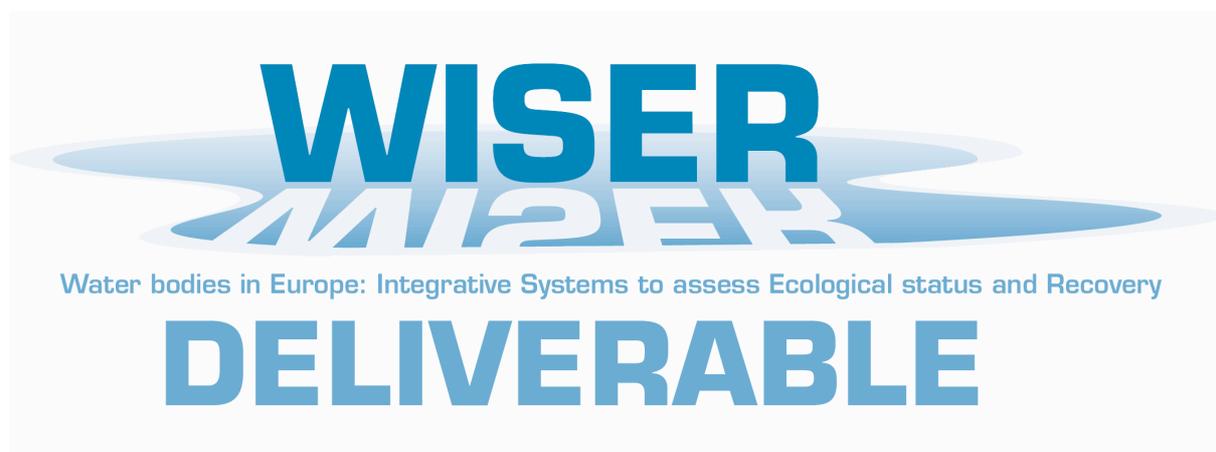


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Non-technical summary

Europe has decided to manage its surface waters with regard to the ecological status they achieve. Here we present an overview of 297 assessment methods for European aquatic ecosystems focussing on the implementation of scientific concepts and standards of aquatic bioassessment. Twenty-eight countries reported mostly on methods applied to rivers (30 %), followed by coastal waters (26 %), lakes (25 %) and transitional waters (19 %). More than half of the methods assessed macroscopic plants or benthic invertebrates. Other methods assessed phytoplankton, fishes and phytobenthos. Method availability was highest in countries of Central and Western Europe. Among different sampling practices two main strategies were discernable: Small-scale sampling of the taxonomically diverse groups of benthic invertebrates and phytobenthos that require elaborate processing, and large-scale sampling of vast, species-poor plant stands or the mobile fish fauna. About three-quarters of methods identified organisms to species-level while especially phytoplankton-based methods referred to class- or phylum-level, or to no taxonomic information. Out of the nine metric types distinguished, river methods featured more sensitivity and trait metrics while for the other water categories abundance metrics prevailed. Fish-based methods showed the highest number of metrics used per method. Most methods focussed on the detection of eutrophication and organic pollution. Habitat loss was mainly assessed by methods applied to rivers and transitional waters. The pressure-impact relationship of about one-third of methods was not tested empirically with methods for transitional waters being the least validated. Status boundaries were mostly defined using statistical, non-ecological approaches. The existing method diversity clearly obstructs comparable status classification among European surface waters. We advocate better reflection of the necessary sampling effort and precision, full validations of pressure-impact relationships and an implementation of more ecological components into classification. The success of European aquatic bioassessment will significantly depend on necessary improvements resolving the issues highlighted in this review.

1. Introduction

Bioindication of aquatic ecosystems dates back to the late 19th century (Kolkwitz and Marsson, 1902). Aquatic bioindicators are organisms which either accumulate toxic substances or are responsive to environmental stress, such as pollution, nutrient enrichment, habitat loss or overexploitation (Adams, 2002). The methods used in bioassessment are usually composed of a chain of subsequent steps: Biological data are ideally generated in a standardised way and derived from field sampling, sample processing and identification of collected organisms. Biological information is then summarised using biological metrics (Karr and Chu, 1999). Results are then compared against standards or reference values and classified into quality statements to further simplify the complex biological information. Each of these steps could be done in countless ways: sampling can be performed with different equipment, identification of organisms can be to different taxonomic levels, and there is a huge number of assessment metrics.

European bioassessment methods also differ geographically. There are several reasons for making assessment methods specific to different regions or ecosystem types, as organism response to stress varies by region or the size of aquatic system, different species occur for biogeographical reasons, relevant stressors differ and applicable taxonomic resolution varies with the knowledge of the regional fauna and flora. To ensure harmonised approaches at continental scale, however, selected components of bioassessment are standardised on a national or international scale (e.g. EN 27828: 1994, EN 13946:2003).

Over the last ten years biomonitoring of European aquatic ecosystems changed substantially. The development was driven by legal requirements, in particular by the EU Water Framework Directive 2000/60/EC (WFD), which required assessment methods for different ecosystem types ("water categories": rivers, lakes, transitional waters, coastal waters) and different organism groups ("biological quality elements" = BQEs: phytoplankton, aquatic flora, benthic invertebrates, fish). The WFD has changed management objectives from merely pollution control to ensuring ecosystem integrity (Borja et al., 2008). Deterioration and improvement of "ecological status" is defined by the response of the biota, rather than by changes in environmental parameters. This response must be investigated at the level of the "water body" (e.g. a river stretch, a lake or a part of a coastal water), which represents the classification and management unit of the WFD. Water bodies of the same category are grouped into "water body types"; the purpose of these water body types is to enable specific reference conditions for each of the BQEs, against which assessment metrics' results are compared. Such conditions become then the basis for biological assessment against which the observed situation is compared. The result of this comparison is given in five classes: high status (no differences to reference conditions), good status (slight differences), moderate status (moderate differences), poor status (important differences), and bad status (dramatic differences). Good ecological status represents the target value that all surface water bodies have to achieve in the near future. While the WFD indicates what characteristics of the BQEs should be assessed (e.g. "abundance", "community

composition”) it does not specify which indices or metrics of these various elements should be used (Hering et al., 2010). This decision was left to the EU member states.

Just developing methods for the different combinations of BQEs and water categories would have resulted in about 20 methods. However, as many countries preferred developing country-specific methods, either to continue using existing time series by adapting their national methods to the WFD, or to regard for the specific ecoregional and biogeographic situation, a multitude of methods resulted instead of a handful of methods applicable Europe-wide (e.g. Birk and Schmedtje, 2005; Borja et al., 2009).

Here we present for the first time an almost complete overview of bioassessment methods for European aquatic ecosystems which were developed for the implementation of the WFD. We search for commonalities and differences within the full range of methods, focussing on the implementation of scientific concepts and standards of aquatic bioassessment guided by six research questions that address key aspects across the different method components:

- Do the bioassessment methods cover all BQEs and water categories across Europe? Ideally, methods are applied in all EU member states concerned and should allow for an integrated ecological evaluation of the various aquatic systems.
- Is the effort to acquire the biological data sufficient to fulfil the assessment objectives? Sampling procedure greatly influences the results of bioassessment. Thus, sampling must be sufficiently precise and representative of the relevant BQE characteristics in time and space (de Jonge et al., 2006).
- Is the taxonomic resolution appropriate for the assessment method? Taxonomic composition represents a key parameter in the assessment of ecological status. The degree of taxonomic resolution is indicative of the ecological precision, available expertise and associated costs.
- Is the selection of biological assessment metrics well-balanced? Biological metrics should reflect different responses to human disturbances. In total, metrics may address different levels of ecological hierarchy (from individual to landscape) (Karr and Chu, 1999).
- Is the methods’ response to anthropogenic pressure known and quantified? Ecological classification should reflect the degree of human influence on the aquatic ecosystem (Borja et al., 2011).
- Does the quality class boundary setting follow ecological rationales? The class definitions represent qualitative statements on the ecosystem’s structure and functioning. Boundaries shall represent tipping-points in quality defined by thresholds relevant to the ecosystem (Groffman et al., 2006).

2. Material and methods

2.1 Data collection

Data on national assessment methods were collated with a questionnaire. This process was part of the official reporting procedure of the European intercalibration exercise to gain full descriptions of biological assessment methods used in national WFD monitoring programmes (European Commission, 2010). Following basic principles of survey design (Oppenheim, 1998;

Noelle-Neumann and Petersen, 2005) we prepared a questionnaire comprising 66 questions that covered the topics general information, data acquisition and data evaluation (see supplementary data). Addressees included coordinators at national water administrations, members of the WFD Working Group on Ecological Status (ECOSTAT) and leading scientists in the European intercalibration exercise. Expert selection ensured a complete coverage of states implementing the WFD and experts for each water category and BQE in the individual states. We asked the recipients to forward the questionnaire to other experts, if necessary.

Data from questionnaires returned until November 2010 were analysed in this publication. The full data were entered into an online database (Birk et al., 2010) accessible at <http://www.wiser.eu/programme-and-results/data-and-guidelines/method-database/>. To complete data gaps or clarify ambiguous information on specific aspects of reported methods, we consulted the scientific literature cited in the questionnaires.

2.2 Data analysis

We described the frequency distributions of selected parameters using pie charts, and compared the parameter values among BQEs and water categories by statistical analyses (Kruskal-Wallis H-Test). For this we defined analytical units (AU) by assigning the methods to individual water categories, BQE or pressure-responses depending on the research question. A method that is used in coastal and transitional waters, for instance, was assigned to two AUs. For some analyses we aggregated the BQEs freshwater macrophytes, marine angiosperms and macroalgae into “macroscopic plants”. Together with freshwater phytoenthos these BQE represent the “benthic flora”.

In case of multiple modalities per method we applied Multiple Correspondence Analysis (MCA). Homogeneity in the dataset and the absence of rare modalities were checked in order to avoid bias (Escofier and Pagès, 1990). For the multivariate analyses data were analysed as complete disjunctive tables. Since the data basis comprised some incomplete information, the number of methods used in the analyses slightly differs between analysed aspects (Table 1).

2.2.1 Coverage of aquatic bioassessment

To summarise the outcomes of the questionnaire survey we quantified the total number of biological assessment methods, and described the relative shares of BQEs and water categories covered by the methods. Furthermore, we evaluated if all BQEs and water categories across Europe were covered by this survey. In theory, a country holds assessment methods for each BQEs of water categories located in this country. We related the number of methods that were actually described to this theoretical value to gain the percent of method coverage for each country.

Table 1: Number of national methods (N) and analytical units (AU) used in the analyses

| Analysed aspect | N | AU |
|---------------------------------------------------|------------------|-------------------------------------|
| Availability of bioassessment methods | | |
| - General overview | 297 | 324 ^b , 300 ^c |
| - Methods' completeness | 290 ^a | 290 |
| Sampling | | |
| - Sampling season | 279 | 308 ^d |
| - Sample size | 196 | 208 ^c |
| - Sampling site selection | 278 | 305 ^d |
| Taxonomic resolution | 288 | 288 |
| Biological metric selection | 290 ^a | 1170 ^e |
| Pressure-impact relationship | | |
| - Scope of detected pressures | 290 | 495 ^b , 458 ^c |
| - Tested relationship | 269 | 295 ^b , 282 ^c |
| - Strength of relationship | 110 | 119 ^b , 115 ^c |
| - Number of observations to validate relationship | 131 | 139 ^b , 134 ^c |
| Quality class boundary setting | 259 | 259 |

^a including only methods for which biological assessment methods were specified.

^b number of AU among water categories

^c number of AU among Biological Quality Elements

^d "active individuals" (i.e. analytical units) in Multiple Correspondence Analysis

^e biological metrics as "active individuals" in Multiple Correspondence Analysis

2.2.2 Sampling

To investigate the sampling strategies of aquatic bioassessment methods in Europe we analysed the information about sample size, sampling season and site selection. The data provided in the questionnaires were first categorised (Table 2), then tested for differences in sample sizes among BQEs and water categories. For the criteria of sampling season and site selection we applied MCA. Aquatic flora was separated into macroscopic plants and phytobenthos as we expected systematic sampling differences between these elements. Phytoplankton methods were excluded from the analysis of sample size as it was not possible to categorise the sampled volumes using the available data.

Table 2: Categories of the sampling criteria

| Criterion | Categories |
|-----------------------|---------------------------------------------|
| Sample size | Small surface area (<20m ²) |
| | Medium surface area (20-100m ²) |
| | Large surface area (>100m ²) |
| | Entire water body |
| Sampling season | Spring (mid-March to mid-June) |
| | Summer (mid-June to mid-September) |
| | Autumn (mid-September to end of November) |
| | Winter (December to mid-March) |
| | Number of sampled seasons |
| Sample selection site | Expert-based |
| | Random |
| | Stratified |

2.2.3 Taxonomic resolution

By reviewing the list of assessment metrics used by each method (see 2.2.4 *Biological metric selection*) we identified which taxonomic level was required to allow for calculating all metrics applied by the method. If, for example, (1) number of families and (2) total abundance of sampled organisms were used in a multimetric scheme, family-level identification was selected as the lowest taxonomic unit required by the method.

2.2.4 Biological metric selection

Based on existing metric classifications (Karr and Chu, 1999; Hering et al., 2006) we grouped metrics into the following types: 1) metrics that did not account for ecological characteristics but taxonomy (“taxonomy-based metrics”), 2) metrics that account for ecological characteristics (“autecology-based metrics”) and 3) non-biotic metrics (e.g. spatial extent of suitable habitats).

The first category („taxonomy-based metrics“) comprised:

- richness metrics, i.e. number of taxa of a certain organism group (e.g. total taxa richness, richness of Ephemeroptera, Trichoptera and Plecoptera taxa) and proportional richness metrics,
- metrics of abundance and productivity (including age- and size-structure), also expressed as proportion of a total,
- diversity metrics, i.e. a combination of richness and abundance metrics, and
- methods based on taxonomic assemblage comparisons between sites (e.g. multivariate approaches).

The second category (“autecology-based metrics”) included metrics in which taxa were characterised by

- sensitivity to disturbance (whereas disturbance could be nutrient enrichment, organic pollution, acidification, etc.),
- autecological traits,
- individual condition (e.g. individual health, physiological characteristics)
- being native or non-native (alien) taxa.

2.2.5 Pressure-impact relationship

We evaluated the questionnaire replies on the pressures detected by each method, using four categories of pressure: eutrophication/organic pollution, hydrology/morphology (i.e. aquatic habitat deterioration, flow modification, hydromorphological degradation, riparian habitat alteration), other water quality aspects (i.e. acidification, heavy metals, pollution by organic compounds such as DDT, PCB) and unspecific pressure (i.e. general degradation). We checked if the pressure-impact relationship was tested for method development. We reviewed the strength of the relationships (expressed as correlation coefficients) and the number of observations used to empirically validate the relationships. If methods were tested against the same stressor at different water body types we averaged the correlation coefficients per pressure category. Responses to different pressures were kept separately in the analysis.

2.2.6 Quality class boundary setting

We specified five approaches of boundary setting grouped into ecological, statistical and expert-based types of classifications (Table 3). We assigned one classification type to each method according to the approaches specified by the respondent. For multiple statements we followed the hierarchy of setting options established by European Commission (2003, 2010): If ecological approaches were used among others, we classified the assessment method into the ecological group. Any use of statistical setting without ecological approaches resulted in a classification as statistical type. Expert-based setting was only assigned if stated without mentioning ecological or statistical approaches.

Table 3: Types of ecological status class boundary setting

| Type of boundary setting | Approaches specified in the questionnaire |
|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ecological | Using discontinuities in the relationship of anthropogenic pressure and the biological response Using biological metrics that respond in different ways to the influence of the pressure (i.e. "paired" metrics) |
| Statistical | High-good boundary derived from metric variability at near-natural reference sites Equidistant division of the existing gradient of ecological quality |
| Expert-based | Class boundaries calibrated against pre-classified sampling sites |

3. Results

3.1 Coverage of aquatic bioassessment

A total of 28 European countries reported on 297 biological assessment methods being used in ecological status monitoring (see supplementary data). River methods made up 30 % of all methods reported; lake and coastal assessment methods held about 25 % each. Transitional waters were least covered (19 %) (Figure 1). Benthic invertebrates were the most prevalent biological group used in aquatic bioassessment followed by macroscopic plants and phytoplankton. Methods evaluating fish fauna and phytobenthos were least represented (Figure 1), but, contrary to all other BQEs, the monitoring of these biological groups is not required for all water categories.

The overview covered 2/3 (66 %) of the theoretical number of assessment methods per European member state (Figure 2). Methods monitoring invertebrates in rivers and phytoplankton in lakes held the highest coverage (90 % and 82 %, respectively). Least covered were methods using phytoplankton in rivers and phytobenthos in lakes (both 21 %).

3.2 Sampling

Benthic invertebrates and phytobenthos were mainly sampled from small areas of less than 20 m² (Figure 3). Conversely, macroscopic plants and fishes were evaluated based on sampled areas of several 100 m², or even with sampling the whole water body. Phytoplankton was usually sampled in all seasons, while macrophytes were most frequently sampled in summer (Figure 3). MCA results showed that these correlations did not depend on the water category.

The number of sampling seasons specified by the assessment methods was significantly different among biological elements (H-Test, $p < 0.001$). Sampling for phytoplankton covered most seasons (mean number of 2.7), while macroscopic plants were only surveyed at an average of 1.5 seasons. Expert knowledge was commonly used to choose sampling sites (78 % of assessment methods).

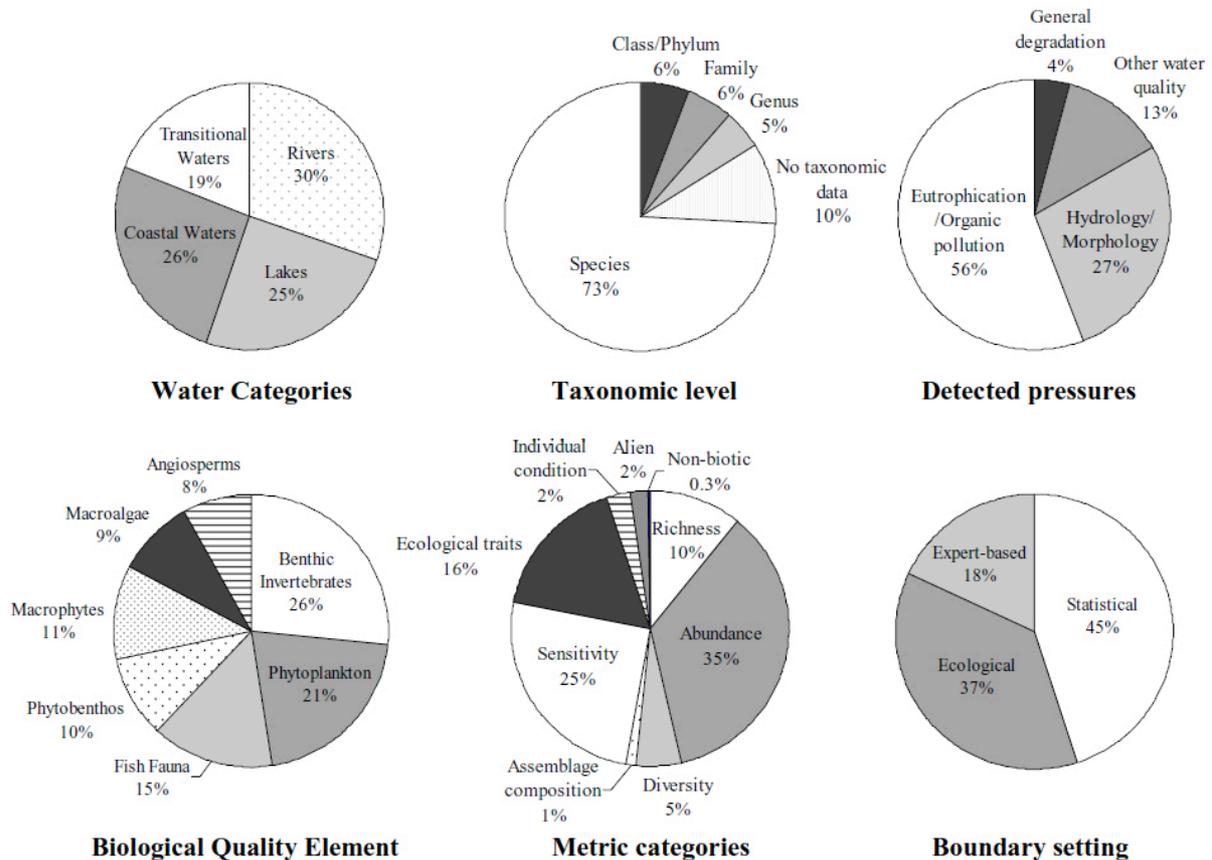


Figure 1: Relative frequencies of selected features of aquatic assessment methods in Europe

3.3 Taxonomic resolution

Almost three quarters of the methods used species-level data for metric calculation (Figure 1). Genus or family level information was mainly used by river methods evaluating the benthic invertebrate communities. Class or phylum data was used in phytoplankton assessment. Only 10 % of methods were using other than taxonomic data, e.g. total biomass or abundance, or morphological and physiological features from monospecific angiosperm stands.

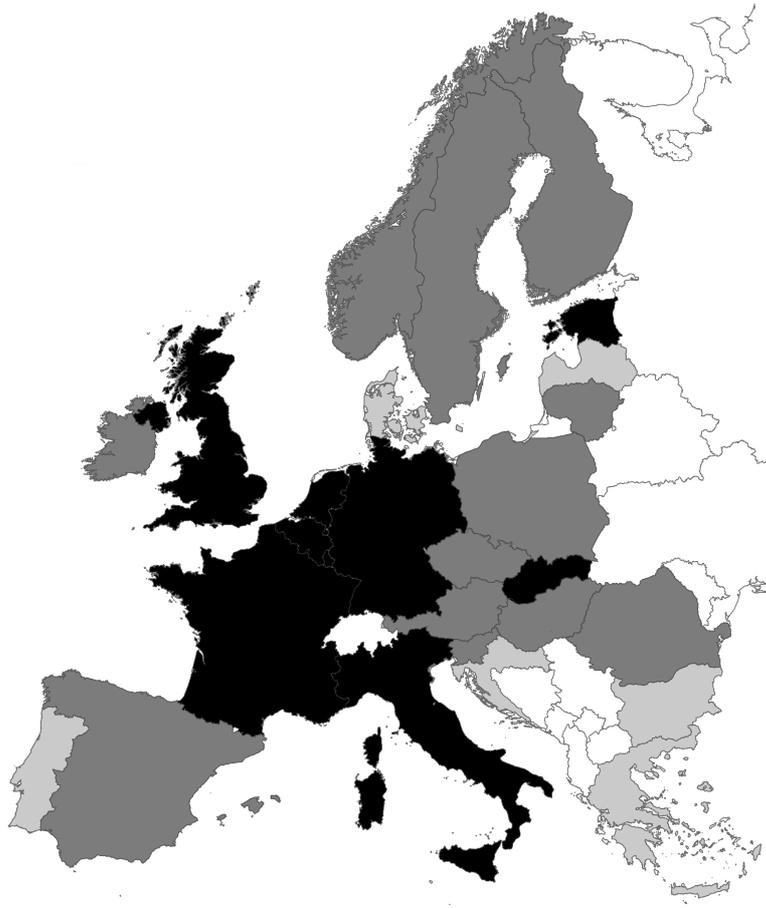


Figure 2: Geographical distribution of the ratio of national methods resulting from this study compared to the number of methods required for national Water Framework Directive monitoring (country * water categories * Biological Quality Elements). Colour codes: black: >75 %, dark grey: 50 % – 75 %, light grey: <50 % (including Cyprus and Malta not depicted on the map), white: not covered by the survey.

3.4 Biological metric selection

Metrics included in the European biomonitoring methods almost equally covered taxonomy-based (about 53 % of metrics) and autecology-based (47 %) metrics, while non-biotic metrics were rarely used (<0.5 %) (Figure 1). Lakes, coastal and transitional waters were most often assessed by abundance metrics (Table 4). The ecological status of rivers was most often assessed by sensitivity and ecological trait metrics, followed by abundance metrics. Sensitivity metrics were also relevant in lake and coastal assessment while the assessment of transitional waters relied more on the assessment of ecological traits. Richness metrics held a considerable share among all water categories. This share was generally smaller for diversity metrics, and according to the MCA results these were usually applied by invertebrate-based methods. MCA results also revealed other patterns: Non-native taxa were used for freshwater assessment, mainly within fish-based methods, and rarely in transitional waters. Metrics targeting individual condition were often associated with angiosperm methods, sensitivity metrics with phytobenthos methods, and abundance metrics with phytoplankton methods.

The number of metrics used per method differed significantly between BQEs (H-Test; $p < 0.05$). Fish assessment methods had the highest number of metrics (mean: 7.8). There were no

differences between water categories (H-Test; $p = 0.15$). In rivers, for instance, the fish methods used on average 7.5 different metrics, in lakes 7.2 and in transitional waters 8.2.

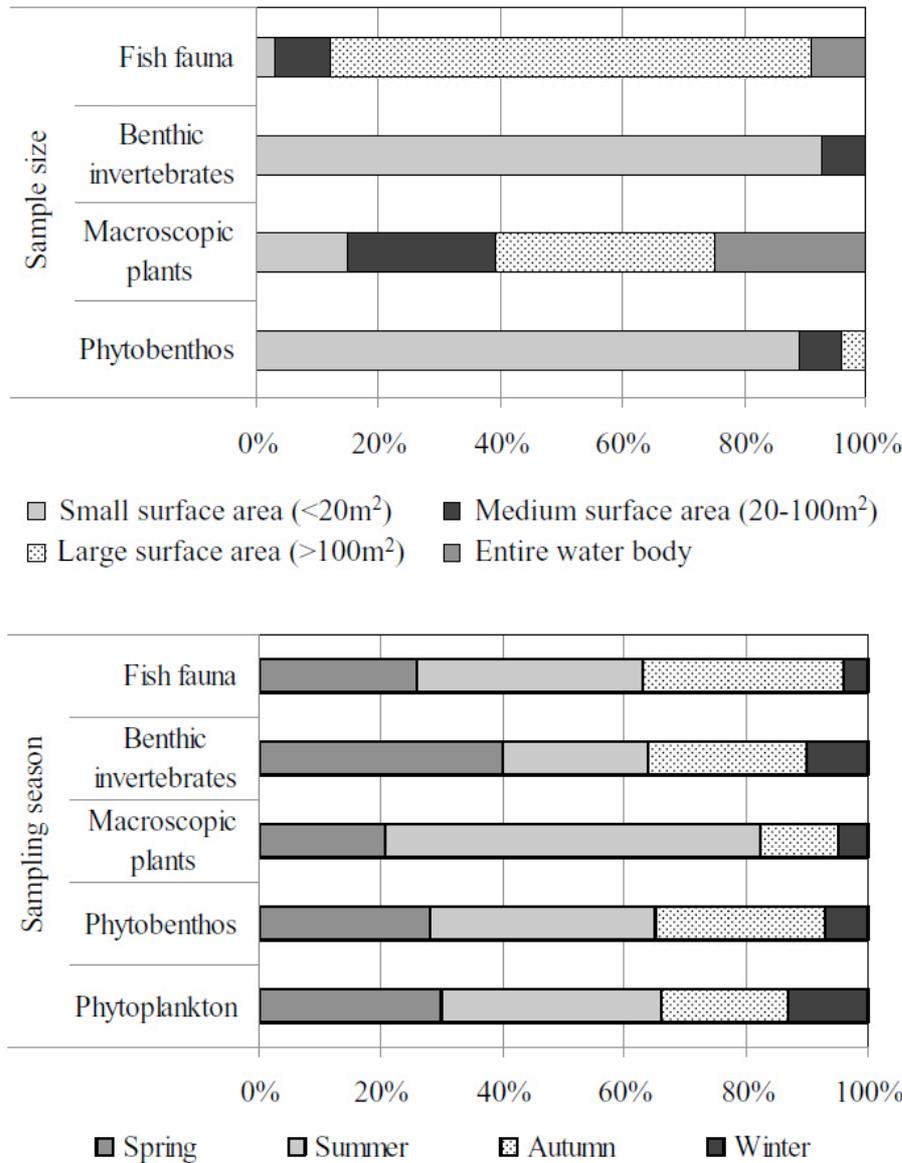


Figure 3: Share of sample sizes and sampling seasons among biological quality elements

3.5 Pressure-impact relationship

More than half of Europe’s assessment methods appraised the impact of eutrophication or organic pollution (Figure 1). This especially applied to methods evaluating lakes and coastal waters where almost 2/3 of methods focused on these stressors. Hydrological or morphological deterioration was detected by 27 % of methods, most apparent for transitional waters. Other water quality aspects were addressed by 13 %. More than half of the river methods focused on pressures other than eutrophication or organic pollution.

The share of methods detecting eutrophication or organic pollutions was markedly decreasing from autotrophic to heterotrophic elements: Phytoplankton > Phytobenthos > Macroscopic plants > Benthic invertebrates > Fish fauna. An almost reverse order was revealed for

hydrological or morphological deterioration: Fish fauna and Macroscopic plants > Benthic invertebrates > Phytoplankton > Phytobenthos. About 20 % of the methods for each biological element (except phytoplankton) addressed other water quality aspects.

Table 4: Percentage of metric types used in water categories. See text for type descriptions.

| | | Coastal Waters | Lakes | Rivers | Transitional Waters |
|-------------------------|-------------------------------|----------------|-------|--------|---------------------|
| Taxonomy-based | Richness | 13.1 | 7.2 | 12.1 | 11.4 |
| | Abundance | 47.3 | 46.2 | 16.4 | 42.4 |
| | Diversity | 5.7 | 5.5 | 4.5 | 4.7 |
| | Assemblage composition | 0.4 | 1.0 | 0.5 | 3.0 |
| Autecology-based | Sensitivity | 20.8 | 25.7 | 37.3 | 10.2 |
| | Ecological traits | 5.3 | 11.0 | 25.7 | 21.6 |
| | Individual condition | 7.3 | 0 | 0.5 | 4.7 |
| | Alien | 0 | 3.4 | 3.0 | 0.8 |
| Non-biotic | | 0 | 0 | 0 | 1.3 |

The pressure-impact relationship had not been tested or documented for one-third of the methods with clear differences between water categories: For 46 % of the methods applied to transitional waters the relationship had not been checked, followed by methods for coastal waters (37 %) and rivers (31 %), while lake methods were tested in 81 % of cases. Methods using fish fauna or macroscopic plants were checked least frequently (44 % and 43 % unchecked, respectively) while phytoplankton, phytobenthos and benthic invertebrates were the best validated biological elements in European bioassessment (more than 75 %).

The number of case-studies to empirically validate the pressure-impact relationships was unequally distributed between water categories and biological elements (see Table 5). Most studies were reported for lakes (especially phytoplankton), and least for transitional waters. Across water categories most case-studies referred to benthic invertebrates, and least to fish fauna. The majority of studies tested the response to gradients of nutrient enrichment or organic pollution.

The strength of relationships differed significantly between biological elements and water categories (H-Test, $p < 0.05$). The correlation coefficients generally covered a broad range (<0.4 to >0.8), but on average with the pattern: Phytoplankton > Macroscopic plants > Benthic invertebrates > Phytobenthos and Fish fauna (Table 5). In terms of water categories (Figure 4) the following order resulted: Coastal waters > Lakes > Transitional waters > Rivers.

The number of observations used to empirically validate the pressure-impact relationship differed between water categories and biological elements (Table 5). On average, relationships were established based on 250 samples (median value).

Table 5: Median coefficients of correlation and number of observations to validate the pressure-impact relationships per biological element (N = number of case-studies).

| Biological element | Correlation coefficient | N | # Observations | N |
|-----------------------|-------------------------|----|----------------|----|
| Phytoplankton | 0.76 | 30 | 400 | 34 |
| Phytobenthos | 0.56 | 18 | 202 | 17 |
| Macroscopic plants | 0.71 | 24 | 104 | 32 |
| Benthic Invertebrates | 0.64 | 33 | 103 | 36 |
| Fish Fauna | 0.55 | 7 | 484 | 12 |

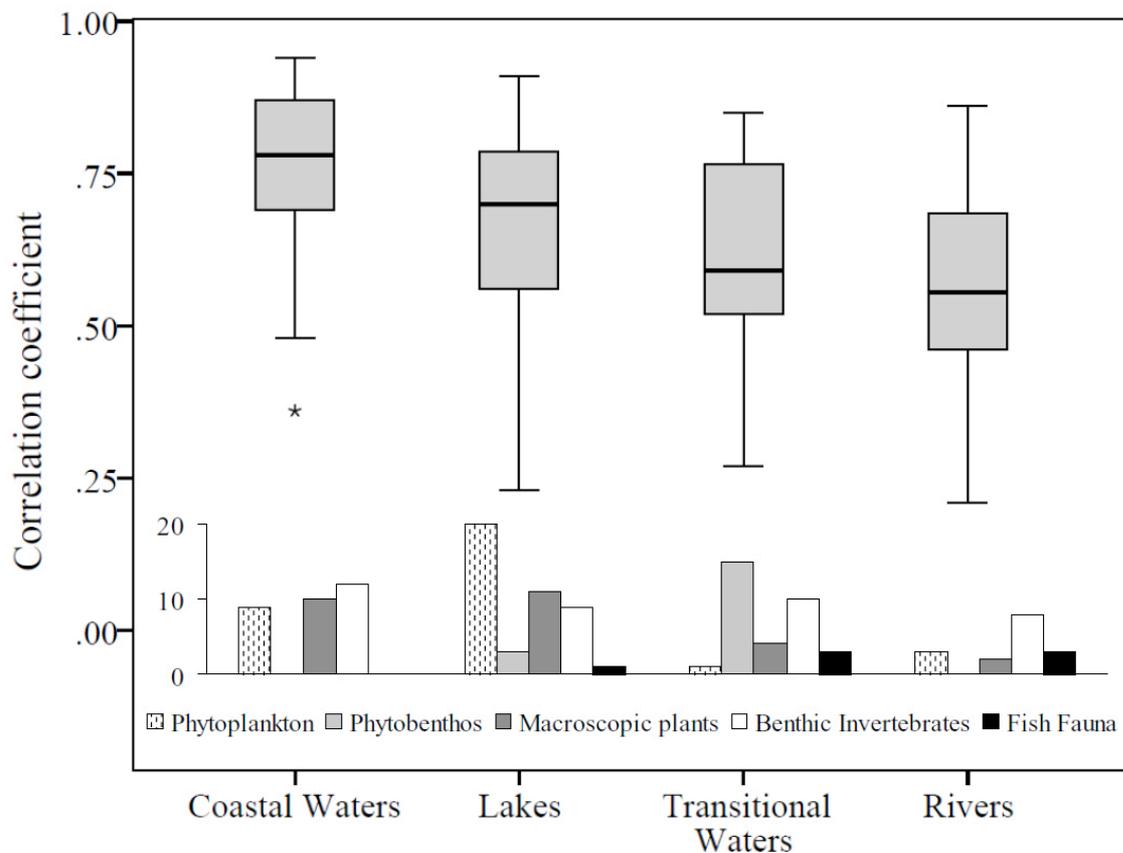


Figure 4: Range of correlation coefficients gained from pressure-impact analysis in different water categories. The number of case-studies per water category is specified below (total number=110).

3.6 Quality class boundary setting

The class boundary setting of European assessment methods was mostly based on statistical principles (45 %), i.e. use of statistical approach without any ecological approach, and 37 % of assessment methods used ecological approaches alone or together with other approaches to define boundaries. Expert judgement alone was used in 18 % of cases (Figure 1). For the development of lake assessment methods mostly ecological approaches were applied (58 %), especially in the assessment of phytoplankton (82 %) and benthic flora (60 %). Statistics-based boundary setting dominated the classifications of rivers and transitional waters (56 %). In these

categories only 24 % of methods were based on ecological boundary setting. This especially applied to benthic fauna and flora in rivers, and benthic flora in transitional waters, where an ecological approach was only used in about 20 % of cases or less. In coastal waters the classification of phytoplankton and benthic flora was mostly based on statistical definitions and expert judgement (approximately 70 %) while the classes in invertebrate assessment were predominantly set based on ecological principles (61 %).

Boundary setting approaches were considerably different between biological elements and water categories (Figure 5). Overall, the highest share of ecological boundary setting applied to phytoplankton methods (47 %) and the lowest to fish and benthic flora assessment methods (31 %). However, conspicuous differences were observed among water categories. The frequency within phytoplankton methods ranged from 82 % for lakes to only 28 % for coastal and transitional waters; within benthic flora methods from 60 % for lakes to only 6 % for transitional waters.

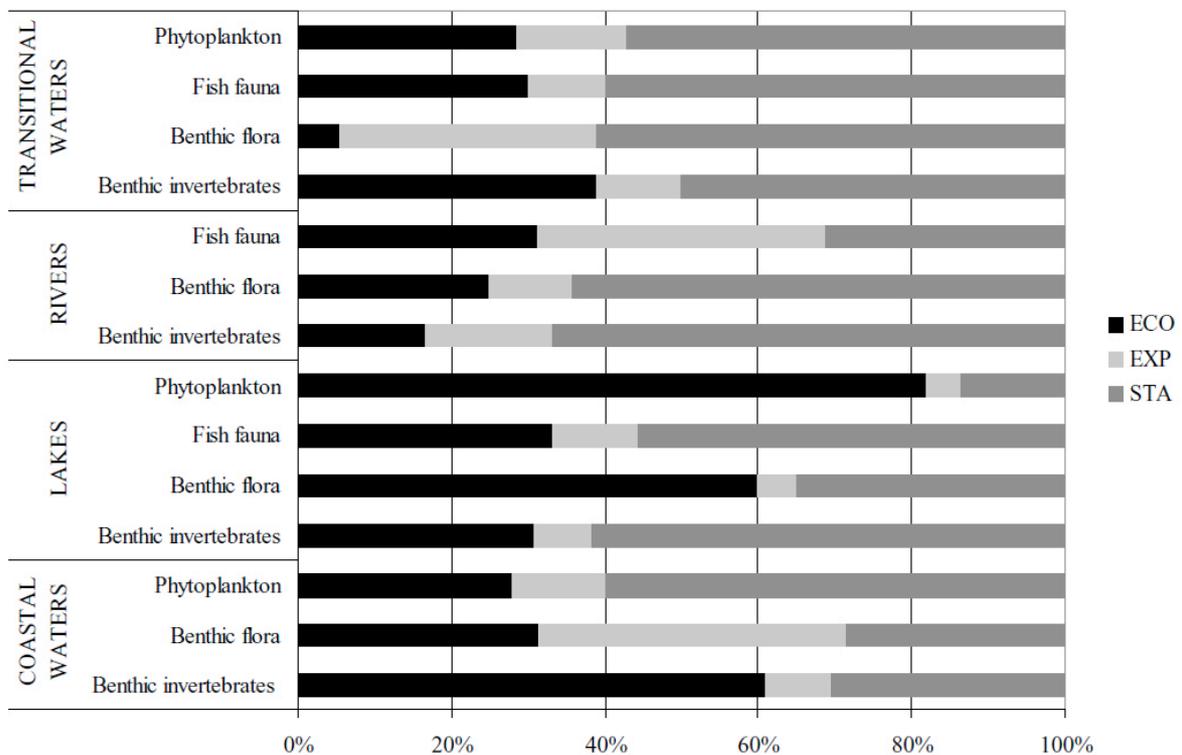


Figure 5: Boundary setting approaches used in ecological assessment methods for water categories and Biological Quality Elements. STAT-Statistical; EXP-Expert; ECO-Ecological

4. Discussion

Europe has decided to manage its surface waters with regard to the ecological status they achieve. The bioassessment methods reviewed in this paper act as principal indicators of this status. They form the link between the actual condition of the environment and its interpretation that leads to management actions of either conservation or (often costly) restoration. With such a responsible role, aquatic bioassessment methods should ideally provide highly reliable outputs

from an integrated appraisal of relevant structural and functional key variables of the ecosystem at low costs (e.g. Kurtz et al., 2001; Bonada et al., 2006). Since these all-in-one solutions are illusive, in practice aquatic bioassessment seems always to be a trade-off between the legislative requirements, best scientific knowledge and socio-political emphasis.

To characterise how this balance is accomplished in Europe we compiled essential data on bioassessment methods developed for the purpose of WFD monitoring. From earlier publications we expected a large number of methods existing (e.g. Birk and Schmedtje, 2005; Borja et al., 2007; Poikane, 2009) and thus chose a survey design based on mail questionnaires. The ease of data accessibility and verification through pertinent networks (e.g. ECOSTAT, GIGs) compensated for probable disadvantages of this design (e.g. ambiguous replies, refusal of information, lack of face-to-face communication; Galpin and James, 1984). Key aspects investigated in our study were extensively discussed among bioassessment experts participating in the European intercalibration exercise. Contents of the data basis are publicly available on the internet which further helped in assuring a maximum level of data quality (Birk et al., 2010).

At first glance the multitude of aquatic bioassessment methods used for European surface waters is perplexing. One is tempted to query if this methodological patchwork allows for comparable status classification across the continent. A Europe-wide record of ecological status, and the practice of river basin management in particular, demand for harmonised assessment concepts and approaches. Comparability is currently addressed by an extensive intercalibration exercise (e.g. Birk and Hering, 2009; Borja et al., 2009). Our findings point out the generally demanding character of such an exercise regarding the high number of different methods.

But looking beyond intercalibration, is this methodological diversity necessary for a successful WFD implementation, or does it rather obstruct any pan-European management objectives? The outcomes of our research questions reveal distinct patterns among methods assessing the same quality elements or water categories. We thus discuss these findings individually with regard to their chances and drawbacks before we conclude on the major issues highlighted by this review.

4.1 Coverage of aquatic bioassessment

The low availability of methods in Eastern and Southern Europe reflects the different monitoring traditions and the fact that in the early 1990s, even for rivers, only half of the European countries were assessing biological parameters in addition to their physicochemical monitoring (Hering et al., 2003). Furthermore, it does not come as a surprise that there are only few methods for transitional waters since only 16 countries have designated water bodies in this category. Additionally, lagoons and estuaries are particularly challenging water bodies for development of ecological assessment methods due to their dynamic and naturally stressed character (Elliot and Quintino, 2007). Zaldivar et al. (2008) emphasised that the implementation of the WFD is particularly problematic in transitional waters and listed several reasons including the difficulty to distinguish between natural and man induced stress (see also Dauvin and Ruellet, 2009).

The high availability of benthic fauna methods reflects the considerable tradition (Rosenberg and Resh, 1993) of macrozoobenthos based aquatic biomonitoring due to the limited mobility, variety of traits and adaptations of benthic animals. The assessment of macroscopic plants is also quite well established as they are mostly fixed and sensitive to biotic and abiotic changes (Orfanidis et al., 2001).

4.2 Sampling

Sampling is fundamental to aquatic bioassessment as it provides the base data on which water bodies are classified. An ideal strategy combines the aspects of high precision and representativeness to detect relevant changes in ecological status of the entire water body. Since sampling represents a considerable part of the costs of bioassessment existing strategies often represent tradeoffs between these aspects. Sampling practices applied in Europe are manifold, but our outcomes reveal two main strategies related to the organism groups used in bioassessment.

The sampling of small surfaces is used in the assessment of benthic invertebrates and phytobenthos. Organisms of these groups are characterised by small body sizes, high taxonomic diversity, and their distribution is related to microhabitat-structures. Single samples can contain many and/or highly abundant taxa whose identification often requires laboratory treatment and specific expertise (Kelly et al., 1998; Bonada et al., 2006). Processing invertebrate or phytobenthos samples is elaborate and thus only performed for limited surface areas (Friberg et al., 2006). Basic assumption of this strategy is that the sample reflects the condition of the whole water body.

Macroscopic plants and fishes are surveyed at larger spatial scales. Both groups feature smaller numbers of taxa relevant in bioassessment, and their organisms are usually larger in size (i.e. detectable by the "naked" eye). The plants are themselves important structural components of the ecosystem and can form vast stands (e.g. seagrass meadows). Such formations often contain only few taxa, but their extension is of ecological relevance, hence requiring large-scale surveys (e.g. Juanes et al., 2008; García et al. 2009). The fish fauna shares similar integrative features that demand extended sample surfaces: Fish habitats are considerably large, and most fish taxa are highly mobile, thus indicating conditions at larger spatial scales (e.g. Schiemer, 2000). Here, spacious sampling, e.g. done by boat survey or aerial photography, seems to come at the cost of a lower sample precision.

For an effective management of the aquatic resources representative sampling is a fundamental, yet highly theoretical concept. Only few studies exist that investigate necessary sampling area and efforts in relation to the size of water body, its spatial heterogeneity or temporal dynamics (e.g. Carstensen, 2007). As sampling strongly determines the results of status classification its strategy shall ideally be aligned to the individual biological indicator used in monitoring (de Jonge et al., 2006). But in practice many assessment methods were build on the basis of already existing data (Beliaeff and Pelletier, 2011).

4.3 Taxonomic resolution

According to the niche-concept in ecology each species is adapted to an individual suit of environmental conditions (Hutchinson, 1957; Devictor et al., 2010). Since anthropogenic pressures affect particular conditions, their impact is best indicated by monitoring at species-level (e.g. Resh and McElravy, 1993; Lenat and Resh, 2001; Lavoie et al., 2009). This level is used by most assessment methods reflecting the well-established taxonomy and good expertise generally existing across Europe.

While species-level is commonly attainable for the less diverse groups of macroscopic plants and (freshwater) fishes the benefits of identifying benthic invertebrates to higher taxonomic levels is a recurring theme in scientific literature (e.g. Stubauer and Moog, 2000; Dauvin et al., 2003; Bertasi et al., 2009). Besides sufficient bioindicative capacity the supporters advocate the reduced risk of wrong determination (and, hence, misclassification of ecological status), less expertise required and, ultimately, lower costs of biomonitoring (e.g. Cochran, 1963, Pik et al., 1999; Mistri and Rossi, 2001).

However, when investigating the response of multimetric methods to gradients of human pressure, methods using species-level could probably be more sensitive in evaluating the impacts than those using higher taxonomic levels, especially in multi-pressure environments (Borja et al., 2011). This still needs additional research to be confirmed. But for those methods that operate at class- or phylum-level, or without any reference to taxonomic information, we see room for improvement by considering data at a finer taxonomical scale (e.g. Tuvikene et al., 2010).

4.4 Biological metric selection

The metrics used differ considerably between BQEs and between water categories. An important factor in the use of metrics seems to be the scientific tradition. For example, in lakes, the metric chlorophyll-a, a measure of algal abundance, is present in all assessment method, resulting in a higher frequency of abundance metrics in lakes. For rivers, there is a long tradition in using benthic invertebrates for assessing the effects of organic matter (i.e. Saprobic Systems, e.g. Birk and Schmedtje, 2005). Further method development has built to a large extent on this tradition, resulting in a relatively high frequency of sensitivity metrics for benthic invertebrates. For the BQE fish, much development of assessment methods has taken place recently in the framework of scientific projects specifically aimed at supporting the WFD (Schmutz et al., 2007), resulting in a more balanced, multimetric approach.

The WFD defines ecological status as “an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters” (WFD art. 2), but relies mostly on metrics for taxonomy and ecosystem structure to quantify it. The current interpretation of art. 2 is that metrics directly quantifying ecosystem functioning are not specifically required as it is implied that good structure resembles good functioning (Solimini et al., 2009), discouraging their development. One can argue that autoecology-based methods are a

proxy for real functional metrics – but this has not been properly tested yet (Sandin and Solimini, 2009).

Given the complex requirements of the WFD, it is not surprising that multimetric indices are common as BQE level assessment methods. The number of metrics per method is approximately three for all BQEs, except for fish where on average eight metrics are combined. There is no obvious ecological reason for this difference, it is most likely caused by the difference in research tradition discussed above. The choice of metrics has been driven to a large extent by the need to fulfil the very detailed requirements of the WFD, leading to multimetric indices covering the main aspects of the WFD Annex V – abundance, species composition, and autecology-related aspects. Other relevant aspects, most notably ecosystem functioning, have been underrepresented as a result.

4.5 Pressure-impact relationship

Main emphasis of Europe's aquatic bioassessment is still on the classical burdens of nutrient and organic pollution. This reflects the status-quo especially of the receiving water bodies such as lakes and coastal waters: excessive nutrient inputs from diffuse sources continue to pose major threats for the European environment (EEA, 2010). Densely populated areas in the European lowlands and especially at estuaries suffer from direct human activities causing morphological alteration and habitat loss (Borja et al., 2006; CIS, 2006; López y Royo et al., 2009). Hence, these stressors are primarily addressed in rivers and transitional waters appraising medium- to large-scale habitat quality as indicated by the fish fauna or macroscopic plants. The variety in pressure focus seems to mirror the multidimensional perturbation generally acting on European rivers.

The high amount of methods with untested pressure-impact relationships is alarming. What do these methods actually assess? They often were developed based on theoretical concepts including a large deal of expert knowledge (e.g. Teixeira et al., 2010). Empirical testing was never conducted, justified by a lack of data or the general conditions unfavourable for bioassessment (see e.g. the Estuarine Quality Paradox; Elliott and Quintino, 2007). Our results seem to reveal a relationship between the degree of the system's natural variability (from transitional waters to lakes) and the percentage of methods untested. However, empirical validation of the pressure-impact relationship is indispensable for any method's development (Borja et al., 2011). Environmental management relies on bioassessment to detect anthropogenic impacts and their causes (Cairns, 2003). In return, the effects of measures to enhance the ecological status need to be indicated, given the high economical and socio-political relevance of aquatic resource management (Feld et al., 2011).

Our summary of validated pressure-impact relationships demonstrates successful linkages of human disturbance and biological responses. We are aware of the limited representativeness of these results owing to the possible bias introduced by the questionnaire replies and the enormous coefficient ranges caused by the heterogeneous conditions covered in the case-studies. Nevertheless, some general patterns clearly emerge: Similar to the findings discussed above, best relationships are shown for methods of the naturally less disturbed ecosystems (lakes,

coastal waters) and the classical indicators (phytoplankton). Coefficients above 0.7, for instance, suggest that more than half of the biological variability is explained by pressure effects. The small datasets underlying most analyses highlight the preliminary character of these studies. In summary, outcomes are already promising but efforts need to increase towards a more comprehensive understanding of the human pressures detected by the individual methods. In particular there is a need to better understand cause (human pressure) - effect (metrics or indicators) relationships, especially for highly integrative BQEs such as fishes or plants. This understanding is required to decide which management actions should be implemented regarding these BQEs.

4.6 Quality class boundary setting

Boundary setting is one of the most critical steps in the design of assessment methods as it defines the target values for environmental management. Common concepts promote the target of stable and healthy ecosystems (e.g. Karr and Chu, 1999). At the desired state the long-term capacity to supply ecosystem goods and services is sustained (Rapport et al. 1998). Ecological thresholds, i.e. small changes in an environmental driver that trigger major changes in the ecosystem, shall play a key role in boundary setting (Groffman et al., 2006; Lyche Solheim et al., 2008). Furthermore, target values shall reflect environmental conditions that are socially desirable or acceptable (Smyth et al., 2007).

But in practice boundary setting often follows non-ecological principles. Statistical approaches, for instance, in which the gradient of biological condition is divided into equidistant classes (Karr and Chu, 1999; Erba et al., 2009) allow for a convenient mapping of the ecosystem status but lack biological significance (Kelly et al., 2007; Brenden et al., 2008; Grenier et al., 2010). Prerequisites for the definition of ecologically meaningful boundaries are well-established pressure-impact relationships (Karr and Chu, 1999; Davies and Jackson, 2006). However, as outlined above, these do often not exist due to limited data availability or significant knowledge gaps. We found that in such cases expert judgement remained the last resort in national boundary setting. Moss et al. (2003, 2008) even argue that boundaries should generally be based on expert opinion since degrees of ecological status are not absolute entities but matters of judgement.

We recommend more efforts to include ecology-based elements in the boundary setting of all methods. This comprises investigating the existence of ecological thresholds, e.g. indicated by non-linear pressure-impact relationships (Lyche Solheim et al., 2008). Especially the selection of biological metrics determines to a large degree whether such relationships are detectable. Non-linearities in the pressure response are most likely picked up by single metrics sensitive to a specific pressure, but may remain undetected by a multimetric index sensitive to multiple pressures. Also the choice of appropriate tools can support the detection of ecological thresholds. However, there is an apparent gap between the prominence of present theoretical frameworks involving ecological thresholds and regime shifts, and the paucity of efforts to conduct statistical tests on the actual appearance of such phenomena in ecological data. A wide range of statistical methods and analytical techniques have been proposed (e.g. Denoël and

Ficetola, 2007; King and Richardson, 2003; Muggeo, 2003; Qian et al., 2003; Lougheed et al., 2007; Grenier et al., 2010). Yet, their application has been surprisingly sparse and disturbance thresholds are mostly identified through visual inspection of data series plots (Lyche Solheim et al., 2008; Penning et al., 2008; Marchetto et al., 2009; McFarland et al., 2010).

Since most classifications rely on non-ecological principles there is no guarantee that ecological class boundaries correspond to meaningful changes in ecosystem functions and biological communities. This results in European ecological targets being built on the basis of statistical distributions rather than ecological impacts. We strongly advocate an enhancement of national boundary setting rationales by implementing ecological components into quality classification.

5. Conclusions

The almost 300 ways to assess Europe's surface waters provide a remarkable account of the continent's natural and cultural diversity, the latter comprising political, social and scientific differences that often promoted unilateral approaches in national method development. The existing diversity clearly hampers to compare the classifications between countries. But more significant are the common drawbacks partly attributable to the specific preconditions of the WFD, for example the emphasis on ecosystem structure not function (Sandin and Solimini, 2009), or the status classification implying a stable, non-dynamic notion of ecosystems (Steyaert and Ollivier, 2007; Hatton-Ellis, 2008). The untested pressure-impact relationships, poor reflection of necessary sampling effort and precision, and, not addressed here but equally important, the lack of uncertainty estimation represent further shortcomings.

Drawing upon a long tradition the importance of bioassessment in Europe increased tremendously in the recent years. Due to the WFD's central focus on good ecological status, the assessment methods received a central role in ecosystem management. The success of this new paradigm will significantly depend on necessary improvements resolving the issues highlighted in this review.

6. References

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