 "Good status" community:** Composition-metrics were not developed for phytoplankton quality assessment. Good status for the chlorophyll-metric in Type 1a: 3.5-7 ug/L (Eastern Cantabrian Coast), 5-10 ug/L (Gulf of Cadiz) and 1-2 ug/L (Canary Islands). Good status for the chlorophyll-metric in Type 1e: 6-9 ug/L (Western Cantabrian Coast) and 8-12 ug/L (Iberian Upwelling Coast). Good status for the bloom-metric: 20-40% (Type 1a) and 30-50% (Type 1e).

## **Uncertainty**

- 3.13 Consideration of uncertainty:** No (to be done)

- 3.14 Comments:**  
none