Collaborative Project (large-scale integrating project)
Grant Agreement 226273
Theme 6: Environment (including Climate Change)
Duration: March 1st, 2009 – February 29th, 2012

SEVENTH FRAMEWORK PROGRAMME

WISER

Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery

DELIVERABLE

Deliverable D3.2-1: Overview and comparison of macrophyte survey methods used in European countries and a proposal of harmonized common sampling protocol to be used for WISER uncertainty exercise including a relevant common species list

Lead contractor: **Institute of Environmental Protection (IEP)**

Contributors: **Agnieszka Kolada, Seppo Hellsten, Antti Kanninen, Martin Sondergaard, Bernard Dudley, Peeter Noges, Ingmar Ott, Frauke Ecke, Marit Mjelde, Vincent Bertrin, Thomas Davidson and Harm Duel**

Due date of deliverable: **Month 6**
Actual submission date: **Month 4**

Project co-funded by the European Commission within the Seventh Framework Programme (2007-2013) 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)	

Content

Overview and comparison of macrophyte survey methods used in European countries and a proposal of harmonized common sampling protocol to be used for WISER uncertainty exercise including a relevant common species list

Agnieszka Kolada[1], Seppo Hellsten[2], Antti Kanninen[2], Martin Sondergaard[3], Bernard Dudley[4], Peeter Noges[5], Ingmar Ott[5], Frauke Ecke[6], Marit Mjelde[7], Vincent Bertrin[8], Thomas Davidson[9] and Harm Duel[10]

Partner institutions:

[1] Institute of Environmental Protection (IEP)

[2] Finnish Environment Institute (SYKE)

[3] Aarhus University – National Environmental Research Institute (AU)

[4] Natural Environment Research Council Centre for Ecology & Hydrology (NERC)

[5] Estonian University of Life Sciences (EMU)

[6] Swedish University of Agricultural Sciences (SLU)

[7] Norwegian Institute for Water Research (NIVA)

[8] French Research Institute for Agricultural and Environmental Engineering (CEMAGREF)

[9] University College London (UCL)

[10] Deltares (DELFT)

I. Review of the field methods for macrophyte survey in lakes applied in the European countries

Introduction

The assessment of the structure and functioning of an aquatic ecosystem, expressed as a deviation of the existing status from the undisturbed (reference) conditions, is the requirement set for the monitoring systems by European Directive 2000/60/EC, called the Water Framework Directive [WFD; EC 2000]. The ecological status of an ecosystem is assessed on the basis of the condition of the aquatic communities, the so-called biological quality elements (BQEs). Macrophytes are one of the basic elements used in addition to phytoplankton, macrozoobenthos and fish in the assessment of ecological status and classification. In many European countries, this element has not been monitored on a regular basis to date or such monitoring has been conducted to a very limited extent. Implementing the Water Framework Directive has made it necessary for all the Member States of the European Community to adapt the existing or develop new monitoring methodologies for water assessment on the basis of biological elements.

The monitoring of all the biological elements, also including macrophytes, requires the development of two complementary methodologies: (i) the methodology of a field survey, i.e. the manner of material sampling; (ii) the methodology for the water assessment based on the material collected in the field, i.e. the manner of data analysis and calculation of biological indicators.

As a group of organisms strongly connected with the aquatic environment and vulnerable to changes taking places in aquatic ecosystems, due to eutophication for example, macrophytes attracted the interest of many researchers and for at least several decades they were the object of research carried out primarily by academic centres and scientific research institutions. Different methods were used in macrophyte surveys: from the simplest one, based on species lists, through more complex ones, such as the transect method, or the method of phytosociological surveys [Braun-Blanquet 1964, Tüxen 1974], adapted to the research on aquatic vegetation, to the most exact method of full vegetation mapping in the entire littoral.

The aim of this paper was to present the macrophyte field survey methods which have been used to date in the different European countries and to analyse their practical aspects in the light of the biological monitoring of waters consistent with the Water Framework Directive.

A detailed review of the different methods used for taxonomic composition surveys and measurements of aquatic macrophyte abundance is presented below. Based on this compilation and comparison a harmonized common methodology for macrophyte sampling for the WISER uncertainty exercise was proposed.

1. Floristic inventories

The simplest method for describing the taxonomic composition of aquatic vegetation applies **floristic inventories** (lists of species present in a lake), based on observations and/or sampling by a rake or grapnel from the shore or a boat [Palmer 1989, Palmer et al. 1992, Toivonen and Huttunen 1995, Heegaard et al. 2001]. This method was used e.g. in the Great Britain, where official methodologies for macrophyte surveys in both running and standing waters were developed and published in the late 1980s [*Methods for the Use of Aquatic Macrophytes for Assessing Water Quality* - MEWAM 1987]. In accordance with this document, the manner of investigating macrophytes depends on the size of the lake: for small lakes the whole phytolittoral inventory should have been applied, whereas for large lakes, transects (the number of transects should be proportional to the size of the lake and no less than three) or quadrats method was recommended. The basic drawback of the species inventories is their low information value (the absence of data on the qualitative relationships, abundance, the area occupied etc.) and the species list itself may not be sufficient to allow the further assessment.

2. Phytosociological approach

The **phytosociological method** has been developed and commonly used to investigate terrestrial vegetation [Braun-Blanquet 1964, Tüxen 1974], although it was adapted also for research of aquatic vegetation [Podbielkowski i Tomaszewicz 1996, Best 1988, Matuszkiewicz 2002]. Among three basic phytosociological methods, developed in western Europe, French-Swiss school has most followers globally [Best 1988]. This classification system, based mainly on floristic composition and plant sociology, describes vegetation within a survey unit of homogenous vegetation pattern (called 'relevé') in both quantitative and qualitative way. The fundamental vegetation unit, the association, is defined as a plant community with more or less constant floristic composition, characterized by faithful species and constant accompanying species. Comparing with the terrestrial plant communities, aquatic phytocenoses are relatively poor in floristic terms but are seldom monospecific.

They are most often single-species associations, with a small or even incidental share of other species. Thus, their identification and classification are based on the dominant species rather than a characteristic combination of species [Best 1988, Matuszkiewicz 2002]. Therefore, most water quality assessment methods based on aquatic macrophytes use the indicative features of the species themselves rather than the communities which they build. Moreover, although phytosociological surveys present the quantitative relationships within a vegetation plot (phytocenosis), they do not show the quantitative relationships in the individual phytocenoses throughout the phytolittoral. For this reason, the phytosociological approach can be used to a limited extent in the monitoring methods. Nevertheless, the method has been widely used for scientific purposes, e.g. in Poland [Dąmbska 1966, Podbielkowski and Tomaszewicz 1996, Matuszkiewicz 2002] or Finland [Mäkirinta 1978].

The phytosociological method applied at the higher level of landscape complexity is the **method of sigma-associations** (phytocomplexes) [Géhu 1976, 1977, Tüxen 1979], where the area of the relevé is the total area occupied by the lake vegetation, i.e. the phytolittoral. The share of the individual vegetation associations are defined on the six-point Braun-Blanquet cover-abundance scale. Although, the method was used sometimes in scientific research [Kłosowski et al. 2004, 2006] it has not found a practical application in monitoring programmes.

3. Total phytolittoral mapping

The most exact method for macrophyte surveys is the **mapping of lake vegetation** (phytolittoral mapping). According to this method, the taxonomic composition, distribution and quantitative relationships of aquatic vegetation are investigated within the phytolittoral as a whole. Such an approach was applied e.g. in the macrophytoindication method developed in the 1980s in Poland [Rejewski 1981]. Vegetation is mapped at the peak of the vegetation season (June-August), both from a boat, by using a rake or a grapnel together with bathyscope survey and from the shore. The distribution and the horizontal and vertical ranges of all the phytocenoses should be plotted on a bathymetric plan, which enables to determine the spatial structure and the quantitative relationships of aquatic and rush vegetation within the phytolittoral. In order to improve the method and to reduce the measurement error caused by the surveyor's subjective judgement, it is recommended to use the Global Positioning System for mapping purposes [Jäger et al. 2004, Ciecierska 2008]. The phytolittoral mapping method has been used to date at a local scale e.g. in lake surveys in Poland [Rejewski 1981, Ciecierska 2003, 2004a, 2004b, Ciecierska and Żurawska 2004]. In Finland this methodology was applied with relatively coarse methodology during 30ies combined with transect surveys [Vaarama 1938].

A slightly different approach to macrophyte mapping is recommended in Belgium. The method for assessing standing waters in Flanders was developed in 2004, for the purposes of the implementation of the Water Framework Directive [Schneiders et al. 2004, Leyssen et al. 2005]. Macrophytes are examined throughout the whole phytolittoral divided into sections of homogenous vegetation. For shallow lakes the phytolittoral is considered as a strip reaching from the shore to the 2-meter isobath, whereas for deep lakes it is assumed to extend to 4 m depth. The maximum colonisation depth is recorded as additional information and where it exceeds 2 and 4 m, respectively, the vegetation beyond this zone is not taken into account in the course of water assessment. Within each homogeneous section of the vegetation strip, all species of submerged and emerged plants are recorded, along with an estimation of their percentage shares in the cover. As a result, this produces a map of the lake vegetation in the designated depth zones.

The mapping of the vegetation by a spot-sampling of the phytolittoral is used also in a Dutch monitoring system [Vlek 2006]. The investigation is carried out in depth zones (strata): shallow (<1,5 m depth, with natural or artificial shore separately) and deep (between 1,5 and 3,0 m depth, 5 m only in some lakes according to expert judgement). Number of sampling sites vary from 6 per zone in lakes smaller than 100 ha to 20 per zone in lakes of the area >500 ha. Selection of sites within a stratum is pseudo-random. Each sample site, consisnts of five sub-samples taken with a rake. All species within a stratum are recorded and coverage is estimated in percentage scale or by using Tansley method. Additionally, coverage of the different growth forms (emergent, submerged, floating) is estimated.

4. Transect method

The most universally applied method for aquatic vegetation surveys in both lakes and rivers is the **transect-based method**. In general it can be divided to simple transects and belt transects. Former is applied when there is an interest to data only of depth distributions without estimations of abundance and frequency of different species. Latter method is recommended by the European Committee for Standardisation CEN (*Comité Europeén de Normalisation*) [CEN 2002, 2003]. The method consists in establishing transects (sectors) perpendicular to lake shoreline, with a length covering the complete depth range of the macrophyte occurrence, and in estimating the quantitative share and sometimes also maximum colonisation depth of each species identified within a transect.

It is relatively easy and not very time-consuming to investigate the vegetation in transects; at the same time, it provides reliable detailed information also of depth distribution of different species. For this reason, this method has been commonly applied in monitoring methodologies in many European countries.

In Finland, the monitoring methodology based on transects origins late 30ties [Vaarama 1938] and was further developed and applied by Mäkirinta [1978], Toivonen and Lappalainen [1980], Keskitalo and Salonen [1994] and Hellsten [2001]. This quadrat transect method consists of fixed transects of 1 m wide, perpendicular to the shore of the lake and covering the whole width of the phytolittoral zone (from the shore to maximum colonisation depth). The number of transects was calculated from a formula, taking into account the surface area of the lake and the length of the shoreline [Jensén 1977]. Within each transect, at 1 m intervals, all the plant species, the depth, bottom substrate and other additional observations were recorded. At present, in Finland, 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 (in continuous percent scale) of all detected species are 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].

Similarly, the transect method was used **in Germany**, where intensive surveys on macrophytes were carried out at local scale, e.g. in the Alpine lakes in Bavaria, already in the early 1980s [Meltzer et al. 1986, 1987, 1990, Harlacher et al. 1991, 1993, 1995, all after Schaumburg 1996]. At each lake, the vegetation was mapped in 20 m wide transects, laid out from the shore to the maximum boundary of macrophyte occurrence. Macrophytes were investigated in four depth zones (0-1 m, 1-2 m, 2-4 m and >4 m) and their abundance was estimated on the semiquantitative five-point Kohler scale [1978] (from 1 – a very rare species to 5 – a very abundant species). A slightly modified and specified version of this method was adapted in the most recent German monitoring methodology, consistent with the assumptions of the Water Framework Directive [Schaumburg et al. 2004]. At present, macrophyte surveys are also conducted using the transect method in four depth zones, with the number of transects depending on the size of the landuse variability in the catchment.

The method based on transects, established at all the places of different landuse, is traditionally applied also in lake macrophyte surveys **in Austria**. The surveys were carried out in depth zones and the abundance of individual taxa was estimated on the five-point Kohler scale [1978] [Janauer 2001, 2002]. At present, for the regular water monitoring purposes in Austria, a more detailed field survey methodology was developed, which specified; the transect width (2-5 m), the depth zones where the survey is conducted (0-1 m, 1-2 m, 2-4 m, 4-8 m, >8 m) and the manner of assessing the macrophyte abundance, based on the estimation of the area occupied by a given species in three dimensions (the so-called PME- *Plant Mass Estimates*) on the Kohler scale. Thus, PME is a measure of the volume of the water column occupied by each species rather than the measure of cover abundance [Janauer 2003].

In the United Kingdom, a new guidance for the monitoring of the British standing waters [*Common Standards Monitoring Guidance for Standing Waters*, JNCC 2005] was published in 2005. According to the guidance, a macrophyte survey is performed by wader- and boat survey within a 100 m wide sampling sectors. The wader survey for each 100 m sector includes 20 regularly spaced sampling points. Five transects from the shore to deeper water should be spaced at 20 m intervals along the 100 m sector. On each transect, a 1 m^2 sampling point are surveyed at 0,25 m, 0,5 m, 0,75 m and >0,75 m depth, using a bathyscope and a grapnel, if necessary. In addition, a grapnel haul of 4 m length should be undertaken parallel to the shore, at 0,25 m, 0,5m and 0,75 m depth. At >0,75m depth, a 4 m grapnel haul should be taken at a direction perpendicular to the sector. From each of the 1m^2 sampling points and from the grapnels all species present and an estimate of total vegetation biomass (scoring 0 - 3) are recorded. Where lakes macrophyte communities occupy a deep water zone or there is a characteristic macrophyte zonation, or where access to shallow water is difficult, a boat survey is considered. In such cases, a boat is used to undertake 100 m transects, from deep water to shallow water. Each 100 m transect is located at the 50 m point on the 100 m sector. The transect should begin at the maximum depth of macrophyte colonisation. At each of 20 regularly spaced sampling points, an area of water body bed of 1m^2 is examined, or if visibility is poor, a 4 m grapnel haul is carried out. At each sampling point water depth, all species present and an estimate of total vegetation biomass (scoring 0 - 3) should be recorded. The maximum depth of macrophyte colonisation is also noted. The combination of 100m shore sectors with short transects, and 100 m boat transects, should not total less than four, unless the water body is small and species-poor. Where necessary (e.g. large, rich sites), the number of transects per shore sector may be reduced in order to increase the number of sectors examined. However, there should not be less than three transects per sector.

In Denmark, in addition to phytoplankton, zooplankton and fish, the regular biological monitoring also covered macrophytes since 1993 [Jeppesen et al. 2000, Baattrup-Pedersen et al. 2001]. From 1993 to 2003 an "area investigation" was used where each lake was divided into a number of areas defined by water depth (0.5-1m meter depth zones) in a number of subareas covering the whole phytolittoral zone. In each depth zone a minimum of 10 observation points were used for each subarea to estimate macrophyte coverage and plant volume inhabited. A transect sampling approach has been used in Denmark for macrophyte monitoring purposes since 2004. In each lake a number of transects are defined which represent the whole lake area. On each transect a number of observations points are made. The total number of observation points in a lake depends on lake size, but varies from 30 to 375. In lakes larger than 5 ha at least 150 observations are used. On each sampling point GPS position, water depth, species composition, macrophyte coverage (for each species using the 0-100% Braun Blanquet method) and mean plant height is recorded. The data is used to produce detailed maps of macrophyte distribution, calculate mean coverage of the whole lake, maximum colonization depth and mean plant volume inhabited for the whole lake.

Also **in Poland**, sampling methodology, introduced into routine monitoring in 2006, is based on the belt-transect method. For each lake a minimum number of transects depending on the area and the shape of the lake is calculated [Jensen 1977, Keskitalo and Salonen 1994]. Normally it goves one transect for approximately 500 m shore length. The transect is about 20-30 m width and of the length from the shoreline to the maximum colonisation depth. The macrophyte survey within a transect is conducted according to the phytosociological approach [Braun-Blanquet 1964], therefore no species but syntaxonomic composition is determined. Each transect is sampled with a rake to identify all plant communities, share of each plant community in seven point scale, total plant cover within a transect and maximum depth of plant growth. Data from all transect is then recalculated for the whole lake.

In France a common framework for macrophyte sampling in standing waters for the WFD application was currently established on a basis of CEN standard EN 15460:2007 and on current European methods adapted to the French context [Dutartre and Bertrin 2008]. The method is a compilation of phytolittoral mapping and transect method and involves counting of the species present on sections of shore and along profiles set up perpendicularly to the shore. The counting is carried out from points along the shores which are located, in a preliminary step, by applying Jensen's protocol [Jensen 1977]. The location of the observation units is based on the characteristic of the shore in the way that the main types of riparian zone around the lake are represented. The number of observation units can never be less than 3 for a body of standing water of 50 to 250 ha and should reach 8 for a lake of over 10 km^2. An observation unit involves a record of the vegetation of the littoral area (done in waders then by boat until the bottom is no longer visible; the shore section over which the vegetation is recorded should be at least 100 meters long) and records of three profiles perpendicular to the shore in the same section (one profile at each end and one in the centre of the plot). For each of the profiles, approximately thirty samples are taken by using a rake or a grapnel depending on the depth. The width explored for each profile is about 2 meters. The relative abundance of the plants collected in each sample is evaluated on a 5-point scale: 1 corresponding to just a few stems and 5 where the rake head is full of a single taxon. At the end of each profile, the observed maximum colonisation depth of macrophytes is noted.

In Estonia, when surveying aquatic macrophytes in small lakes (except of the two largest lakes Võrtsjärv and Peipsi) a profile method with combination of phytolittoral mapping method is used .The phytolittoral along the entire lake perimeter is examined from a boat. Within each shore section of the 200-500 m length (depending on lake size), profile is investigated. The more developed or geologically variable the shore stretch, the more profiles should be studied. Each profile starts from the edge of water and reaches to the maximum depth of macrophyte occurrence. The width of the profile is not fixed and extends to both sides of the boat to a distance where the species can still be well recognized. In profiles, the composition and coverage of visible emergent and floating-leaved plants are estimated from the boat and their growing depth is measured by the scaled rope of the grapnel. Composition of submerged plants and their depth limits are studied using random grapnel sampling (in very shallow water also rake) at every 1-10 m (depending on coverage and diversity of plants) along the profile. The abundance of species is based on Braun-Blanquet [1964] scale that was modified by condensing it to five points. Species abundances are estimated separately in three groups: emergent plants (helophytes) and hygrophilous plants; floating and floating-leaved plants (lemnids and nymphaeids) and submerged plants (isoetids, mosses, charophytes, elodeids, ceratophyllids).

In the two largest Estonian lakes: Peipsi (3555 km^2) and Võrtsjärv (270 km^2), macrophyte survey is carried out based on a combination of transects and sampling quadrats. The shore is divided into large apparently homogenous sections (e.g., seven sections in Võrtsjärv) an four to five approximately 2-m wide transects are made in each of those reaching from the water line to the maximum colonisation depth of macrophytes. In transects the depth boundaries of all species and ecological groups (helophytes, floating-leaved, submerged plants) are registered. Every 20 meters a 1 m^2 (or 4m^2 in case of sparse vegetation) sampling quadrat is made where additional parameters (the depth, total coverage, species composition, species coverage, shoot density, Percent Volume Infested [PVI] for submerged macrophytes) are described.

As it follows from the above review, although the transect method is commonly used for macrophyte monitoring purposes in many countries, it can vary substantially in technical details such as the number of transects in the lake, the width of a single transect, the number and width of the intervals at which the survey is carried out etc. The transect/profiles/sections width may be different, varying between 1 m [Keskitalo and Salonen 1994, Baattrup-Pedersen et al. 2001], through 2-5 m [Janauer 2002, Leka et al. 2002, CEN 2003, Dutartre and Bertrin 2008], up to 20-30 m [Schaumburg et al. 2007] or even 100 m [JNCC 2005]. Species inventories can be made in each square metre of the transect [Keskitalo and Salonen 1994, Baattrup-Pedersen et al. 2001], at intervals of several metres and also in the individual depth zones, e.g. every 0,5 m [Baattrup-Pedersen et al. 2001] or at depths of 0-1 m, 1-2 m, 2-4 m, 4-8 m, >8 m [Janauer 2002; CEN 2003] or counting whole littoral as a one zone [Kuoppala et al. 2008] . The number of transects can be fixed but most often depends on the size of the lake, the development of the shoreline and the variability of the landuse round the lake [Jensén 1977, Keskitalo and Salonen 1994, Schaumburg et al. 2004] and transects are distributed so as to cover the full diversity of vegetation patterns.

The transect method allows for the identification of the basic vegetation systems dominating in the phytolittoral, the ranges of the individual vegetation zones and the maximum growing depth of submerged vegetation as well as for long-term observations on changes in vegetation systems. A drawback of the method is the possibility of neglecting certain rarer species which do create autonomous systems (a floristic list can be incomplete). However, the transect method seems to be the most economical in terms of the time and effort needed to ensure the required quality of data; hence, it is also recommended for monitoring surveys.

5. Aerial photos

In certain countries, e.g. Finland or Denmark, the analysis of **aerial photographs** is also recommended to complement the field surveys on aquatic vegetation [Baattrup-Pedersen et al. 2001, Leka et al. 2002, Valta-Hulkkonen et al. 2003, Kanninen et al. 2003, Leka 2005]. Aerial photographs are recognised to be a good source of certain information on aquatic vegetation, e.g. on the distribution and abundance of helophytes and floating-leaved or the depth range of submerged vegetation [Partanen and Hellsten 2005]. However, in the light of the high implementation and application costs of this method as well as the substantial time and effort needed for data interpretation, the scope of the general use of aerial photographs as complementary materials in routine monitoring may turn out to be limited. On the other hand it provides valuable and cost efficient information of historical changes of vegetated area.

6. Measurement of macrophyte abundance

Besides from the taxonomic composition, also abundance is an important aspect of aquatic vegetation which must be considered (under the Water Framework Directive) in macrophyte surveys. The abundance measurement scales applied in survey practice vary significantly. The estimation of the quantitative relationships between individual species (phytocenoses) can be carried out with different accuracy. The simplest scale is the **descriptive** one, such as the five-point Kohler scale [1978] (5 – a very abundant species, 4 – abundant, 3 – common/frequent 2 – rare, 1 – a very rare species) or the DAFOR scale (*Dominant, Abundant, Frequent, Occasional, Rare*) [Palmer et al. 1992, CEN 2003].

A much more exact method involves the estimation of the percentage share of the area occupied by the individual phytocenoses in the survey unit (releve, belt-transect, entire phytolittoral). The percentage share is more often represented on a **point scale,** although percentage scale is easy to transfer into other scale. One of the best known scales used in lake vegetation survey is the seven-point Braun-Blanquet scale [1964]. Alternatively, a more exact 20-point DOMIN scale is used [Rodwell et al. 1995]. More detailed scales tend to be used rather for river ecosystems where the degree of vegetation cover is much lower, including e.g. a 14-point scale at intervals of every 3-10 % [Londo 1974], a five-point scale in the range between <0.1 % and >10 % [Holmes and Whitton 1977], or a nine-point scale between <0.1 % and >75 % in the *Mean Trophic Rank* method [Holmes et al. 1999]. However, particularly in the case of large standing water reservoirs, too detailed estimation of the cover involves a very high error and is very subjective.

The most exact method for measuring abundance involves the estimation of the area occupied by the individual phytocenoses in **absolute units** (m^2 or ha). It seems, however, that since this method requires much time and effort and also entails a large estimation error (as it is very subjective) its scope of application in routine water monitoring is limited

A slightly different approach to the quantitative estimation of vegetation involves the estimation of the volume occupied by plants in the unit space of a water column (the so-called PME - *Plant Mass Estimates*) rather than the extent of surface cover; it is carried out on a five-point subjective scale (from 1 - rare to 5 – very abundant) [Janauer 2002, 2003]. This method works very well in the case of transects (the unit volume is the unit space set out by a transect section and water depth); however, it has a limited scope of application as a method for the quantitative assessment in mapping the whole lake vegetation.

Conclusions

The ultimate choice of a macrophyte survey method is affected by many factors. The manner of plant examination in the field depends on the size of the lake, its shape, depth and water transparency. A shallow, 5-hectare lake the vegetation of which can be mapped in 2-3 hours is examined in a different way than a lake of several hundred hectares, with a well-developed shoreline and large depth. For the latter one, the survey method must be simplified, given the time and effort required. Different equipment is needed for shallow and transparent waters, where submerged plants can be seen in practice with a naked eye or using bathyscope, and in deep and/or turbid waters, where different types of plant catchers (e.g. rakes, grapnels etc.) must be used. Moreover, the manner of examination depends on its purpose. It is different in the case of scientific research (where data collected must be as detailed as possible) and in the case of a monitoring survey.

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II. Common macrophyte sampling procedure for WISER uncertainty exercise purposes and common taxa list

The main aim of the WISER uncertainty exercise is to demonstrate and to estimate the uncertainty in aquatic macrophyte survey methods caused by the spatial variability (vertical and horizontal) of aquatic vegetation. This exercise is therefore not focused on applying the best available methodology for aquatic macrophyte surveys. We are fully aware of the existence of better and more comprehensive methods. We do not investigate variability in macrophyte sampling among different observers. The survey is performed by one and the same person per lake. Based on the overview of survey methods used in European countries it was realised that the transect method is the most widespread and commonly used one. Thus, the WISER approach for lake macrophyte survey is a modification of a transect method, which is recognized as a widely used in several European countries and compliant with CEN standard (although CEN gives only a very rough description leaving much space for the interpretation and details)

According to the WISER sampling scheme for the uncertainty exercise purposes in each lake six localities with three transects each (three replicates) will be surveyed

1. Preparatory phase at office

1.1 Use a map or bathymetric plan to determine the locality for the transects. In each lake six localities are surveyed:

- The position for the first locality is selected randomly - select any place at the shoreline; it does not matter whether you select this place randomly or subjectively.
- Measure the length of the shoreline in meters in a geographic information system (GIS).
- Using a statistical software you randomly select a number between 0 and the maximum shoreline length.
- The selected number is the position (in meters) of the first locality counting in clockwise direction from the place you selected in step 2.1.
- Distribute the remaining five localities evenly along the shoreline.

1.2 You can use also a map grid and number all points where gridline is crossing shoreline – use random function of Excel to select six random localities.

1.3 Make minimum six copies of field protocol (Appendix 1) – one protocol for one locality. Take some additional copies of protocols.

1.4 Copy all additional protocols, plans, maps, papers etc. to be used during the field work.

1.5 Collect all needed equipments (see list below).

General equipments for field survey

- High resolution maps of survey area, preferably waterproof
- Plastic bags for collection of samples, small hard plastic containers for fragile species (*Chara* sp. etc.) with additional waterproof labels
- Newspapers to dry plant samples, some paper sheets (A4), plant press
- Pencils or pens, with indelible ink
- Field protocol in sufficient number of copies with field pad (Appendix 1)
- Floras, relevant field keys and identification guides and preliminary check list of macrophyte species
- Personal protective clothing including boots or sandals for wading survey
- First aid kit
- Notebook, preferable with hard back and water repellent paper to make notes.

Equipment for boat surveys

- Boat suitable for local conditions, with appropriate safety equipment
- Ropes and anchors for staying at right place
- Accurate bathymetric data are preferable in delineating littoral zones, bed slopes etc.
- GPS (Global Positioning System), preferably with differential correction
- Hand-held or fixed echo sounders
- Any device appropriate for surveying macrophytes of a given types, e.g. rakes with extendable rod, multipoint grapnel or double-sided Luther-rake with soft rope marked by depth readings
- Underwater viewing tube aid (bathyscope, aquascope, i.e. bucket or box with clear Perspex base)

- Floating rope and/or measuring tape
- Other distance measuring unit (laser style)
- Sticks for transect marking

Additional equipment (useful but not necessarily required)

- Polarising sunglasses
- Digital waterproof camera
- Underwater ‘drop’ camera, waterproof camera mounted to a cable, that can lowered into the water
- Wipes
- Hand lens, ×10 and ×20 magnification
- Conductivity meter
- pH meter
- Secchi disc

2. Preparatory phase in field

The position of the first locality is also the position of the first of three transects per locality. The other two transects are located to the left and right, respectively, of the first transect. The minimum inter-distance between two transects per locality is 5 m.

2.1 Mark the beginning of the selected transect by stick and take the GPS-position. Mark also direction of the transect in the map.

2.2 It is recommended to take photos of the site in order to give a general overview of the site. Take first photo to the direction of open water. Second photo should be taken from the open water to the direction of shore.

2.3 Fill heading of the first field sheet and make a draft of the situation. Use the protocol given in Appendix 1.

3. Vegetation survey within a transect

3.1 A transect is a belt perpendicular to the shoreline, of the length from the shoreline to the maximum depth of plant growth and of the width of approximately double boat width.

3.2 Starting point of a transect should be situated in the beginning of supralittoral vegetation (wetland) or at the shoreline when easily recognizable.

3.3 In case of very dense rush belt, use waders and survey rush by wading as far as possible. Survey rush stands (even theoretically making estimation) using the same pattern of sampling as in open water area (see below).

3.4 Individual belts are rowed or waded when necessary.

3.5 Divide a transect into 1 m depth zones (= of the interval of 1 m depth). In each depth zone five sampling sites, evenly distributed within the zone length should be determined (according to the pattern as presented in Fig. 1). If the deepest part of the phytolittoral does not cover the whole depth zone, the number of sampling sites should be determined proportionally to the vegetated part of the zone.

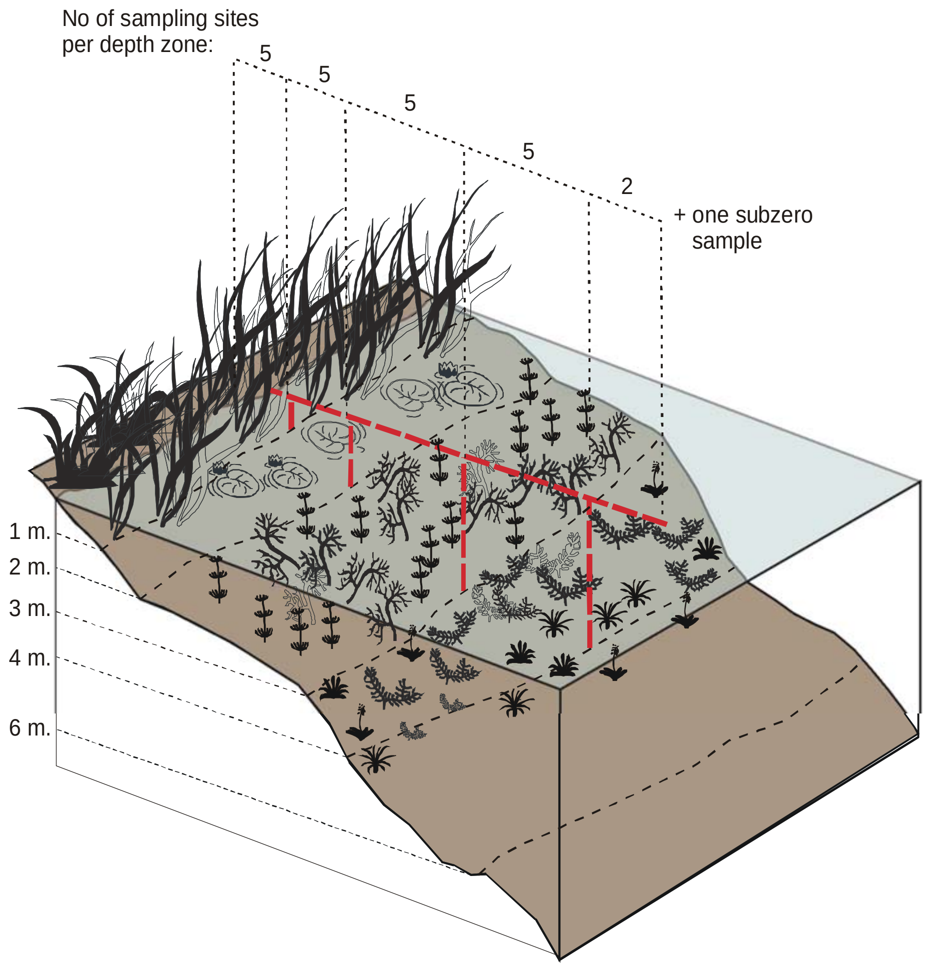

Fig. 1. The scheme of phytolittoral division into depth zones and sampling sites location in belt-transect method used for WISER uncertainty exercise

3.6 To avoid drifting by wind and waves it is recommended to use a long rope with the first end is bind to shore (tree or stone) and second end to heavy anchor. Boat is moved along rope, which keep it at right depth zone. In the case of very calm weather or very gentle slop it not necessary to use rope.

3.7 To assure that the maximum depth of plant growth is defined properly, at the end of the transect the subzero zone should be examined, i.e. a subzero sample is taken.

3.8 Going along the transect, at each sampling site within a depth zone take <u>two macrophyte samples,</u> i.e. one sample from each side of the boat (Fig. 2). When water transparency is sufficient enough use a bathyscope. In case of turbid water and in deeper zones use any kind of macrophyte sampler appropriate for a vegetation of the lake type, e.g. rake or grapnel for dense *Chara* or *Ceratophyllum* stands, dense rake for isoetids. Note that you should not mix different sampling methods at same site!!!

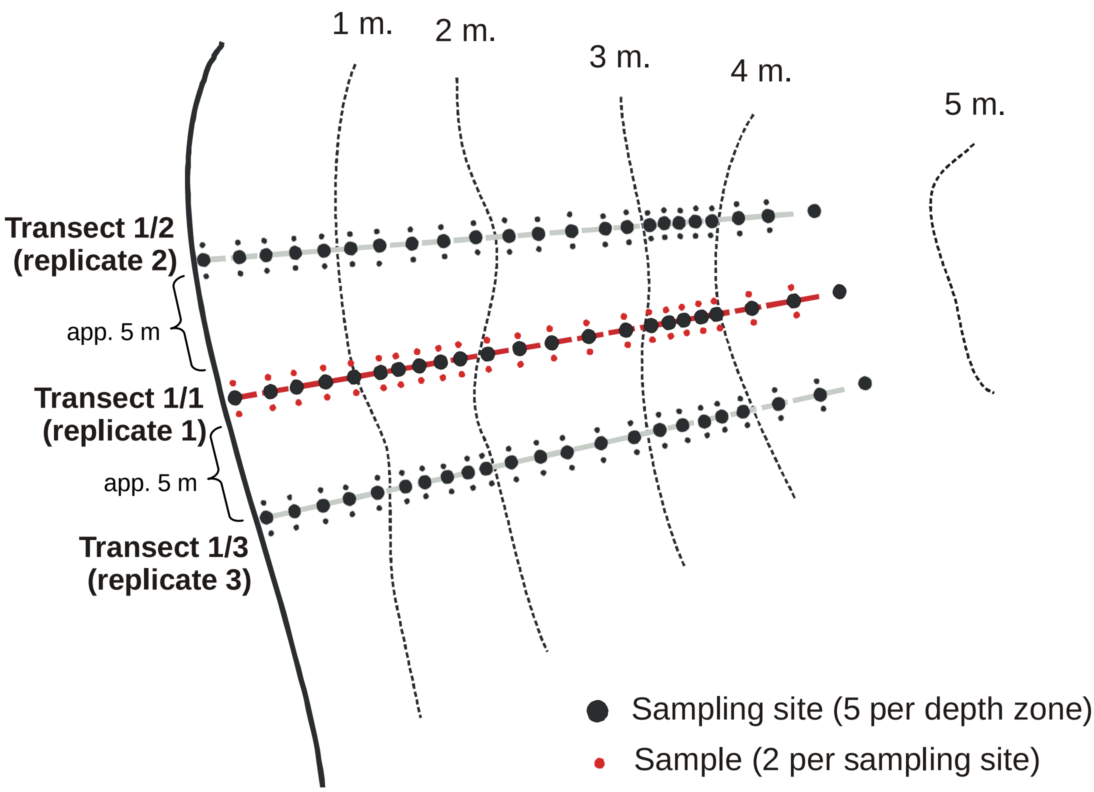

Fig.2. Sampling pattern within a sampling location (transect in three replicates)

3.9 To help comparability of samples with rake approach, use standardized length of each "throw". Depending of your capability train first and use same length – this means relatively "short" sample at deep water. One sample is one look through a bathyscope or one amount of rake/grapnel.

3.10 Drifting plants are only recorded if they can be assigned to a certain sample.

3.11 Record the water-depth for each sample.

3.12 Based on two samples within a sampling site all species are identified and their abundance is estimated in percentage continuous scale (easy to be recalculated to various point scales).

3.13 Overall abundance of the macrophyte cover within a sampling site should be also determined.

Species identification and common taxa list

- During the survey all vascular plants including sedge rush, rush, floating leaved and submerged species, and also Characeans should be identify
- The identification level should be the lowest possible, preferably species level
- In case of mosses and structural algae only overall abundance is estimated (not identified to the genus/species level; only very few exceptions, eg. *Fontinalis* sp.).
- All difficult species for further investigation should be stored in plastic bags or containers; bigger plants can be dried with newspaper; small thin leaved or fragile plants can be preserved in alcohol.
- For the WISER uncertainty exercise common taxa list with the unique coding was established. All the species identified should be coded according to the common taxa list (Appendix 2 – deliver as an extra file)

4. Additional surveys within a transect

In order to search macrophyte-environment relationships, during the macrophyte survey many other parameters describing environmental conditions should be determined, e.g. bottom substrate, dominating land use , pressures

- For WISER uncertainty exercise it was decided to use Lake Habitat Survey procedure. Thus, LHS results should be available for all lakes included in the common sampling site list.
- Because the complete LHS procedure is performed within 10 hab-plots established randomly around the lake shore (not necessarily close to the macrophyte survey sites), for all macrophyte locations (transects) it is recommended to fulfill the rapid LHS protocol (Attachment 1, page 4/4) to collect environmental data on the macrophyte transect level

Appendix 1. Field protocols

FIELD PROTOCOL FOR SURVEYING AQUATIC MACROPHYTE				Page 1/4
GENERAL INFORMATION				
Lake name:	**Lake type:**	**Lake code:**	**Surveyor:**	**Date:**

Lake information			
Surface area (km^2)		Lake altitude (m asl)	
Maximum depth (m)		Mean alkalinity (meq)	
Mean depth (m)		Water colour (mg/Pt/L)	
Lake perimeter incl. islands (km)		Dominating geology in catchment (S=siliceous, C=calcareous, O=organic)	
Retention time		Dominating land use in cathcment (F=forest, A=agricultural, U=urban, O=other)	

P of locality		
Locality number:	**Starting time:**	**Ending time:**
Start-point (GPS)		
End-point (GPS)		
Locality direction (degrees)		

Littoral habitat *(width 25 m both side in the beginning of transect, 15 m from shoreline)*			
Frequency 1=rare (<10%) 2=moderate (10-40%) 3=abundant (40-75%) 4=very abundant (>75%)			
Dominating types	Frequency	**Dominating types**	Frequency
Steepness of shore bank	mild > 30° ☐ average 30-60° ☐ very steep or eroding ☐		

FIELD PROTOCOL FOR SURVEYING AQUATIC MACROPHYTE	Page 1/4 continued
Freehand drawing of transect	
Additional notes (water level, another pictures, non-standard way of working, other circumstances affecting work)	

FIELD PROTOCOL FOR SURVEYING AQUATIC MACROPHYTE MACROPHYTE SURVEY		Page 2/4		
Transect no:		**Transect .../1**	**Transect …/2**	**Transect …/3**
Vegetation spatial structure within a transect				
GROWTH FORM TYPES: he = helophytes, ny = nymphaeids, el = elodeids, le = lemnids , ce = ceratophyllids , is = isoetids, ch = charids				
Growth form dominating				
Start- point depth (m)				
End-point depth (m)				
Maximum depth of plant growth (m)				
Max. observed depth of rush (m)				
Max. observed depth of elodeids (m)				
Max. observed depth of charids (m)				
Max. observed depth of isoetids (m)				
Bottom quality: *total cover of whole zone as percentage*				
Rock (>4000 mm)				
Boulders (250-4000 mm)				
Stone (16-250 mm)				
Gravel (2-16 mm)				
Sand (0.06-2 mm,)				
Silt (smooth between fingers)				
Clay (elastic, grey)				
Mud				
Peat				
Detritus (tree leaves, trash, etc.)				
Other (specify)				

Species composition on a belt transect (abundance/cover; 0.5,1 ,3 ,5 ,7 ,10 ,15, 20, 30…100%)

Depth zone	Species list: Sampling sites within the depth zone:	**Transect .../1**					**Transect …/2**					**Transect …/3**				
		1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
0-1 m																

Species composition on a belt transect (abundance/cover; 0.5,1 ,3 ,5 ,7 ,10 ,15, 20, 30…100%)

Depth zone	Species list: Sampling sites within the depth zone:	**Transect .../1**					**Transect …/2**					**Transect …/3**				
		1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
0-1 m continued																
1-2 m																

FIELD PROTOCOL FOR SURVEYING AQUATIC MACROPHYTE MACROPHYTE SURVEY																Page 3/4
Depth zone	Species list:	**Transcect .../1**					**Transect …/2**					**Transect …/3**				
	Sampling sites within the depth zone:	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
2-3 m																
3-4 mm																
4-5 m																

Depth zone	Species list:	**Transect .../1**					**Transect …/2**					**Transect …/3**				
	Sampling sites within the depth zone:	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
4-5 m continued																
5-6 m																
6-7 m																

FIELD PROTOCOL FOR SURVEYING AQUATIC MACROPHYTE				Page 4/4
LAKE HABITAT SURVEY (rapid protocol)				
Transect no:		**T.../1**	**T…/2**	**T…/3**
Water quality				
Water clarity (clear, turbid, peaty)				
Secchi depth (cm) (if possible)				
Surface algal blooms (+/-)				
Riparian attributes (15 m inland of banktop) (0=<1%, 1=1-10%, 2=10-40%, 3=40-75%, 4=>75%)				
Canopy cover > 5 m, 0 - 4				
Understorey cover 0,5 - 5 m (shrubs and tall herbs) 0 – 4				
Ground cover <0.5 m (herbs / grasses etc) 0 – 4				
Dominant land cover (F=forest, G=-grassland, A=agricultural, U=urban, O=other)				
Shore zone (banktop to water edge)				
Distance HWM to water edge (lateral m)				
Raised or lowered water level/visible trash line? (height in m)				
Shore vegetation cover 0 – 4				
Shore vegetation structure (CL = canopy layer, US = understorey, GC = Ground cover, MI = mixed)				
Evidence of erosion (+/-)				
Human pressures (+/-)				
Commercial activities				
Residential developments				
Roads or railways				
Parks and gardens				
Docks, marinas, jetties or boats				
Walls, dykes or revetments				
Litter, dump or landfill				
Quarrying or mining				
Pasture (ring if observed grazing)				
Coniferous plantation (ring if logging)				
Tilled land				
Pipes, outfalls				
Riparian / Aquatic macrophyte cutting				
Other (specify)				

Visible pressures – whole lake level (not restricted to sample sectors) +/-

Notable invasive species		Shore modifications (hard/soft)	
Water body type (natural / res. etc)		Upstream activities/impoundments	
Fish farming		Hydro-power	
Boat angling		Shore angling	
Water sports		Riparian weed control	
Road drainage		Drainage from nearby buildings	
Litter problems		Coniferous plantations/logging	
Impacting agricultural activities (poaching)		Other (provide details):	

Shoreline characteristics - estimate extent (0= <1%, 1=1-10%, 2=10-40%, 3=40-75%, 4=>75%)

Wetland habitats		**Pressures (list as above)**	
Fringing reeds			
Wet woodland			
Alder Carr			
Bogs			
Quaking banks			
Other (e.g. fen, marsh)			

Other natural habitats		**Notes**
Broadleaf/mixed woodland/plantation		
Shcrub and shrubs		
Moorland / heath		
Open water		
Rough grassland		
Tall hear / rank vegetation		
Rock / scree / dunes		
Other (specify):		
NV Not visible, BL Broadleaf wlnd., CW Conifer wlnd., SH Scrub / shrubs, WL Wetland. MH Moorland / heath, OW Open water, RP Rough pasture, IG Improved grazing, TH Tall herb, RD Rock / scree, TL Tilled land, PG park / gardens SU Urban		