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Report on the most suitable lake macrophyte based assessment methods for impacts of eutrophication and water level fluctuations

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Extended summary

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The report summarizes the progress made within the WISER project in the following areas:

- Developing and improving methodologies for assessment of eutrophication impacts in lakes based on species composition, abundance and community structure of aquatic macrophytes. For this purpose a set of metrics was elaborated, their sensitivity as indicators was tested within countries, geographic regions, and lake types, and the potential of the metrics for using them as common metrics for intercalibration was assessed.
- Elaborating of palaeoecological approaches based on plant macrofossil records in lake sediments to define reference conditions and to assess ecological status of shallow lowland lakes for which modern reference sites are almost missing.
- Developing relevant metrics to assess the response of lake macrophytes to water level fluctuations, and quantify the uncertainty in their use.

Chapter 1 gives an overview of the data sources, database structure and contents, and describes the common taxa list. Much of the data in WISER was collected as part of the REBECCA project, but supplemented with more data provided by both WISER partners, and participants in the WFD Intercalibration Exercise. The WISER common database in Microsoft Access[®] consists of a series of hierarchical linked tables containing the bulk of the data, and accessory tables containing descriptions of fields in the main tables. Selecting, aggregating, and compiling of data in WISER database is achieved through Microsoft Access[®] forms and queries. A common list of 1289 plant taxa has been compiled in order to standardise naming and coding of taxa. The list has been made available on the internet at: http://www.freshwaterecology.info/TaxaDB_mphSearch.php. It is also maintained and published on the internet at: <http://www.aqplants.ceh.ac.uk/> where it is available to anyone for download

Chapter 2 explores, based on data from the UK, the potential of using palaeolimnological techniques for defining reference condition for shallow lowland lakes for which modern reference sites are scarce or missing in most parts of Europe.

A total of 74 sediment cores from 61 lakes throughout the UK was collated for the analysis. The taxonomic nomenclature of different sub-fossil species and types (so-called “pseudo-species”) was harmonized and a scoring system developed for their quantification. Selected pseudo-species data were plotted using the stratigraphic software C2 and subject to principal components analysis (PCA) to assess patterns of change within a core. Detrended canonical correspondence analysis (DCCA) was used to assess the overall variation in pseudo-species data from all the dated cores and to quantify the shifts in pseudo-species through time as

beta-diversity, which measures the dissimilarity of species between sub-samples. A clear trend in beta-diversity could be seen along the cores, with higher values tending towards the more recent layers. To help interpret the aquatic plant sub-fossil data, the mean beta-diversity of the pre-1850 sites (1.44 SD) was used as our estimate of reference condition. The growing trend in beta-diversity moving away from this value can be interpreted as an overall increase in species turnover since 1850 due to increased pressures on the freshwater environment.

It is impossible to make any direct relationship between fossil numbers and species abundance. There were however, distinct patterns seen in the palaeolimnological record of many sites where the relative abundance of certain fossil remains increased or decreased through time. Many of the sites showed clear trends from species rich mixed plant communities in the past, to fewer and more typically 'eutrophic' species towards the present.

In conclusion, aquatic plant macrofossils provide an insight not only into the past species assemblages that might have populated 'reference' communities in lakes, but also it can be demonstrated that these assemblages have in many cases shifted away from the relatively stable conditions of pre-1850. Beta-diversity of sub-fossil plant remains can be used to assess the direction and magnitude of change away from pre-defined reference conditions and therefore this palaeo-limnological tool represents a valuable asset for both defining reference conditions and for setting restoration targets.

Chapter 3 explores macrophyte metrics for assessment of eutrophication impacts in lakes. The main goal of the work was to elaborate metrics responding strongly to eutrophication and being applicable in different countries that would allow using them further for intercalibration of existing national methods. For testing the response of macrophyte metrics in a pressure gradient, the mean seasonal concentration of total phosphorus (TP) was used as a pressure proxy. The values of determination coefficient $R^2 \geq 0.30$ and Pearson's correlation coefficient $R \geq 0.55$ (linear relationships), and Spearman correlation coefficient $R_{sp} > 0.60$ (non-linear relationships) were assumed as criteria for good performance of a metric. Since the species abundance measurements in different countries were extremely diverse and incomparable, all metrics were tested using the presence/absence data only.

Three groups of **metrics on taxonomic composition** were tested: i) indices based on trophic scores, ii) indices based on species richness, and iii) indices based on proportion of functional groups. The results showed that

- The ICM_LM was proved as a good metric giving strong ($R^2=0.52$, $p=0.000$) and almost linear overall relationship with the pressure variable (logTP) over all lakes analysed.
- The metric values had significant differences between lake types being on average <5 in Nordic lakes and >5 in the Central-Baltic lake types.
- The strength of the ICM_LM:TP relationship differed by countries whereas the metric performed best in Nordic and Atlantic lakes and slightly worse in Central-Baltic lakes. No significant relationship between ICM_LM and TP was found for the lakes in the Eastern-Continental GIG.

The pressure-response curve (TP vs. ICM_LM) exhibited systematic differences by alkalinity (Alk) indicating at equal TP levels worse status in lakes with higher alkalinity. This may have implications for the applicability of the metric for lake types with broad alkalinity ranges (e.g. in Central-Baltic GIG). The ICM_LM:TP relationship was stronger (R^2 close to 0.4) in lakes with moderate and high Alk, and weaker in less buffered lakes ($R^2=0.26$).

Due to good performance of the ICM_LM in Nordic and Atlantic lakes, it was suggested there as a common metric for intercalibration.

The Ellenberg Index (EI) based on the Ellenberg indicator scores for nitrogen (N-score) was calculated for all lakes in the database both using total number of taxa (EI_TT) and only submerged taxa (EI_ST). The results indicated that including helophytes improved the strength of the general relationship with TP. Therefore, only the EI_TT was explored further. The missing N-scores for 37 important aquatic taxa were calculated from the regression with LTR ($R^2=0.64$). Although the Ellenberg N-score was related purely to nitrogen and LTR-score to general nutrient requirements, the two indices calculated on their basis (EI and ICM_LM) were strongly related ($R^2=0.85$). The EI_TT had a similarly high predictive power for TP ($R^2=0.47$) as the ICM_LM showing it as a well performing metric. Similarly to ICM, the best performance of EI_TT in detecting lake eutrophication was found in NO, UK and IE. In most of the remaining countries the diagnostic value of the index was considerably lower or the relationship non-significant.

The TP vs. EI_TT relationship was slightly modified by lake depth and alkalinity classes. Within the three alkalinity types the correlations were significantly weaker than within the depth types. Strongest relationships were found among shallow and deep lakes ($R^2=0.52$; 0.46) and slightly weaker in very shallow lakes. Ellenberg index did not perform well in any of the common lake types.

Among variables characterizing **species richness**, the total number of taxa (N_TT) and the number of submerged taxa (N_ST) had a unimodal distribution relative to TP whereas in different countries the correlations with TP were positive, negative or non-significant. In eutrophic ecosystems the increase of the number of helophyte taxa along with increasing TP levels compensates the decrease the number of submerged taxa. This diminishes the metric diagnostic value. Due to the poor metric response, the use of species richness for assessing eutrophication process is very limited. To some extent the total number of taxa may be used in some Nordic countries where the trophic state of lakes is generally lower, and the number of submerged taxa in some Central-Baltic countries or lake types. However, the potential use of these metrics for IC purposes is doubtful.

Since the overall relationships of TP with the number of characeans (N_char) and number of isoetids (N_iso) over all lakes were but very weak (although statistically significant), these metrics cannot be considered as promising for the IC purposes as well.

Chapter 4 describes the abundance of submerged macrophytes as potentially good candidate metric for describing the eutrophication pressure. Many lakes have experienced a decrease in the

abundance of macrophytes because increased nutrient availability has increased phytoplankton in lakes, which led to increased turbidity and impoverished light conditions. Changes in the abundance of submerged macrophytes may be expressed both by coverage and maximum colonization depth (C_max). The analysis in this report focuses mainly on C_max, which has been widely used in the monitoring of lakes and therefore provides a good opportunity for data analysis. The absence of a commonly agreed definition of C_max, however, adds to the uncertainty of the results.

Among single factors, Secchi depth explained the largest part of C_max variability ($R^2 = 0.43-0.58$) compared to both TN and TP ($R^2 = 0.0-0.22$). In the multiple regression, Secchi depth and water colour explained 49-55% of the C_max variability. Humic substances reduce light penetration similarly to chlorophyll, which increase is caused by eutrophication. Ecological quality ratios can be developed also for humic lakes by setting the average value of maximum growing depth as reference and dividing other status classes evenly. The euphotic zone describes more the production of phytoplankton whereas penetration of red light describes better C_max of aquatic macrophytes. C_max may preferably be used in deep lakes with maximum depth above 5-6 m, as in shallow lakes C_max often corresponds to max depth. Latitude affects C_max and earlier studies with charophytes have estimated an average decrease in C_max of 0.12 m per degree increase in latitude. Preliminary results from the WISER project show that the correlation between Secchi depth and C_max seems to vary with latitude, especially for *Nitella* species, and for isoetids. Analyses of 18 Danish lakes have shown that the year-to-year variability of C_max can be quite considerable.

Coverage of macrophytes represents mean macrophyte per-cent coverage relative to the whole lake area. Quantitative data on coverage are scarcer and have been analysed in less detail and mostly in Danish lakes. Mean macrophyte coverage in shallow lakes (mean depth <3 m) shows a clear decrease with increasing nutrient and chlorophyll a concentrations. Chlorophyll a concentration of 30 ug/l, TP of 50 $\mu\text{g l}^{-1}$, and TN of 1.5 mg l^{-1} are the thresholds above which the coverage of macrophytes is usually low. As for C_max there is a considerable year-to-year variation in the coverage of submerged macrophytes in individual lakes.

Chapter 5 gives a literature overview of potential metrics to assess the **response of lake macrophytes to water level (WL) fluctuations** for which there is a general growing demand.

Biodiversity indices are not very promising indicators for hydromorphological pressures in heavily regulated lakes and rivers, because most of the species are also present in modified parts as long as regulations are not too extreme. Several studies have shown that the diversity declines with increasing range of WL fluctuations. Extensive literature survey of Scandinavian lakes showed that general biodiversity correlated mainly with draw-down of water level, but regulation amplitude between 1 and 3 meters supported highest biological diversity. In lakes with natural WL fluctuations, regulating that decreases the amplitude may decrease biodiversity as a slight disturbance could create suitable habitats for aquatic macrophytes. A sudden increase of water level will initiate erosion processes, which lower biodiversity. Taxonomic composition

is a poor indicator of water level increase, because most of the species are still present after water level increase, although abundance may differ significantly. Lowering of water level will lead to increased diversity, as newly exposed littoral zone or general shallowness allows the sublittoral zone to cover the entire water body.

Several studies indicate that **abundance is a much more sensitive indicator** for hydrological change than species composition. The effect of water level fluctuation on zonation patterns depends on the bathymetry of a lake and thus is a lake-specific reaction. In addition to the range of water level fluctuation, the dynamics of the fluctuation affects significantly the abundance of macrophytes. Still there are few classification schemes related to relationships between seasonally distributed hydro-morphological factors and macrophytes. In the Netherlands the abundance of nymphaeaides has been related to fetch, waterlevel fluctuation, soiltype, depth distribution and lake age. Lowering of the water level while a lake is ice-covered will have significant effects, especially on large sized isoetids such as *Isoetes lacustris* and *Lobelia dortmanna*. The direct response of *Isoetes lacustris* to ice penetration enables its distribution to be used for classification purposes. The distribution of other large isoetids such as *Isoetes echinospora*, *Lobelia dortmanna* and *Littorella uniflora* can also be used for classification purposes, because they are all relatively weak against ice erosion and changes in sediment structure.

The chapter introduces a **new water level index for Nordic lakes (Wlc)** elaborated on macrophyte data from 79 mainly low alkalinity, oligotrophic lakes from Finland, Norway and Sweden. Of these, 37 were storage lakes (H3), 20 other regulated lakes (H2) and 22 natural or semi-natural lakes (N2, sN2). Water level fluctuation varied between 0.1 and 6.8 m. The main difference between natural lakes and storage lakes is in the WL regime in winter. In natural lakes the WL is generally low in winter whereas in storage lakes the level is kept high or passes a drawdown phase. Winter drawdown was calculated as the average difference between highest water level in October-December and lowest level during the following April-May as an indicator of water level regulation amplitude.

The Wlc was based on dividing macrophyte species into sensitive and tolerant species regarding water level fluctuations. All countries include species composition, frequency and abundance of aquatic macrophytes. Helophytes were excluded from the analysis.

Sensitive species were defined as species which prefer reference lakes or only appear in those, and which frequency and abundance decrease (or the species disappear) with increasing WL fluctuations.

Tolerant species were defined as species which occur with increased frequency and abundance at increased WL fluctuations and are often less frequent in reference lakes. Identification of sensitive and tolerant taxa was based on the species occurrence along the winter drawdown gradient. Based on this method, the most sensitive species were defined as those with the 75th percentile ≤ 1.6 m (winter drawdown), while the most tolerant species as those with the 75th percentile > 2.6 m (winter drawdown). The species in “middle group” were considered less

sensitive than the sensitive ones. Within the analysed data 46% of the aquatic macrophytes were characterised as sensitive, 25% as tolerant, and 29% as less sensitive.

W_{Ic} was calculated as the percentage of sensitive minus tolerant species in the total number of aquatic macrophytes. In case there are more tolerant than sensitive species, the index value becomes negative. W_{Ic} correlated very well with winter drawdown in the storage reservoirs for all countries with respective R² values of 0.77, 0.67, and 0.73 for Finnish, Norwegian and Swedish lakes. The slope of the regression for the Swedish lakes was very different from that for the Finnish and Norwegian lakes. Until this dissimilarity is further investigated, the index and suggested boundaries will only be applicable for Finland and Norway.

The reference value and the high/good boundary were set based on natural and semi-natural lakes. The good/moderate boundary was set at W_{Ic} value of -20, which corresponds to a winter drawdown of 3.4-3.5 m at which stands of *Isoetes lacustris* usually disappear. In addition the boundary setting is related to clarity of the water. Clear water lakes provides wider ecological niche for large isoetids although dense stands are disappearing.

For lakes in countries from the **Central Baltic region**, there is a huge gap in the availability of data of the water-level regime. For the development of a metric of water-level fluctuations in this region, only data were available for floodplain lakes and main channel of the River Rhine and Meuse in The Netherlands. Because these data are restricted to a small geographic area with only shallow, eutrophic lakes, the applicability to other areas and lake types in the Central-Baltic is unknown.

In the 100 floodplain lakes sampled, water-level fluctuations during the growing season ranged from 0.10 – 2.30 meter. Macrophyte species richness peaked at water-level fluctuations of 0.4 – 0.6 meter, and was significantly lower at fluctuations of < 0.2 meter and > 1 meter. Because regulation of the water level regime may result in either stabilization or increased fluctuations in lakes, two separate indices have been developed: one for the impact of stabilization of water-levels (< 0.2 meter), and one for the impact of large water-level fluctuations (> 1 meter). Similar to the approach used for the water level index for Nordic lakes (W_{Ic}), macrophyte species were classified as sensitive or tolerant regarding water fluctuations of respectively < 0.2 meter and > 1 meter. Subsequently, the metrics have been calculated separately for ‘presence’ and ‘abundance’ data of macrophyte species.

W_{Ic} for water-level stabilization (fluctuations < 0.2 meter) correlated significantly with the proportion of summer drawdown in the floodplain lakes (Spearman R = -0.55 (abundance-based metric) and -0.45 (presence-based metric); both with p < 0.01). However, there is still a huge scatter around the regression line, especially for lakes with no drawdown.

W_{Ic} for increased water-level fluctuations (> 1 meter) correlated significantly with the amplitude of water-level fluctuations in the lakes (Spearman R = 0.31 for both ‘presence’ and ‘abundance’-based metric; both with p < 0.01) but also for these metrics there is a huge scatter around the regression lines.

Introduction

The Annex V of the Water Framework Directive requires that assessment of the ecological status of lakes based on aquatic vegetation should include taxonomic composition and abundance of macrophytes.

The main objectives of our study presented in the report were to validate and supplement macrophyte metrics based on species composition, abundance and community structure for assessment of impacts of eutrophication, and to determine and evaluate their sensitivity and usefulness as indicators. Our aim was also to develop relevant metrics to assess response of lake macrophytes to water level fluctuations. Moreover, the use of palaeoecological approaches (plant macrofossil records) to define reference conditions and to assess ecological status for selected lake types was addressed.

As most of the Member States have developed and intercalibrated their assessment systems or are in the process of intercalibration, suggestions about the most suitable assessment systems could be valuable for those MS who have not developed their assessment systems yet, but also for those who would like to improve them. The developed common metrics for eutrophication and hydromorphological pressures give an opportunity to evaluate the current assessment systems in the course of intercalibration (metrics included, assessment concept etc.).

When searching the best responding macrophyte metrics for eutrophication and hydromorphological alterations the procedure recommended by Hering et al. 2010 in Guideline for indicator development (Deliverable 2.2-2) was applied. The issue of uncertainty in macrophyte metrics was also addressed and the results are included in complementary report by Penning et al. 2011 (Deliverable 3.2-2; *in prep.*).

1. WISER macrophyte database

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1.1 Background

At the outset of the WISER project, it was decided that all data used in the project should be converted and stored in a standardised format, so that all WISER data would be interoperable. The format chosen was that of a Microsoft Access[®] relational database. Although, at the time of writing, WISER data exists in multiple databases, the intention is that all data will be included into a single database of the same design. This chapter describes the common structure of WISER databases, the extent of the data used by workpackage 3.2, the common taxa list used by the workpackage, methods used for converting data from other formats, and methods used for extracting and compiling data.

1.2 Database structure

The WISER common database structure consists of a series of hierarchical linked tables containing the bulk of the data, and accessory tables containing descriptions of fields in the main tables. The main data tables and a short description of each are listed in Table 1.1. More detail of the structure is shown in Figure 1.4.

Table 1.1 Main data tables in WISER common data structure and number of records in these tables in the workpackage 3.2 data.

Table name	Description	Records
t_Waterbody	Waterbodies, as per WFD definitions	2408
t_Station	Points within waterbodies where sampling has occurred	2592
t_SampleBio	Samples taken for biological analysis	9066
t_SampleEnv	Samples taken for measurement of physical and chemical parameters	11922
t_ValueBio	Values of measured biological parameters	43673
t_ValueEnv	Values of measured environmental (physical and chemical) parameters	103508

Except for t_Waterbody, each of the main tables is linked to one of the others in a hierarchical structure. Each value in t_ValueEnv or t_ValueBio is linked to a sample in t_SampleEnv or t_SampleBio, each sample in the samples tables is linked to a station, and each station in t_Station is linked to a waterbody in t_Waterbody.

1.3 Database content

Data has been collected from various sources and is ongoing. Much of the data was collected as part of the REBECCA project (Moe et al. 2008), but this has been supplemented with more data provided by both WISER partners, and participants in the WFD Intercalibration Exercise. Tables 1.2 and 2.3 show a distribution of data held by Workpackage 3.2, grouped by country,

and Figure 1.1 shows a map of Europe which shows the locations of waterbodies for which biological data are held.

Table 1.2 Countries for which data is held by WISER Workpackage 3.2, the number of lakes for which data is held, and separation of the lakes into lake types.

Country	Lakes	A1	A2	A3	CB1	CB2	CB3	N1	N2	N2a	N2b	N3	N5	N6	N8	U
Belgium	8				4	4										
Germany	3															3
Denmark	2															2
Estonia	48				17	14	15									2
Finland	655							27		52		149	13	31	90	293
France	5					3										2
Ireland	136	2	11	6				1	7							109
Italy	4															4
Lithuania	9				6	3										
Latvia	296				124	101	70									1
Netherlands	57				18	39										
Norway	228							21		13	9	4	1	1	2	177
Poland	183				58	48										77
Romania	17					17										
Sweden	270															270
United Kingdom	103	2	3		17	39	8	1		4	2	1				26
Total	2024	4	14	6	244	268	93	50	7	69	11	154	14	32	92	966

Table 1.3. Total number of taxa recorded from each country in the WISER WP3.2 database, the total number of taxa records held, and separation of taxa into different growth forms, where these are known.

Country	Taxa	Records	Terrestrial	Helophytes	Charophytes	Isoetids
Belgium	43	88	2	5	6	1
Germany	24	717	0	8	3	0
Denmark	23	428	2	6	2	0
Estonia	115	2112	25	34	13	5
Finland	153	14642	29	36	6	14
France	17	483	2	2	1	3
Ireland	51	888	2	3	2	7
Italy	18	1019	1	4	3	0
Lithuania	53	282	2	8	9	1
Latvia	72	2702	4	10	11	7
Netherlands	76	707	7	12	15	0
Norway	102	3298	10	17	12	15
Poland	131	4322	36	42	18	2
Romania	36	219	0	1	5	0
Sweden	154	7144	27	40	16	15
United Kingdom	197	2446	34	59	17	18

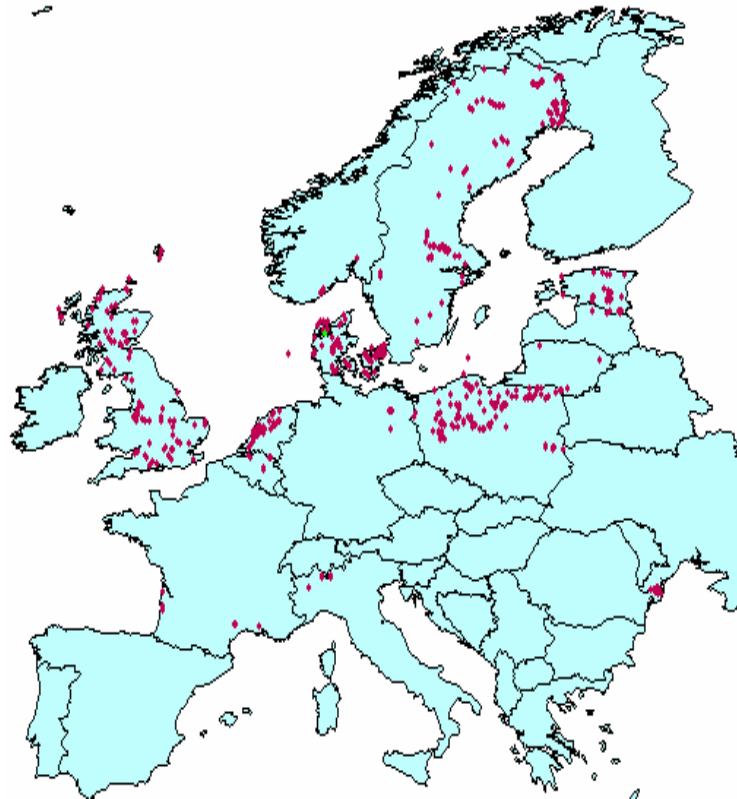


Fig. 1.1 Map of Europe showing locations of sampling stations with biological data used by WISER Workpackage 3.2. Note that some sites are not shown on the map because coordinates were not supplied by the data providers (for example, there are 655 lakes in Finland).

1.4 Common taxa list

A common list of plant taxa has been compiled, in order to standardise naming and coding of taxa. The list is maintained as a separate relational database, stored on a GNU-Linux (Fedora Core) server using mysql server software. The design of the database is hierarchical, and reflects Linnaean taxonomy, so there are tables for Kingdoms, Divisions, Classes, Orders, Families and Genera, each of which is linked to its parent. Data for each defined taxon is stored in a 'Taxa' table, which is linked to the Genera table, but may be defined at any taxonomic level. The database allows for multiple coding systems, so that a single taxon name may be referred to by an unlimited number of codes, or none. This allows for easy translation between different coding systems. At the time of writing, this list included 1315 unique names. Of these, 71 were synonyms.

The content of the list has been gathered by many collaborators inside and outside of the WISER project. Notable among these are the REBECCA project and CEMAGREF. Although initiated as part of the WISER project, maintenance of the list by the NERC project partner is considered to be an ongoing project, which will continue beyond the life of WISER.

The list has been made available on the internet at: http://www.freshwaterecology.info/TaxaDB_mphSearch.php. It is also published and maintained on the internet at: <http://www.aqplants.ceh.ac.uk/> where it is available to anyone for

download, and where registered users may add or modify records, either singly using online forms, or in bulk, by uploading a list of additions and/or changes. A screenshot of the list is shown in Figure 1.2.

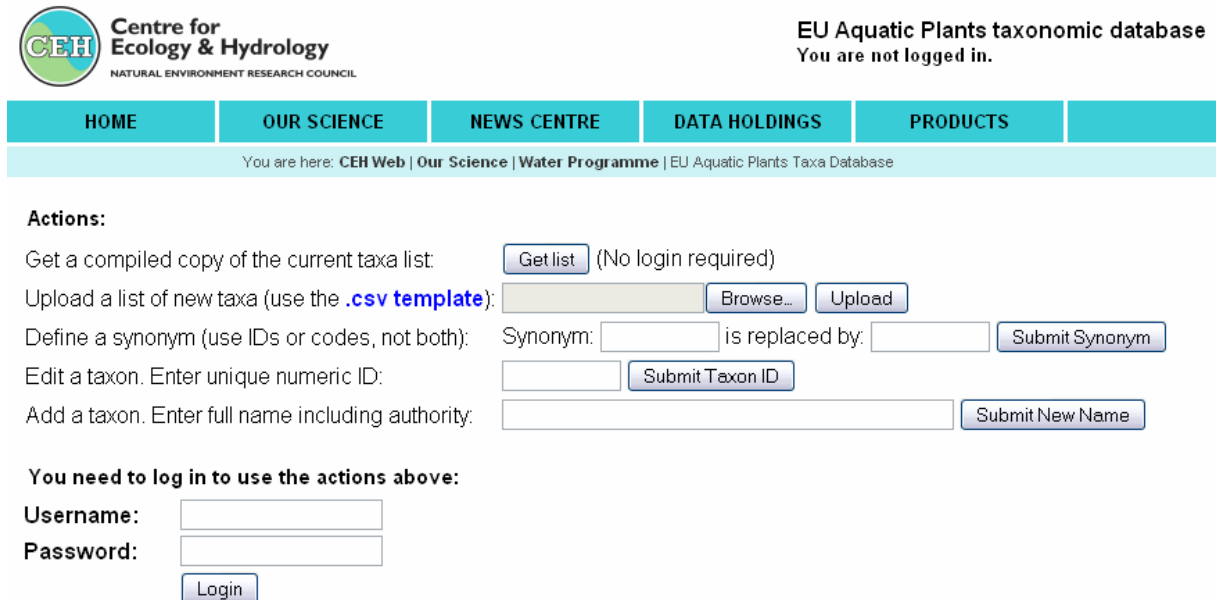


Fig. 1.2. Image of online aquatic plant taxa database website, <http://www.aqplants.ceh.ac.uk/>

1.5 Data conversion

In all but one case (France), data were provided as Microsoft Excel[®] files. Conversion of these files into the common database format followed these general principles:

- supplied data should be kept in its original form
- a documented and repeatable system of conversion should be built for each dataset
- details of the origin and appropriate intellectual property rights should be maintained and linked to the data.

These principles were achieved by the following general methods:

- database tables were constructed to hold the data in as close to its original form as possible
- a set of queries was built to separate the data into the tables of the common structure
- tables were constructed to translate both biological and environmental parameters into the standard codes.

1.6 Data extraction

A tool for selecting, aggregating, and compiling WISER database data was developed by Workpackage 2.1. This is achieved through Microsoft Access[®] forms and queries. A graphical representation of the main form used is shown in Figure 1.3, and a diagram showing the structure of queries used is in Figure 1.4.

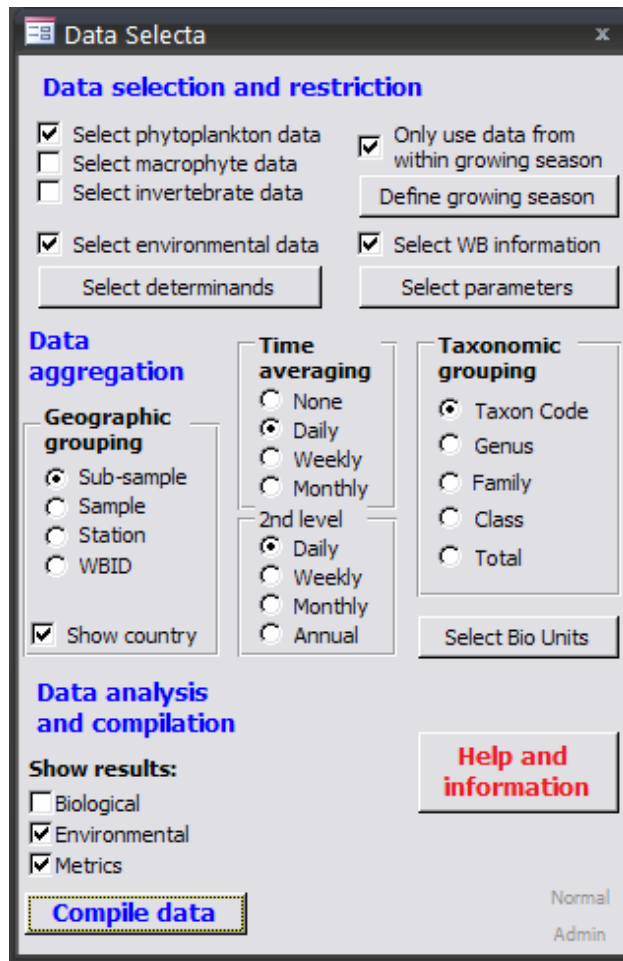


Fig. 1.3 Main Microsoft Access[®] form used by WISER data extraction tool

Although this structure might appear to be overly complex, it was found to be necessary to accommodate the needs of all WISER partners who wished to use it. The system of aggregating by time twice, for example, was deemed necessary by partners working with phytoplankton data, as they wished to know the annual means of monthly means, for selected months (growing season) only.

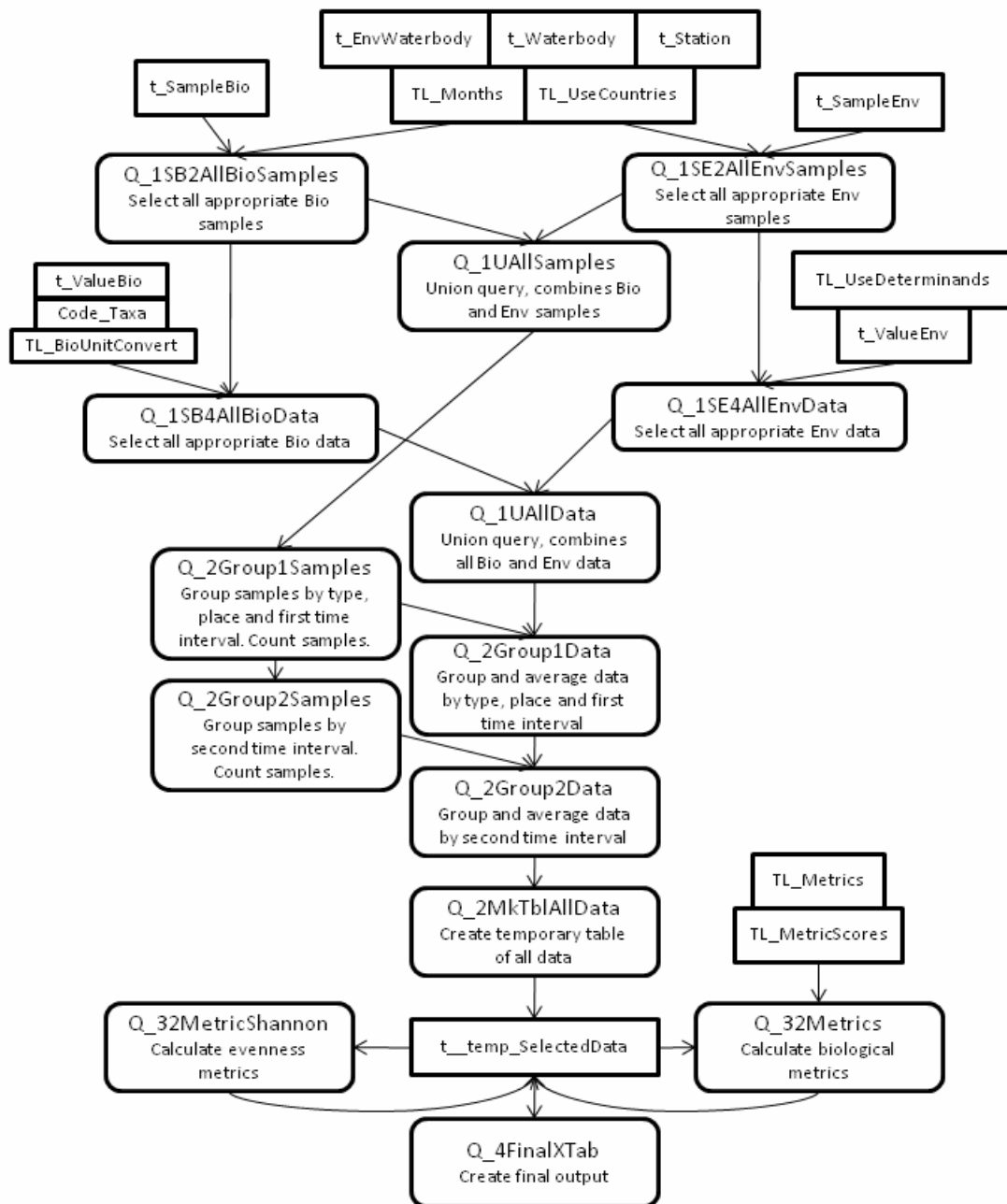


Fig. 1.4 Data selection, aggregation, and compilation flow diagram for the WISER/GIGs data extraction tool. Boxes with squared corners represent tables and boxes with rounded corners represent queries

1.7 References

Moe S.J., Dudley B., Ptacnik R. 2000. REBECCA databases: experiences from compilation and analyses of monitoring data from 5,000 lakes in 20 European countries. *Aquat. Ecol.* 42: 183-201

2. Paleo-derived reference conditions

Responsible: Ben Goldsmith, Contributors: Thomas A. Davidson, Helen Bennion

2.1. Background

With the primary aim of the EU WFD (European Union, 2000) being to achieve “good ecological quality” in all qualifying waters by 2015, there is a need to define ‘good’ in terms of not only the physico-chemical and hydromorphological environment, but also the aquatic biology. The WFD identifies these biological quality elements (BQE) to include fish, invertebrates, macrophytes, phytobenthos and phytoplankton with overall ecological quality judged by the degree to which the present-day assemblages deviate from those in the past, prior to anthropogenic influence. These so-called ‘reference conditions’ are a key element in defining the ecological status. A lake with BQEs which differ little from the reference condition will be classed as having “High” status, whereas increasing deviation from this reference will result in categories of Good, Moderate, Poor and Bad status being attributed.

Within each of the European ecoregions, the Member States have been tasked with the characterisation of water bodies into distinct ecotypes; each with differences in their ecological communities which are sufficient to allow the description of type-specific reference conditions. The determination of reference conditions and a system for setting ecological status boundaries is crucial for the implementation of the WFD. A number of methods for defining reference conditions are given within the directive, including expert judgement, spatial-state schemes, historical data and modelling. The directive explicitly states that hindcasting methods may be employed and palaeolimnology is named as one such approach (Pollard and Huxham 1998; European Union 2000; Bennion and Battarbee 2007).

Palaeolimnological techniques have been employed extensively over the past few decades with a view to defining baseline conditions and setting restoration targets for lakes and often have particular success in identifying the causes of change (see reviews in Battarbee 1999 and Smol 2008). These methods therefore have a potential role in the determination of reference conditions as defined within the WFD for European lakes. In particular, the assessment of site-specific reference conditions from the sediment record may be the only feasible approach for some water body types for which too few (if any) examples exist in a pre-disturbance, reference condition. Shallow, lowland lakes are one such example where palaeolimnological methods may be the only way to determine pre-impact conditions (Bennion *et al.* 2003, Bennion and Battarbee 2007, Bennion *et al.* 2010a). Furthermore, although long term monitoring data-sets do exist, they rarely extend to pre-disturbance times and the methods used for collecting physico-chemical and particularly biological data have changed greatly over time (Madgwick 2009), thus making interpretation of these records difficult.

A number of recent studies have illustrated the value of the palaeolimnological record for defining chemical reference conditions in the context of the WFD for a range of water body types (see Bennion and Battarbee 2007). These include diatom-inferred total phosphorus (TP)

and pH in Scottish freshwater lochs (Bennion *et al.* 2004; Battarbee *et al.* 2005), Finnish lakes (Kauppila *et al.* 2002; Miettinen *et al.* 2005; Kauppila 2006) and Irish lakes (Leira *et al.* 2006), and diatom-inferred nitrogen in Danish and Finnish coastal systems (Weckström *et al.* 2004; Kauppila *et al.* 2005; Clarke *et al.* 2006; Weckström 2006). The geochemical record has also been used to establish reference conditions of trace elements and metals as demonstrated by Renberg *et al.* (2001) for lead in Lake Mälaren, Central Sweden, by Grahn *et al.* (2006) for Ag, Be, In, Ga, Sb, Tl in four Swedish boreal lakes and for a suite of elements by Leira *et al.* (2006) for lakes in the Irish ecoregion. There are also attempts to establish reference conditions for sediment accumulation rates (Rose and Morley 2006, Rose *et al.* 2010) and organic carbon (Cunningham *et al.* 2010).

With the focus of the WFD on ecological quality rather than chemical water quality, there has been an impetus to move away from transfer functions and instead to place greater emphasis on retaining ecological information. An analysis of the biological components of the palaeolimnological record can provide valuable information on ecological reference conditions as well as helping to define lake ecotypes where reference sites cannot be found in the current population. In addition to a wide range of fossil indicators from both the micro-flora (e.g. planktonic and benthic diatoms, pollen, pigments) and fauna (chironomids, ostracods, cladocerans, fish scales) the analysis of aquatic plant macrofossils (Birks 1980; Birks 2001) greatly enhances our understanding of the palaeo-record and potentially allows the structural and functional characteristics of pre-impact aquatic ecosystems to be reconstructed. This technique has been put to good effect on a core from Loch Leven in Scotland (Salgado *et al.* 2010), which clearly demonstrates the ecological shifts in the aquatic macrophyte community associated with eutrophication and changes in catchment and lake management.

With an increased understanding of modern community ecology, multiproxy records from lake cores can be further interpreted. Studies at Felbrigg Lake, England, have characterised reference conditions not only in terms of high species richness species, but go as far as identifying the site as having had low spring and autumn phytoplankton biomass, relatively high aquatic macrophyte density with long seasonal duration of plant dominance and zooplanktivorous fish (Sayer *et al.* 2010a; Sayer *et al.* 2010b; Davidson *et al.* 2010a). These conditions were seen to deteriorate with the onset of eutrophication in c. 1870 with a whole community shift observed. A loss of plant species richness and a change from perennial to annual species was coupled with increased planktonic diatoms and *Daphnia* spp. suggesting shorter periods of plant dominance. Recent sediments reflect the current eutrophic conditions of the lake with few plants and high algal biomass. These studies combined a number of different approaches to achieve a reference condition based on habitat and lake function rather than a dependence on just species composition.

The palaeolimnological approach to defining reference condition, be it species- or community-based, may potentially be further enhanced by the employment of novel statistical methods. Analogue matching (Flower *et al.* 1997; Simpson *et al.* 2005; Bennion *et al.* 2010b) can be used to compare the fossil assemblage from a degraded site (i.e. from when the site was un-impacted and therefore in reference condition) with modern assemblages from un-impacted

lakes and thus determine which modern sites are closest to reference condition. This has been put forward as a potentially ideal solution to defining reference conditions as it is informed by both spatial and temporal data. (Bennion *et al.* 2010a)

2.2.1 Macrofossil analysis in lake sediments

Aquatic macrophytes form an important component of the sub-fossil record in many lakes and well established protocols exist for their analysis in lake sediments (Birks 2007). While there are some species which leave very few, or no remains (e.g. *Elodea* spp., liverworts), the majority of submerged aquatic plant species leave some form of identifiable remain: e.g. seeds, leaves, oospores, leaf spines, turions, megaspores, trichoscleroids and flower parts. In addition, many wetland and terrestrial plant remains are incorporated into the sediments of lakes and while possibly less relevant to defining reference conditions are nonetheless often used to infer information about marginal and local terrestrial habitats (Birks 1973; Birks 2001). The taxonomy of many sub-fossil remains is relatively straight forward for the well preserved remains (e.g. seeds, propagates, leaves (see Birks (2007) for an extensive list of identification sources)), but can become increasingly complicated due to fragmentation, poor preservation and the contamination by terrestrial material. The taxonomic complexity of some plant remains is such that they cannot currently be identified to species level, but in a novel study by Davidson *et al.* (in prep), attempts have been made to address this for charophyte oospores by using morphometric characters to increase the taxonomic resolution of this otherwise complex group. With a few exceptions, species level determination is unlikely to be possible for many *Chara* spp. oospores, but progress has been made in identifying several species groups which exhibit similar characteristics (Fig. 2.1).

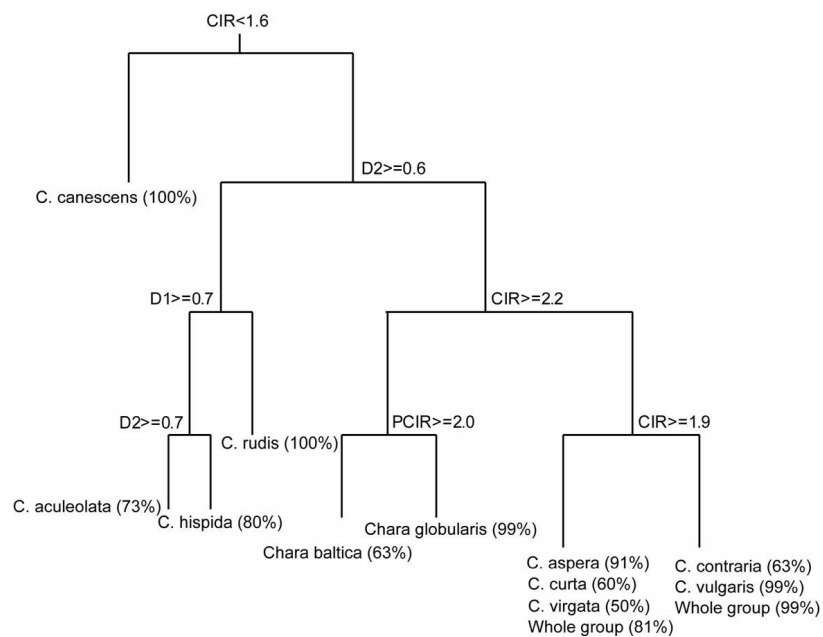


Fig. 2.1. Classification tree for *Chara* species oospores based on key morphological characteristics (from Davidson *et al.* in prep.)

Unlike microfossils (e.g. diatom frustules) which can generally be assumed to focus towards the deeper parts of a lake, plant macrofossils tend to accumulate closer to their origin and thus representivity from a single core can be problematic (Birks 1980; Zhao *et al.* 2006). In an attempt to minimise this effect, a single core is often taken from the littoral zone of a sheltered bay, preferably at a point towards the windward shore where plant remains are most likely to accumulate. Multiple cores can also be used, but this greatly increases the analytical effort and throws up other issues, such as cross-core correlation, which cannot always be overcome. Zhao *et al.* (2006) concluded that while a single core taken from a littoral location often underestimates the overall species richness of a site, it nonetheless reflects, in most cases, the dominant components of the aquatic flora. A similar study by Koff and Vandiel (2008) also reported a good relationship for some remains with the modern vegetation for two Estonian lakes, but 50% of the aquatic species were not recorded in the sediments.

While the representation of sub-fossils in the sediments is relatively good, the relationship between the plants and their representation in the sub-fossil record is less well understood and is highly complex (Abernethy and Willby 1999). For example, charophytes oospores accounted for 97% of the reproductive remains observed by Zhao *et al.* (2006) in the multiple surface sediments from a small eutrophic lake in England, yet this group was no longer abundant in the flora of the site. Conversely, in the same study, *Potamogeton* spp. achenes were very scarce in the surface sediments, despite them typically yielding high numbers of fruit and being common in the modern flora; possibly this is a result of temporary seed buoyancy (Abernethy and Willby 1999) or even due to their reported palatability to ducks (De Vlaming and Proctor 1968; Van Wijk 1989).

For the purposes of assessing historical reference conditions, the ability to determine the dominant components of the past flora may in many cases be enough to establish a baseline for the site. Even without abundance data and the likelihood of an incomplete species list for a site, many studies have been able to demonstrate significant changes in the flora through time, and often with an associated reduction in species richness where eutrophication was the primary pressure on the system (Birks 2001; Davidson *et al.* 2005; Salgado 2010). In the UK a number of recent studies have been commissioned by the regional conservation agencies to use aquatic plant sub-fossils to inform restoration targets and help identify the timing and cause of lake habitat degradation (Davidson *et al.* 2008a; Bennion *et al.* 2009a; Bennion *et al.* 2009b; Davidson *et al.* 2009; Bennion *et al.* 2010c). These, and many other studies, undertaken at University College London have resulted in an increasing wealth of macrofossil data which has yet to be analysed as a whole. Many of the sediment cores that have been analysed have also been dated using either radiometric or SCP techniques (Rose *et al.* 1995) and thus provide an excellent opportunity to examine plant community shifts on a temporal scale reaching back to 1850; a period generally considered to approximate be pre-impact conditions in many UK lakes (Bennion *et al.* 2010a). The majority of the existing data are derived from shallow eutrophic lowland lakes in the UK with medium to high alkalinity. This lake type is particularly difficult to define reference conditions for, as few, if any undisturbed examples occur in the UK and they are rare throughout most of Europe. In terms of assessing macrophyte communities,

palaeolimnological methods may therefore potentially provide a powerful tool for defining realistic reference conditions. This is particularly poignant for ecoregions which lack modern examples of un-impacted lake types.

2.1.2 Objectives

Using existing data from the UK this chapter explores the potential of using palaeolimnological techniques for defining reference condition for shallow lowland lakes.

- Collate available palaeo data from dated sediment cores
- Develop a scoring system for different sub-fossil species and types
- Examine the relationships between surface sediment remains and c.1850 remains
- Assess the potential of palaeolimnological data for defining reference conditions.
- Compare palaeo-derived reference conditions with Spatial state schemes. (To be explored further in the future)

2.2. Methods

2.2.1 Data sources

The data used in this paper have been collected mainly within the past 10 years, with a total of 74 cores taken from 61 different lakes throughout the UK (Table 2.1). With the original reason for taking and analysing cores being varied, the first stage of the analysis has been to collate the data and assess its suitability for the purposes of defining reference conditions. Not all the cores have been subject to dating, and for those that have, there is a subset where the dating has been unreliable or shows the sediments not to extend back as far as the 1850 marker arbitrarily chosen to represent pre-impact conditions in the UK (Bennion *et al.* 2010a).

Table 2.1 Sites for which existing plant macrofossil data are held at UCL. Sediment cores used in the analysis are shaded. H Alk = High alkalinity ($>1 \text{ meuql}^{-1}$), M Alk = Moderate alkalinity ($0.2-1 \text{ meuql}^{-1}$), S = Shallow (mean depth 3-15 m), VS = Very shallow ($<3 \text{ m}$).

Site	Core Code	GB Lake type	Dating	UK WBID	Lat / long
Aqualate Mere	AQUA3	H Alk, VS	Yes	35724	N52°46.85',W002°20.34'
Barnby Broad	BARB4	H Alk, VS	Yes	37536	N52°27.44',E001°38.97'
Barningham Broad	BARN1	H Alk, VS	Yes	34976	N52°52.48',E001°11.52'
Barton Broad	BART3	H Alk, VS	No	35655	N52°44.33',E001°29.67'
	BART5		No		
Berrington Pool	BERR1	H Alk, S	No	36634	N52°39.63',W002°42.25'
Blickling Lake	BLIC2	H Alk, VS	Yes	35249	N52°49.01',E001°13.75'
Bosherston Central Lake	BOSHC1	Marl VS	No	41602	N51°37.01',W004°55.35'
Burntfen Broad	BURF1	H Alk, VS	Yes	35852	N52°42.9',E001°27.64'
Butterstone Loch	BUTT5	M Alk, S	Yes	23531	N56°35.23',W003°32.02'

Site	Core Code	GB Lake type	Dating	UK WBID	Lat / long
Llyn Cadarn	CADA3	Marl S	Yes	32792	N53°18.34',W004°15.85'
Calthorpe Broad	CALT1	H Alk, VS	No	35475	N52°46.53',E001°34.32'
Cromes Broad	CROM1	H Alk, VS	No	35772	N52°43.36',E001°30.86'
Cunswick Tarn	CUNS1	Marl VS	Yes	29394	N54°20.23',W002°47.19'
Felbrigg Hall Lake	FELB1	H Alk, VS	Yes	34827	N52°54.17',E001°15.31'
	FELB2		No		
	FELB5		No		
	FELB6		No		
	FELB7		No		
Filby Broad	FILB1	H Alk, VS	Yes	35981	N52°40.63',E001°38.39'
Loch Flemington	FLEM2	H Alk, S	No	17013	N57°32.55',W003°59.39'
Fritton Decoy	FRIT1	H Alk, VS	No	36989	N52°32.68',E001°39.59'
Groby Pool	GROB4	H Alk, VS	Yes	36536	N52°40.13',W001°13.86'
	GROB2		Yes		
	GROB5		Yes		
Hoveton Great Broad	HGBO1	H Alk, VS	No	35977	N52°41.6',E001°26.01'
Hickling Broad	HICK1	H Alk, VS	No	35640	N52°44.17',E001°34.99'
Hornsea Mere	HORN3	H Alk, VS	Yes	30244	N53°54.32',W000°11.37'
Kenfig Pool	KENF2	H Alk, VS	Yes	42170	N51°31.2',W003°44.13'
Killymackan Lough	KILL1	H Alk, VS	No	50082	N54°8.03',W007°29.72'
Kilroosky Lough	KILR1	Marl VS	Yes	50315	N54°11.59',W007°14.67'
Knockballymore C	KNOC1	Marl VS	No	50429	N54°11.42',W007°15.85'
Loch Leven	LEVE14	H Alk S	Yes	24843	N56°11.89',W003°22.55'
Lough Garve	LGAR1	H Alk VS	No	50381	N54°59.5',W006°6.53'
Little Broad	LILT1	H Alk, VS	Yes	36233	N52°39.53',E001°37.25'
Lindores Loch	LIND2	M Alk, S	Yes	24422	N56°20.04',W003°11.23'
Llangorse Lake	LLAN5	H Alk, VS	No	40067	N51°55.81',W003°15.81'
	LLAN6		No		
	LLAN7		No		
Mill Lough	LMIL1	H Alk, S	Yes	50067	N54°8.43',W007°29'
Martham South Broad	MARS1	H Alk, S	Yes	35738	N52°43.36',E001°38.4'
Mautby Decoy	MAUT1	H Alk, VS	Yes	35772	N52°43.36',E001°30.86'

Site	Core Code	GB Lake type	Dating	UK WBID	Lat / long
Meenantully	MEEN1	M Alk, VS	Yes	50289	N54°32.14',W008°0.1'
Melchett Mere	MELC3	H Alk, VS	No	32787	N53°19.58',W002°22.57'
Lake of Menteith	MENT7	M Alk, S	Yes	24919	N56°10.46',W004°17.53'
	MENT8		Yes		
	MENT9		Yes		
Monzievaird	MONZ2	H Alk, S	No	24171	N56°23.26',W003°52.81'
Loch Nan Gad	NGAD2	M Alk, S	No	26482	N55°45.43',W005°32.01'
Norton's Broad	NORT1	H Alk, VS	No	35974	N52°42.07',E001°23.36'
Ormesby Great Broad	ORMG1	H Alk, VS	Yes	35981	N52°40.63',E001°38.39'
	ORMG2		Yes		
Ormesby Little Broad	ORML1	H Alk, VS	Yes	35981	N52°40.63',E001°38.39'
Over Water	OVER1	H Alk, Sh	Yes	28806	N54°42.3',W003°9.77'
Rockland Broad	ROCK1	H Alk, VS	Yes	36730	N52°35.65',E001°26.51'
Rollesby Broad	ROLL2	H Alk, VS	Yes	35981	N52°40.63',E001°38.39'
Round Water	ROUW2	H Alk, VS	No	n/a	N52°27.56',E001°41.06'
Lough Rushen	RUSH1	H Alk, S	No	50064	N54°32.64',W007°58.25'
Salhouse Great Broad	SALG1	H Alk, VS	No	36043	N52°41.36',E001°25.8'
Comber Mere	SCM14C	H Alk, VS	No	34480	N52°59.86',W002°36.67'
Tatton Mere	SCM41F	H Alk, S	No	32804	N53°19.04',W002°22.12'
Sotshole Broad	SOTS1	H Alk, VS	Yes	36181	N52°40.21',E001°29.27'
Sprats Water	SPRA1	H Alk, VS	Yes	n/a	N52°27.58',E001°41.15'
Sunbiggin Tarn	SUNB3	Marl, S	No	29178	N54°27.81',W002°30.04'
Tangy Loch	TANG2	M Alk, S	No	27234	N55°29.48',W005°39.06'
Lough na Trosk	TROS1	M Alk, S	Yes	50284	N55°29.48',W005°39.06'
Upper Lough Erne	ULET1	H Alk, VS	No	50003	N54°13.15',W007°31.04'
	ULET2		No		
Upton Little Broad	UPTL1	H Alk, VS	No	n/a	N52°39.95',E001°31.99'
Upton Great Broad	UPTO3	H Alk, VS	No	36202	N52°39.95',E001°31.96'
Westwood Great Pool	WGRP1	H Alk, VS	Yes	38586	N52°16.07',W002°10.69'
White Loch	WHIE2	H Alk, S	Yes	23607	N56°34.24',W003°21.15'
Wolterton Hall Lake	WOLT1	H Alk, VS	Yes	35179	N52°50.14',E001°12.06'
Woolners Carr	WOOC1	H Alk, VS	Yes	n/a	N52°27.52',E001°41.02'

2.2.2 Data handling and harmonisation

Due to the rather ad hoc nature in which macrofossil data have been recorded in the past, as well as the complexity of the taxonomy, there is a need to harmonise the data to ensure compatibility. The latter problem has occurred as a result of different analysts ascribing their own names to sub-fossil remains; the species often not being in doubt, but the type of remain within a species can vary considerably. For example, *Potamogeton obtusifolius* might occur in the sub-fossil record as seeds (achenes), leaves or turions, but these can be further broken down for seeds into 'seed fragment', 'seed beak', 'half seed', 'seed husk' or for leaves; 'leaf tip', 'leaf fragment', 'leaf middle' or for turions: 'fragment', 'tip', 'base' etc. The permutations for any one species are many and consistency between analysts often varies and remain types often vary between sites. Thus in the analysis, few species are clearly defined as a single taxon, but instead are assigned 'pseudo-species' status (e.g. *P. obtusifolius* seed, leaf-tip, leaf fragment, turion etc) and coded accordingly. In the combined data-set, pseudo-species have wherever possible been combined to reduce the final number of taxa. For example, *P. obtusifolius* leaf tips and leaf fragments have been treated as a single pseudo-species. Similarly, a pragmatic approach was taken for *Chara* spp. oospore fragments, and seed fragments, which were combined with their whole counterparts at a ratio of 1:10 (whole oospore to fragment).

The fragmented or decayed nature of some sub-fossil remains make accurate identification to species level uncertain. With fine leaved *Potamogeton* spp. leaf fragments, it is often possible to determine identification to either *P. berchtoldii* or *P. pusillus* and thus there a number of pseudo-species present in the combined data-set which are assigned to 'cf.' taxa, e.g. *P. cf. berchtoldii / pusillus* leaf fragments and for some *Chara* species, oospores can be assigned to a species type with some degree of certainty, but not to species level, e.g. *Chara vulgaris / contraria*. While perhaps the treatment of the data in this way may be construed as compromising the taxonomic integrity of the data, these mixed pseudo-species have been left in the analysis with the rationale that they provide a valuable insight into the structure of the aquatic vegetation that existed at the site. This is particularly poignant for macrofossil data as rarely are all aquatic plant species growing in a lake recorded in the sediments (Zhao *et al.* 2006; Koff & Vandell, 2008) and thus any information of vegetation structure is important, even if not at the highest taxonomic level.

Another problem associated with the sub-fossil data is the vast range of numbers of individuals that may be recorded. Rarely in one sample does one find more than ten *Potamogeton* seeds in a single sample of 100 cm³ of sediment, but *Ceratophyllum* leaf spines and charophyte oospores may number several thousand and as many as 86,000 *Nymphaea* trichosleries have been recorded from a single sample. We know that the distribution of sub-fossil remains in sediments is at least in part governed by proximity of the parent plant, and therefore it is impossible to make any direct relationship between fossil numbers and species abundance. There are however, distinct patterns seen at many sites in the palaeolimnological record where the relative abundance of one fossil remain increases or decreases through time (sediment depth) and furthermore, a number of multi-proxy studies have been able to attribute these changes to pressures such as eutrophication (Bennion *et al.* 2010a). To ignore the sub-

fossil numbers altogether risks the potential loss of information, but quantifying the relationship between these remains and the lake flora lies outside the scope of this paper.

With the combined data-set from the 74 cores, attempts were initially made to put the data into abundance classes (0-5) using equal ranges, equal counts and percentages, facilitated in the program C2 (Juggins 2003). In addition, data analysis was performed using square root transformations on the raw species data. The effect of classing the data was to reduce the huge disparity in individual numbers, but none of the resulting analyses differed greatly from when simply using square root transformations, and therefore the latter method was chosen to maintain the maximum structure in the data, while still reducing the influence of high individual counts.

2.2.3 Data analysis

The sub-fossil data from submerged and floating-leaved species were plotted using the specialist stratigraphic software C2 (Juggins 2003) and subject to the unconstrained multivariate technique of principal components analysis (PCA) using the same software, to assess patterns of change within a core. Detrended canonical correspondence analysis (DCCA) (ter Braak 1986; ter Braak & Verdonschot 1995) was used to assess the overall variation in pseudo-species data from all the dated cores using age as a single constraining variable. In so doing, it is possible to quantify the shifts in pseudo-species through time with the added advantage of using DCCA being that it scales the axis scores in units of standard deviation or turnover of beta-diversity (Smol *et al.* 2005). By making the assumption that prior to c.1850 sites were relatively un-impacted (i.e. in reference condition), it is possible to estimate the change in species composition through time to the present day; i.e. deviation from reference conditions. Beta-diversity measures the dissimilarity of species between samples; a low beta diversity score denotes a high level of similarity, whereas a high score indicates a low level of similarity. For samples constrained by age within the DCCA analysis, the technique examines species turnover through time as the primary axis.

2.3. Results and Discussion

The majority (60 out of 74) of the lake sediment cores for which data exist are from lowland regions (Alt. <200 m) with high alkalinity (L_CB1 & 2). Of these, only 30 had adequate dating to be able to ascribe an approximate date of 1850 or older. Dating was also a problem in some of the cores from the lower alkalinity sites, leaving too few of this lake type to analyse separately. Two sites, Loch Leven and White Loch, which are classified as high alkalinity within the GB lakes typology, had sub-fossil remains more akin to lower alkalinity conditions (*I. lacustris*, and were therefore removed from the analysis due to being atypical. The data presented in this report are all from high alkalinity, shallow and very shallow, lowland lakes. The original data-set of 74 sediment cores had a total of 109 aquatic macrophyte pseudo-species present, which was reduced to 82 pseudo-species in the sub-set of 30 dated cores.

2.3.1 Temporal changes in aquatic macrophytes

With eutrophication having impacted the majority of shallow lowland lakes in the UK over the past 150 years, it is to be expected that this will be reflected in the sub-fossil record. Indeed, many of the sites for which there are dated cores show clear trends from species rich mixed plant communities in the past, to fewer and more typically ‘eutrophic’ species towards the present. Ormesby Broad, a shallow, eutrophic water body in Norfolk, England, is a good example of this (Fig. 2.2). Prior to c. 1850 (which has been assigned conservatively here), the site appears to have had a relatively species rich aquatic flora, characterised by a number of different *Potamogeton* species as well as stoneworts and water lilies. There then appears to be a shift in the latter part of the 19th Century, with fewer *Potamogeton* remains and an increase in aquatic *Ranunculus* species and *Callitriche* seeds. Perhaps the biggest contrast comes towards the top of the core, where *Chara* species and aquatic *Ranunculus* disappear, *Potamogeton* remains are less diverse and *Zannichellia palustris* seeds increase dramatically. It should be stressed that the sub-fossil remains are only from species that preserve and this does not represent a full species list for the site.

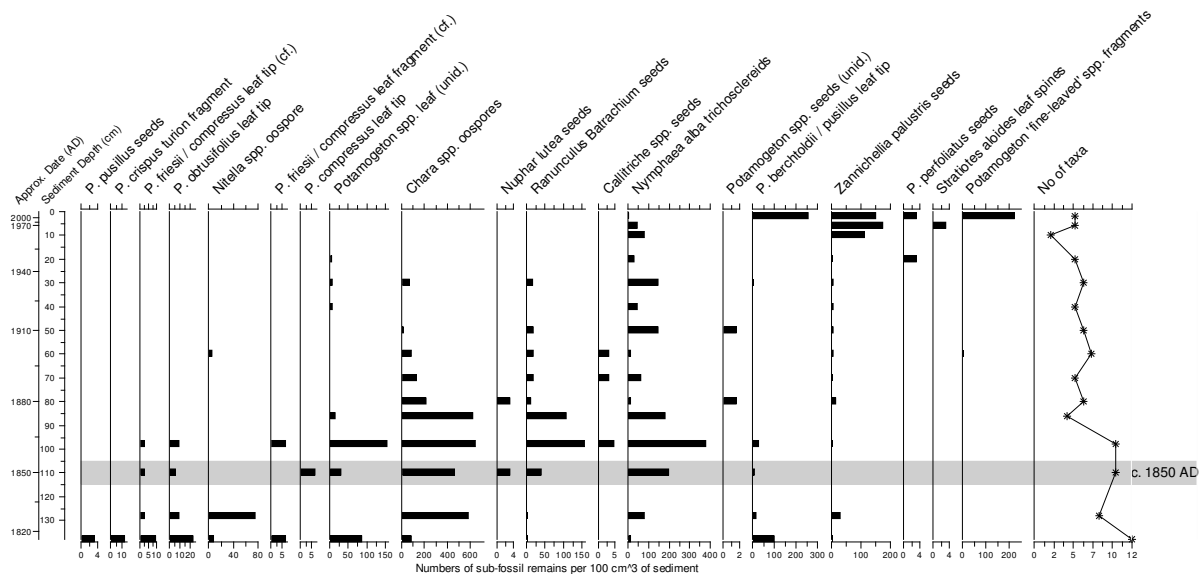


Fig. 2.2 Aquatic macrophyte sub-fossil remains from core ORMG1; Ormesby Great Broad, Norfolk, England (adapted from Davidson et al. 2008b).

This pattern of species loss and turnover is typical of the data collated from shallow lowland lakes in the UK with two further examples presented in Figures 2.3 and 2.4 below. Blickling Hall Lake has rather sparse remains prior to 1850, but there is evidence of a relatively good mixed flora existing at the site in the latter part of the 19th Century, with broad-leaved *Potamogeton* species present, as well as *Nitella* spp., *Chara* spp. and *Myriophyllum verticillatum*. In the first half of the 20th Century, *Chara* species appear still to abound, but there is a shift towards species typical of more eutrophic waters such as *Ceratophyllum* (c.f. *demersum*) and an increase in *Zannichellia palustris*. Most recently there is an increase of *P.*

pectinatus, *Potamogeton* leaf tips (c.f. *P. pusillus*?), *Z. palustris* remains and decline of *Chara* species as well as *Ceratophyllum*. This lake is currently turbid for much of the year and has only a sparse emergent flora and almost no submerged aquatic species.

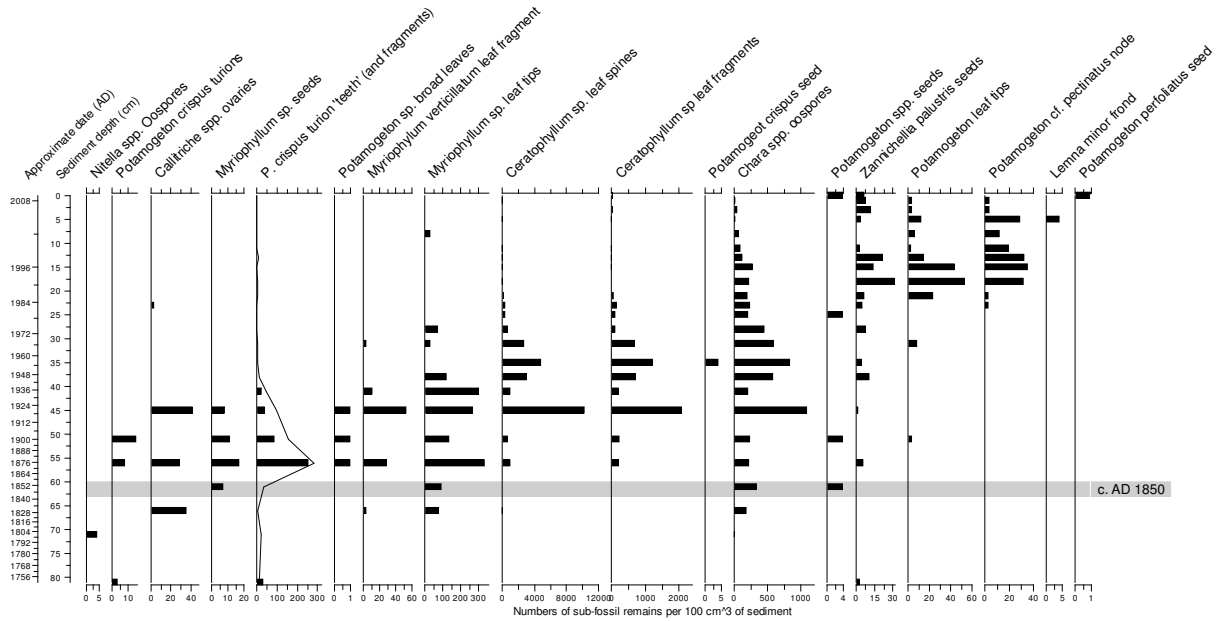


Fig. 2.3 Aquatic macrophyte sub-fossil remains from core BLIC2; Blickling Hall Lake, Norfolk, England (adapted from A. Baker, Unpublished MSc Thesis, UCL, London)

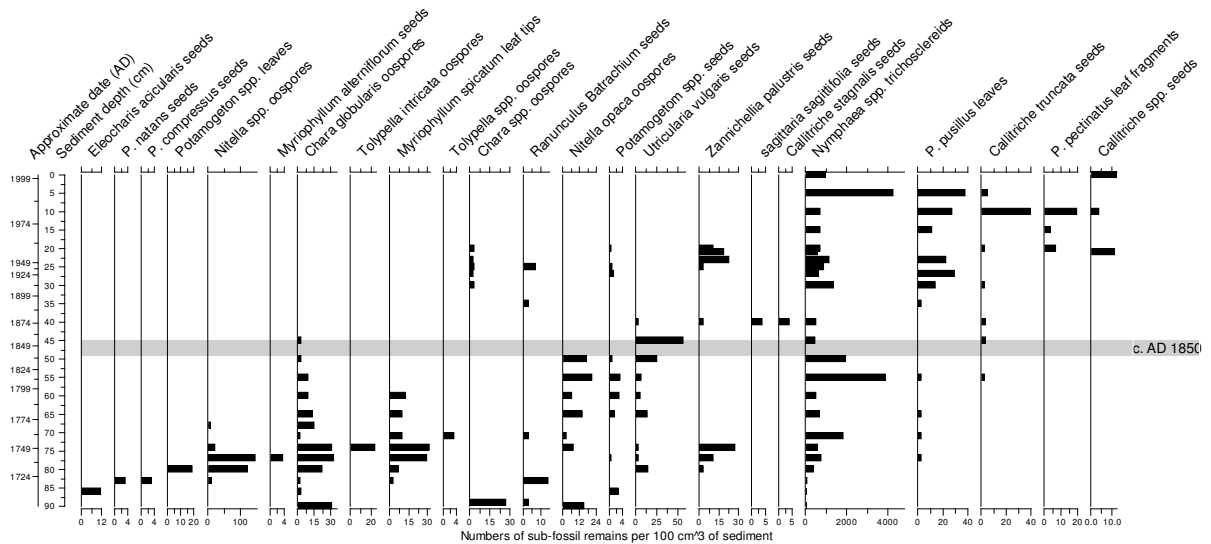


Fig. 2.4 Aquatic macrophyte sub-fossil remains from core GROB4; Groby Pool, Leicestershire, England (adapted from Davidson et al. 2005)

Groby Pool is a shallow lake which is now dominated throughout the site by *Potamogeton pectinatus*, *P. pusillus*, *Nuphar lutea*, *Ceratophyllum demersum* and *Elodea nuttallii* with *Callitriche truncata* occasional. In the past it would appear the site had a much richer flora and one typical of lower nutrient conditions. *Nitella*, *Chara* and *Tolypella* oospores were all

common pre- 1850, and the remains of *Utricularia vulgaris*, *Potamogeton compressus* and even *Myriophyllum alterniflorum* were also present. The site has clearly undergone significant change over the past 200 years and the onset of this even pre-dates 1850. It is possible that the disturbance brought about by documented changes in land use between 1780-90 were actually responsible for some of the observed changes and this period is clearly defined by a coarse mineral layer in the sediments from this time (Davidson *et al.* 2005).

2.3.2 Using plant macrofossils to determine reference condition

The three plant macrofossil examples above are typical of the changes recorded from the sub-fossil remains of moderate to high alkalinity shallow lakes in the lowlands of the UK over the past c.200 years. It is assumed that the remains from sediments pre-dating 1850 were generated under relatively good conditions (in terms of reference condition), and thus it is possible to derive a sub-fossil reference condition for a site type from these remains.

Using DCCA with samples constrained by date the total pseudo-species turnover can be estimated within the sub-set of 30 dated cores, yielding a total range of beta-diversity of 0.00-3.76 (expressed as SD units). A clear trend can be seen in the data, with higher values tending towards the more recent samples (Fig. 2.5). This can be interpreted as an overall increase in species turnover since 1850 due to increased pressures on the freshwater environment.

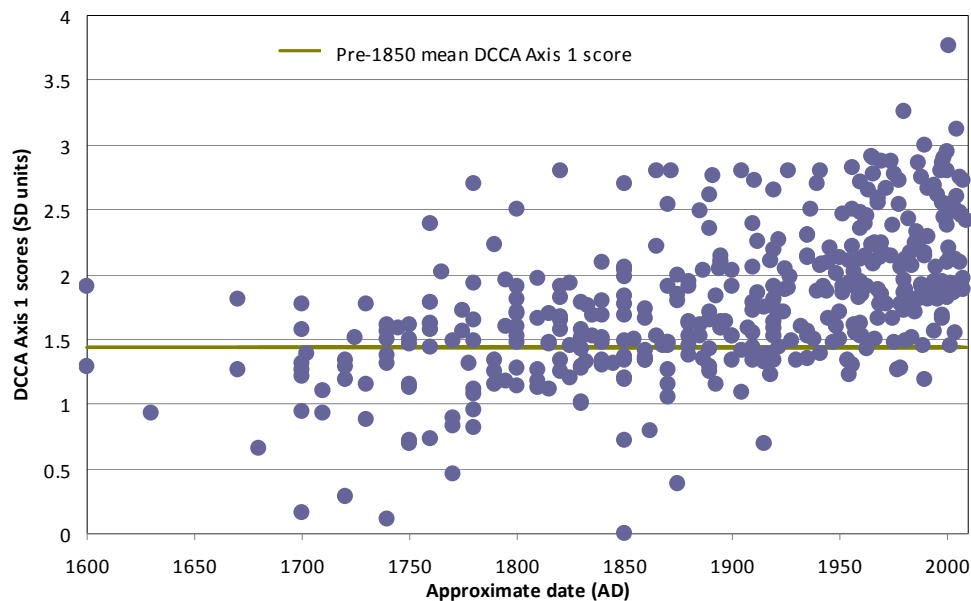


Fig. 2.5 DCCA scores for macrofossil remains, constrained by date

To help interpret the aquatic plant sub-fossil data, the beta-diversity from lakes considered to be in reference condition would ideally be used for comparison. Without such data from modern reference sites, which in the UK are rare for this lake type, we can use instead the mean DCCA Axis 1 scores of the pre-1850 sites as our estimate of reference conditions (mean = 1.43, median 1.45, hence 1.44). Samples with a score greater than 1.44 SD units can therefore be said to be

moving away from reference condition. The extent to which this is significant has yet to be tested, but if samples are examined on a site by site basis, it is clear that since c.1850 there have been considerable shifts in the aquatic plant communities away from the palaeo-derived reference condition.

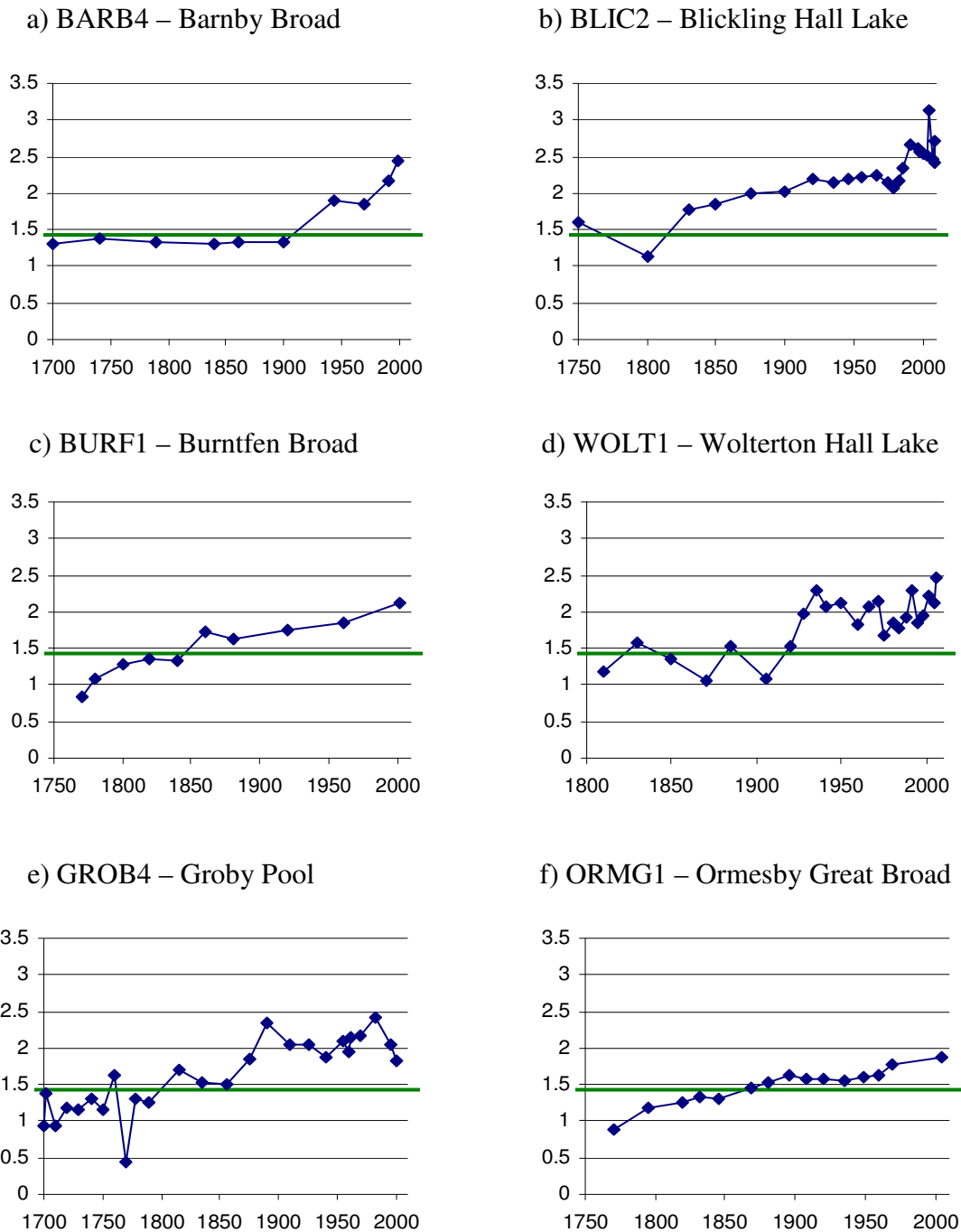


Fig. 2.6 Individual sites showing beta-diversity tracked through time. The green line is the mean beta-diversity based on pre-1850 'reference' samples

Figure 2.6 shows a selection of sites which display this trend with varying degrees of magnitude. Barnby Broad for example, appears to have been very stable up to c.1900, but has undergone rather a rapid community change away from the ‘reference’ condition since then. Groby Pool shows more variation prior to 1850, possibly as a result of catchment disturbance which we know occurred at the site in 1780 (Davidson *et al.* 2005). Ormesby Great Broad shows only a slow trend in increased beta-diversity. This is despite what appear to be relatively major shifts in the plant community in the past 150 years (Fig. 2.2). The low beta-diversity scores suggest that while the site was certainly undergoing major change (as reported by Davidson *et al.* 2008b), it remained relatively close to the reference condition until quite recently.

Without good modern macrophyte data for many of the lakes in the dataset it is not possible to interpret the palaeo-limnological results in terms of their current condition, although the majority of sites are known to be degraded. For the sites that do have recent macrophyte data there does appear to be a relatively good match between the macrofossil-inferred condition and the current status. Kilroosky Lough, a high alkalinity shallow lake in Northern Ireland, is one of the few sites which appears relatively stable throughout the palaeo-derived data (Fig. 2.7a), and recent surveys show it to be in good condition with respect to macrophytes with at least six species of *Chara* recorded in clear low nutrient (Mean TP = 12 $\mu\text{g l}^{-1}$) waters (Goldsmith *et al.* 2007). Kenfig Pool, a medium alkalinity, shallow lake in Wales, appears to have undergone gradual change over the past 200 years (Fig. 2.7b). Today the site is dominated by fine leaved *Potamogeton* species (mainly *P. trichoides* and *P. pusillus*), *Ranunculus circinatus* and *Chara* spp. with *P. gramineus* and *P. x angustifolius* occasional (Goldsmith *et al.* 2010). While not in poor condition, increasing frequency of *Zannichellia palustris* and *P. pectinatus* and periods of high algal biomass in late summer, suggest the site is deteriorating which is consistent with the palaeo-derived beta diversity.

Mill Lough in Northern Ireland and Over Water in NW England are both moderate alkalinity shallow lakes that appear to have undergone recent deterioration (Fig. 2.7c & 2.7d). Mill Lough is exceptional within this lake type in the UK for having at least eight *Potamogeton* species present, four *Chara* species and occasional *Littorella uniflora*. Water quality has possibly deteriorated in recent years, but a more major change has occurred in the macrophyte community due to the site now being dominated by *Elodea canadensis* (Goldsmith *et al.* 2008). *Elodea* species do not leave reliable sub-fossil remains in sediments and are therefore not represented in the palaeo data and are not therefore causing the observed change in beta-diversity. Instead, it is primarily a reduction in the *Chara* spp. oospore numbers at the top of the core that drives the change, possibly as a result of the expansion of *E. canadensis* shading out *Chara* spp. beds from large areas of the littoral zone. This theory is entirely speculative, but if correct it demonstrates a sensitivity of the palaeo-limnological methods for detecting shifts in the community structure without any loss of species.

Over Water, once a notable site for its flora, has undergone significant changes in recent years due to both catchment management and periodic reductions in water level due to increasing abstraction demands. Although still relatively species rich, the current flora is now

dominated by *Elodea nuttallii* and this appears to be reflected by shifts in *Chara* spp. and the appearance of *Myriophyllum* spp. remains in the uppermost sediments. Once again there is no direct link between the observed flora (i.e. dominance of *E. nuttallii*) and the most recent sediments, but changes in beta-diversity appear to show deterioration in the site that is consistent with recent changes.

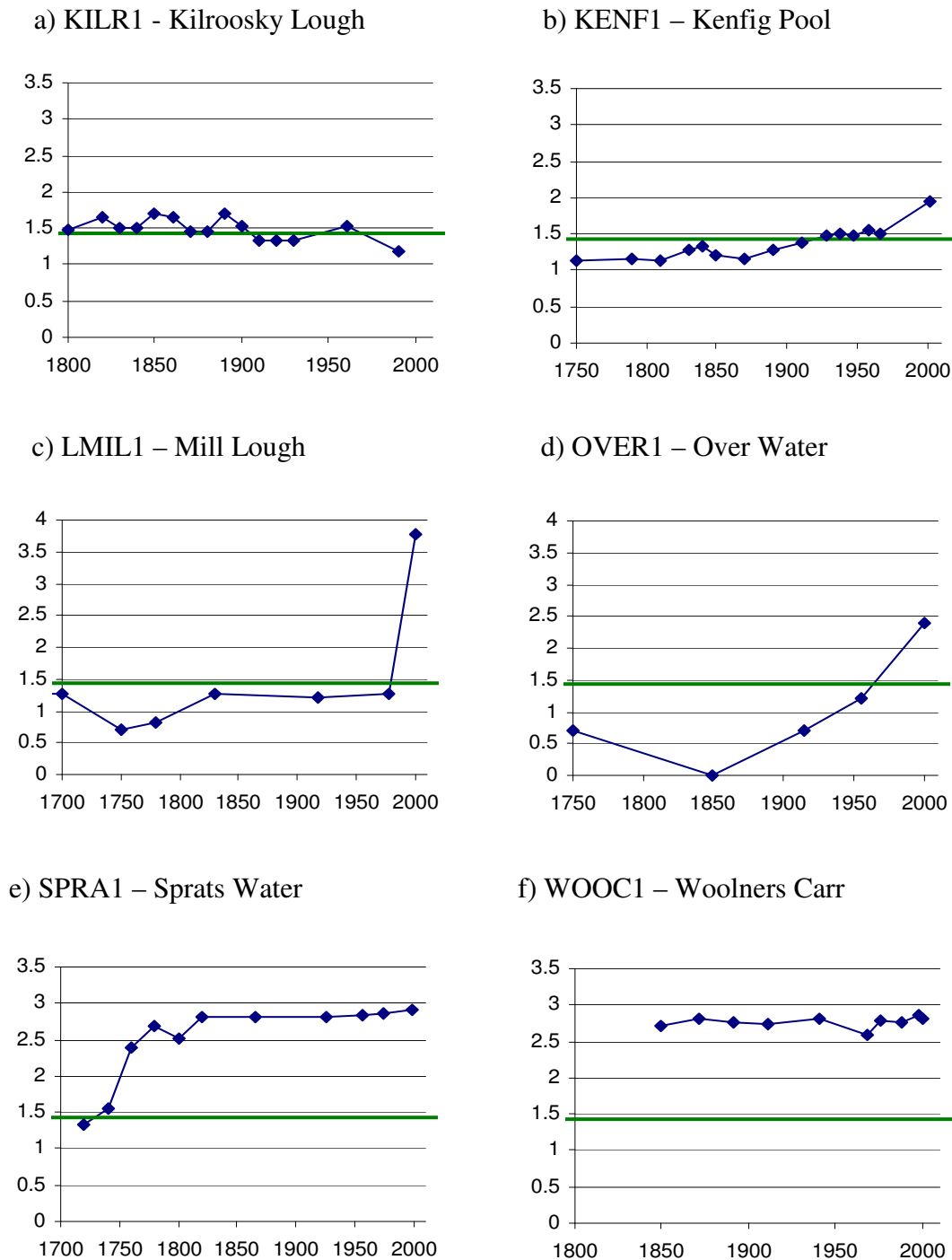


Fig. 2.7 Individual sites showing beta-diversity tracked through time. The green line is the mean beta-diversity based on pre-1850 'reference' samples

Not all sites show a typical response through time, but it is often unclear if this is due to the sites having undergone changes prior to c.1850, or if this is a result of taphonomic issues. The sub-fossil remains of Sprats Water (SPRA1) for example are dominated by large numbers of *Ceratophyllum* leaf spines from low down in the core resulting in high beta-diversity from c.1760 onwards (Fig. 2.7e). Similarly, Woolners Carr is also dominated by *Ceratophyllum* leaf spines throughout the core and consequently has high beta-diversity (Fig. 2.7f). Both cores have very few other remain types and therefore it is possible that had other sub-fossils been better preserved, the beta-diversity may not have been so high or would have exhibited an upward trend. These are small, shallow sites however and lie within a landscape that has been managed and manipulated by man for many centuries and thus may potentially have undergone significant changes prior to 1850, or perhaps never existed as a reference type. This highlights the problems of using a date marker to define reference conditions. The actual timing of anthropogenic disturbance will inevitably pre-date 1850 in the majority, if not all of the UK and much of Europe. While it is generally accepted that 1850 is the period when rapid industrialisation and population growth caused the acceleration of freshwater degradation, there can be no doubt that many lakes in the UK would not have been described as pristine immediately prior to this time (Bennion *et al.* 2010a).

Furthermore, the dating of these littoral cores is not always successful and the data in this report, while drawing on the most reliably dated sediments, does not extend further back than 1850 at best. Earlier dates have been extrapolated from the depth age curves by assuming constant sedimentation rates. It is possible that some cores are not fully contiguous or have been re-worked following water level changes and thus, as with many palaeo-limnological techniques, poor dating can potentially cause errors in interpretation.

Despite inherent problems with dating and taphonomy, the data presented here do show that many moderate to high alkalinity, shallow, lowland lakes have undergone considerable changes in the past 150-250 years. These observations of species change through time not only represent changing plant communities, but for the most part they also display a unidirectional shift from low-beta diversity, typical of pre-1850 ('reference') conditions to higher beta-diversity, characteristic of more impacted sites; mainly due to eutrophication. From this relatively small UK data-set, it is possible to relate sub-fossil remains directly to a reference point, in this case arbitrarily chosen as 1.44 SD units to best represent the conditions occurring prior to c.1850.

The use of this technique is not restricted to a single ecoregion or lake type, but it is suggested that each ecoregion would initially require its own internal calibration data for each lake type. The extent to which these could be used across ecoregions and span different lake types would then need to be further investigated. The limited data from lower alkalinity and mid-altitude lakes, suggests similar patterns exist in the sub-fossil records with beta-diversity increasing at impacted sites. In ecoregions with good modern reference sites it would be prudent to also construct independent data-sets of subfossil beta-diversity from which to define the reference thresholds for each lake type and so preclude the inherent problems associated with sediment dating.

The complexity of aquatic ecosystems means it is rarely only one organism type that is responding to environmental drivers, but instead changes occur at a whole ecosystem level. In addition to aquatic macrophyte remains, the sub-fossils of other groups such as algae, pollen and Cladocera as well as plant pigments can be used together to identify not only the species changes, but also the past habitat structure and ecological processes in the whole community. Various plant and animal macrofossils have been used to infer changes to the composition, architecture and spatial distribution of submerged macrophytes and thus define habitat-based reference conditions for lakes that are not dependent on specific species assemblages (Davidson *et al.* 2005; Davidson *et al.* 2010a & b; Salgado *et al.* 2010). While these latter techniques undoubtedly provide an extraordinary insight into past aquatic communities, their application is currently beyond the scope of this report.

In conclusion, aquatic plant macrofossils provide an insight not only into the past species assemblages that might have populated ‘reference’ communities in lakes, but also it can be demonstrated that these assemblages have in many cases shifted away from the relatively stable conditions of pre-1850. Beta-diversity of sub-fossil plant remains can be used to assess the direction and magnitude of change away from pre-defined reference conditions and therefore this palaeo-limnological tool represents a valuable asset for both defining reference conditions and for setting restoration targets.

Acknowledgements

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3. Taxonomic composition macrophyte metrics for eutrophication

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3.1. Background

According to the requirements of WFD Annex V, taxonomic composition of macrophytes should be considered, supplementary to abundance, in ecological state assessment. In existing methodologies different taxonomic composition metrics are used, from the relatively simple ones, such as diversity indices, or proportion of functional groups, to more sophisticated ones based on trophic scores of taxa along a pressure gradient (Birk 2010 and extensive literature cited there).

In many European countries aquatic plants have been aggregated into categories according to their requirement to trophic conditions. The lists of taxa being tolerant and sensitive to eutrophication are usually elaborated on the national level (country specific lists) and cannot be applied universally. As emphasised by Schneider (2007) if an index is to be applied in a different country from where it was developed, important local indicator taxa are probably neglected and an adjustment of indicator species list to local conditions is most probably necessary. Therefore, the use of indices elaborated on a national level for any international comparisons may be very limited and a more universal approach is needed.

In this study different macrophyte metrics on taxonomic composition were tested against water quality gradient to determine their usefulness for detecting eutrophication in different GIGs, countries and lake types. The main goal of the work was to indicate a metric responding sufficiently strong to eutrophication and being applicable in different countries that can be further used for intercalibration of existing national methods.

3.2. Data availability and methods

The data for metric testing were derived from the WISER common database which includes macrophyte data from approximately 2000 lake-years from 16 countries. Data were compiled from previous REBECCA dataset supplemented with more recent data provided by project partners and GIGs (e.g. Central-Baltic dataset collected for the 2nd round of intercalibration). For testing the response of macrophyte metrics to eutrophication the mean seasonal concentration of total phosphorus (TP) was used as a pressure proxy. In the common WISER database both, biological and TP data for over 1500 lake-years from 12 countries were available (Table 3.1). Database was dominated by Finnish, Swedish and Norwegian lakes followed by Polish, Latvian and Irish ones (Fig. 3.1). From France, Germany and Denmark only three or two lakes were available therefore they were excluded from further analyses.

Table 3.1. Number of lakes with both biological and chemical data in different countries, GIGs and lake types used for testing the response of macrophyte metrics on taxonomic composition in pressure gradient; * - lakes assigned a priori to NO GIG; ** - lakes assigned a priori to ATL GIG; U - unknown

Country	GIGs					IC Lake Types											Sum
	N	CB	ATL	EC	U	N1	N2a	N2b	N3	N5	N6	N8	CB1	CB2	CB3	U	
FI	403					27	52		149	13	31	90				41	
SE	250															250	
NO	52			177*		21	12	10	5	1	1	2				177	
UK	5	20		28		3	3	1	1				3	16	1	28	
IE	7		13	106**												126	
PL		170											105	65		170	
LV		150											62	50	38	150	
NL		54											16	38		54	
EE		35											13	11	11	35	
BE		7											4	3		7	
LT		7											4	3		7	
RO				17												17	
Sum	717	443	13	17	311	48	67	11	155	14	32	92	207	186	50	639	1501

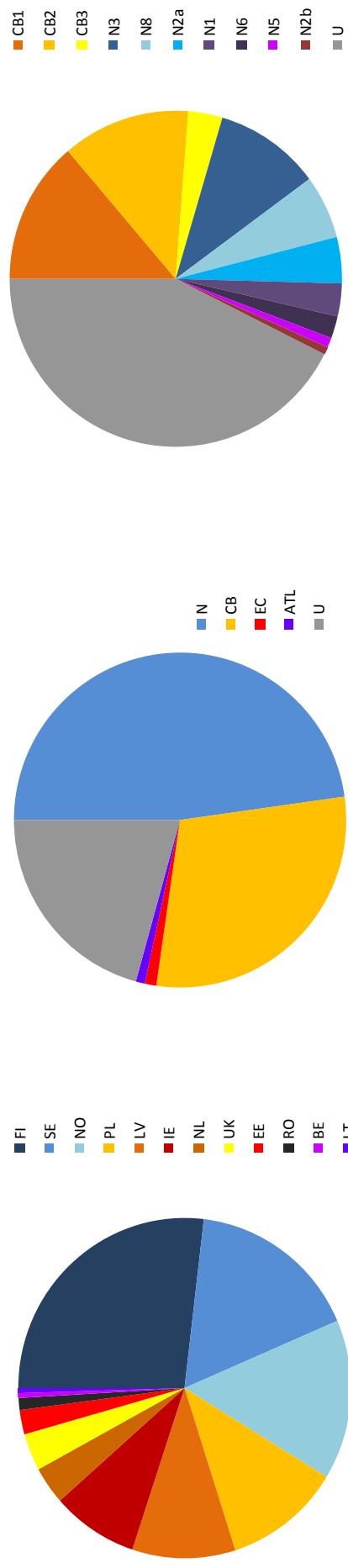


Fig. 3.1. The distribution of lakes used for testing the taxonomic composition metrics in countries, GIGs and common IC types; U - unknown

All the lakes belong to four GIGs (Central-Baltic, Nordic, Atlantic and Eastern Continental), however the EC and ATL GIG were represented by a very few lakes only (17 and 13 respectively). No data from Mediterranean and Alpine GIG were available. Over 20% of the lakes were not assigned to any GIG. All the Swedish, Finnish and Norwegian lakes with no GIG indication were assigned *a priori* to the Nordic GIG and Irish lakes to Atlantic GIG. For only about 60% of lakes (892 lakes) a common IC lake type was clearly indicated. Lakes with no IC type indicated were excluded from the analyses performed for lake types.

Common lake types have been distinguished mainly based on lake geographical distribution (GIGs), altitude, mean depth and alkalinity. In some GIGs also additional specific criteria were applied, e.g. water colour in Nordic GIG (Table 3.2). Thus, the lake type expresses the combination of abiotic environmental conditions.

Table 3.2. The criteria for distinguishing intercalibration common lake types identified in the WISER macrophyte common database

Lake type	Typological criteria
Central Baltic	
L-CB1	Lowland (<200m), shallow (3-15m), calcareous (> 1 meq/l), residence time 1-10
L-CB2	Lowland, very shallow, calcareous, (> 1 meq/l), residence time 0.1-1
L-CB3	Lowland, shallow, small, siliceous moderate alk (0.2-1 meq/l), residence time 1-10
Northern	
LN1	Lowland (<200 m), shallow (3-15 m), moderate alkalinity (0.2-1 meq/L), clear (<30 mg Pt/L)
LN2a	Lowland (<200 m), shallow (3-15 m), low alkalinity (<0.2 meq/L), clear (<30 mg Pt/L)
LN2b	Lowland (<200 m), deep (>15 m), low alkalinity (<0.2 meq/L), clear (<30 mg Pt/L)
LN3a	Lowland (<200 m), shallow (3-15 m), low alkalinity (<0.2 meq/L), humic (30-90 mg Pt/L)
LN5a	Mid-altitude (200-800 m), shallow (3-15 m), low alkalinity (<0.2 meq/L), clear (<30 mg Pt/L)
LN6a	Mid-altitude (200-800 m), shallow (3-15 m), low alkalinity (<0.2 meq/L), humic (30-90 mg Pt/L)
LN8a	Lowland (<200 m), shallow (3-15 m), moderate alkalinity (0.2-1 meq/L), humic (30-90 mg Pt/L)

In the database 400 macrophyte taxa have been recorded including charids, isoetids, elodeids, nympeids, bryids, helophytes and semiterrestrial (supralittoral) plants. Several metrics were used to test the response of macrophyte taxonomic composition to eutrophication gradient in different geographical regions and lake types. It should be stressed that data was collected by applying very diverse procedures, different methodologies of various philosophies, by using different equipment. Also, the abundance estimates used in different countries were extremely diverse. Therefore, the use of presence/absence data in case of all the metrics tested was decided as the most applicable and universal.

Three groups of metrics on taxonomic composition were tested: (i) indices based on trophic scores, (ii) indices based on species richness; (iii) indices based on proportion of functional groups.

- **Intercalibration Common Metric for lake macrophytes (ICM_LM)**

The metric was elaborated by an IC expert (N. Willby) for the purpose of the pan-European intercalibration exercise. Following the procedure described by Birk & Willby (2010) based on an averaging the national assessment results (EQRs) for macrophyte taxa a lake trophic rank

(LTR) has been derived. The LTR scores grade taxa by their response to pressure, mainly nutrient enrichment.

In the WISER macrophyte dataset the LTR for 135 taxa of hydrophytes was indicated ranged from -2.2 for *Tolypella canadensis* to 11.4 for *Lemna minuta* (Appendix 3.1). For all the lakes an Intercalibration Common Index was calculated as an average value of LTRs (based on presence/absence data only).

- **Ellenberg Index (EI)**

Other useful and widely used trophic score system for vascular plants of central Europe was elaborated in 80-ties of XX century by Ellenberg (1988, Ellenber et al. 1991, all in Hill et al. 1999). These trophic scores can be used alternatively to LTR when elaborating macrophyte metric for eutrophication assessment. Since indicator values for phosphorus have not been established by Ellenberg, to calculate the metric, so called Ellenberg Index (EI), macrophyte taxa indicator values for nitrogen (general indicator of environment fertility) were used.

The Ellenberg values for nitrogen (N-score) for 241 macrophyte taxa in the common database both hydrophytes and helophytes were available. 29 taxa were excluded from further analyses as purely terrestrial plants or even trees. For 106 aquatic taxa neither LTR nor Ellenberg scores were available (not included in metric calculating). For 37 aquatic taxa with no Ellenberg value (mainly species from genera: *Callitriche*, *Chara*, *Nitella*, *Tolypella*, *Potamogeton* and *Sparganium*) and with LTR value elaborated, the missing N-scores have been estimated from the LTR-Ellenberg regression equation.

During the intercalibration process the definition of helophytes and its role in lake assessment have been discussed for long. In some countries survey procedures are focused on purely aquatic plants whereas in others emergent vegetation constitutes an important part of the survey methodology. However, the impact of inclusion or exclusion of emergent vegetation on the lake assessment has never been tested. Since the Ellenberg scores were also derived for helophytes, the metric response to TP was tested in two options: when including or excluding emergent vegetation, respectively. For all the lakes in the database the Ellenberg Index was calculated as an average N-score value, both using total number of taxa (EI_TT) and only submerged taxa (EI_ST).

- **Number of taxa (N_TT) and number of taxa submerged (N_ST)**

The species richness was expressed as a number of all taxa identified within a lake (N_TT) and the number of taxa submerged only (N_ST). Since the definitions of ‘real helophyte’ has been discussed for years and a common pan-European list of helophytes has been never agreed, the life form indicated in the common taxa list produced within WISER (available at: <http://www.aqplants.ceh.ac.uk>) was used as a reference.

- **Proportion of characeans (%_char) and isoetids (%_iso) in total number of taxa submerged**

The proportion of taxa from taxonomic groups: characeans (%_char) and isoetids (%_iso) in a number of taxa submerged was calculated. To determine a growth form for taxa the common taxa list (available at: <http://www.aqplants.ceh.ac.uk>) was used as a reference.

Metrics were tested in different countries, GIGs, IC lake types, IC depth types (very shallow <3 m mean depth, shallow 3-15 m and deep >15 m) and IC alkalinity types (low, moderate and high alkalinity).

To improve the data distribution, macrophyte metrics on species richness and TP values were Log-transformed, and metrics on proportion of functional groups square root-transformed. The transformation of ICM_LM and EI did not improve the distribution, thus these metrics remained untransformed. The macrophyte metrics were plotted against TP gradient per country and common lake type. For the linear relationships a linear regression models were applied, and the resulting coefficients of determination (R^2) and correlation (R) were checked. The values of determination coefficient $R^2 > 0.30$ and Pearson’s correlation coefficient $R > 0.55$ were assumed as sufficient to accept a metric as a well performing one. For non-linear relationships a Spearman’s rang correlation was applied and the value of Spearman’s correlation coefficient $R_{Sp} > 0.60$ was considered as acceptable. The differences between various groups of lakes (defined by countries, types, GIGs) in metric values distribution were tested by applying Kruskal-Wallis (linear) or U-Mann-Whitney (non-linear distribution) test.

In the common database 535 lakes indicated by data providers as reference were identified. Out of the 1501 lakes with TP value used for the analyses 487 were declared as reference. They were used to compare metric values in reference and non-reference conditions.

Table 3.3. Number of lake-years in the common macrophyte database indicated by data providers as representing reference conditions; U - unknown

Country	IC Lake Types											Sum	
	N1	N2a	N2b	N3	N5	N6	N8	CB1	CB2	CB3	U		
FI	6	28		55	9	24	28					4	154
SE												97	97
NO	15	10	7	4	1	1	2	7	1			91	139
UK		1	1							3		6	11
IE												46	46
PL								7	1				8
LV									5	1		5	11
NL									5	3			8
EE									1	2			3
BE													0
LT								1	2				3
RO												7	7
Sum	21	39	8	59	10	25	30	15	18	6		257	487

It should be stressed that dataset was collected for the last several years, starting in REBECCA project. The definition and criteria for reference sites have been changed several times. Lakes indicated as reference at different stages of data collection may not meet the final criteria of reference sites, and this information has never been updated. Thus, the results on reference conditions presented in this report should be treated cautiously, as approximate.

3.3 Metrics based on trophic scores

3.3.1 Intercalibration Common Metric for lake macrophytes (ICM_{LM})

The ICM_{LM} values in analysed lakes ranged between 1.75 and 9.47 in total. The overall relationship between ICM_{LM} and logTP in all the lakes analysed was almost linear and reasonably high ($R^2=0,52$, $R=0,72$, $p=0,000$; Fig. 3.2).

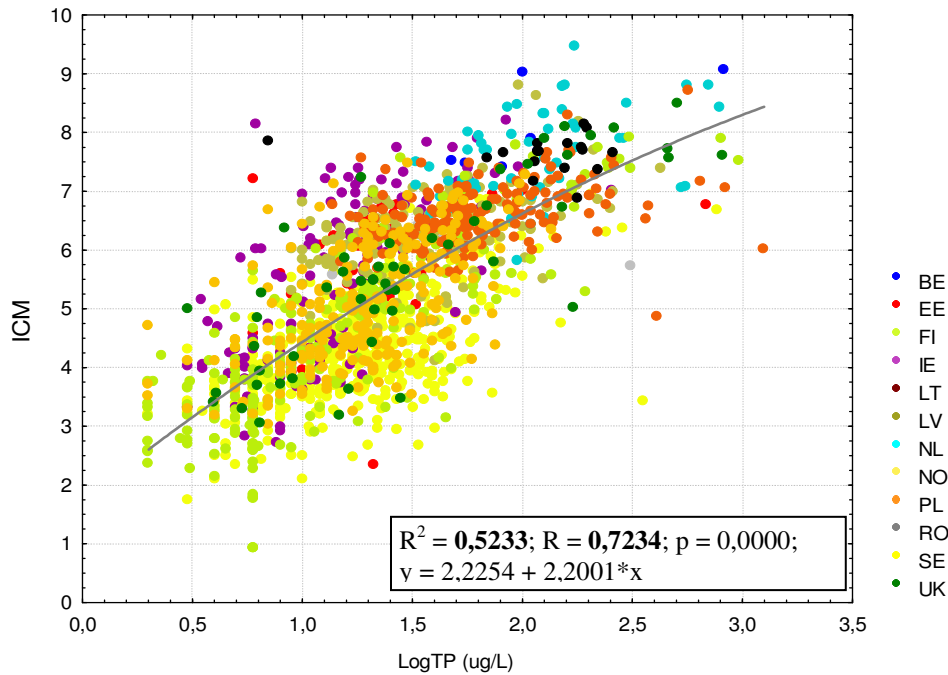


Fig. 3.2. The relationship between ICM_{LM} and TP concentration in the lakes from 12 countries collected in the common WISER macrophyte database (n=1501); the model for linear regression presented

When analysing the ICM_{LM}:TP relationship country by country, the best relationships were found in UK, NO and IE lakes ($R^2>0,6$; $p<0,0000$) and the weakest in PL and EE lakes ($R^2<0,2$). For BE, LT and RO the number of lakes was relatively low and statistically insignificant relationships were found (Table 3.4).

Table 3.4. The relationships between Intercalibration Common Metric for lake macrophytes and TP concentration (ug/L) in lakes in different European countries (in order of strength of relationship); determination coefficient $R^2>0,30$ and Pearson's correlation coefficient $R>0,55$ in linear regression model marked in bold; ns – non-significant at $p>0,05$

Country	n	R^2	R		Regression equation
UK	54	0,668	0,817	$p = 0,000$	$y = 2,6187 + 2,029*x$
NO	230	0,656	0,810	$p = 0,000$	$y = 1,8579 + 2,2484*x$
IE	126	0,450	0,671	$p = 0,000$	$y = 2,417 + 2,653*x$
FI	403	0,332	0,5762	$p = 0,000$	$y = 2,5906 + 1,3551*x$
SE	250	0,312	0,559	$p = 0,000$	$y = 2,9259 + 1,6581*x$
LV	150	0,246	0,496	$p = 0,000$	$y = 4,6197 + 1,0538*x$

Country	n	R ²	R		Regression equation
NL	54	0,207	0,454	p = 0,001	y = 5,5387 + 1,0063*x
PL	175	0,144	0,380	p = 0,000	y = 5,6068 + 0,5525*x
EE	35	0,114	0,338	p = 0,047	y = 4,5953 + 0,9313*x
BE	7	0,533	0,720	ns	ns
LT	7	0,046	0,215	ns	ns
RO	17	0,015	-0,121	ns	ns

When concerning the differences in metric response among GIGs, ICM_LM performed best in Nordic and Atlantic GIG and slightly weaker in Central-Baltic GIG. No significant relationship in Eastern-Continental GIG was found (Table 3.5).

Table 3.5. The relationships between Intercalibration Common Metric for lake macrophytes and TP concentration (ug/L) in lakes in different GIGs (in order of strength of relationship); determination coefficient $R^2 > 0.30$ and Pearson's correlation coefficient $R > 0.55$ in linear regression model marked in bold; ns – non-significant at $p > 0.05$

GIG	n	R ²	R		Regression equation
N	894	0,435	0,659	p = 0,000	y = 2,3088 + 1,8138*x
ATL	119	0,411	0,641	p = 0,000	y = 2,5156 + 2,5108*x
CB	448	0,334	0,578	p = 0,000	y = 4,494 + 1,2068*x
EC	17	0,015	-0,121	ns	ns

When testing the ICM_LM sensitivity in to eutrophication pressure in reference to morphological conditions no distinct differences in metric response in lakes of different depth type were recognised (Fig. 3.3). The correlations, the slopes and the intercepts were very similar in all three depth types (Table 3.5). Therefore, the ICM_LM performs similarly regardless of the lake depth.

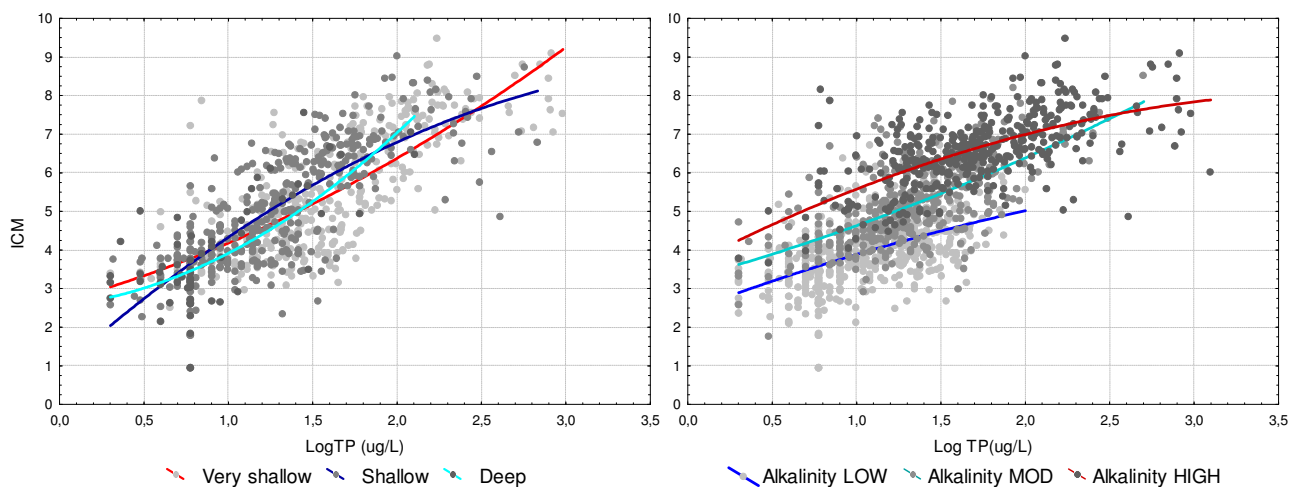


Fig. 3.3. The relationship between ICM_LM and TP concentration in lakes from different depth types (left panel; n=906) and alkalinity types (right panel; n=1389)

For the alkalinity types, the correlations were lower compared to that found for the depth types, the lowest in the case of low alkalinity lakes and slightly higher in moderate and high alkalinity lakes (Fig. 3.3, Table 3.6). In high alkalinity lakes the ICM_LM values were distinctly higher and the intercept was almost twice as high as in low alkalinity lakes. These results indicate that in lakes of a given TP concentration, in high alkalinity lakes ICM_LM indicates worse status than in low alkalinity lakes.

Table 3.6. The relationships between Intercalibration Common Metric for lake macrophytes and TP concentration (ug/L) in lakes of different depth and alkalinity type (in order of strength of relationship); determination coefficient $R^2 > 0.30$ and Pearson's correlation coefficient $R > 0.55$ in linear regression model marked in bold

Type	n	R^2	R		Regression equation
Depth types (VS – very shallow <3 m, S – shallow 3-15 m; D – deep >15 m of mean depth)					
VS	415	0,575	0,759	$p = 0,000$	$y = 1,7937 + 2,3176 * x$
S	423	0,611	0,782	$p = 0,000$	$y = 1,8003 + 2,5119 * x$
D	68	0,523	0,723	$p = 0,000$	$y = 1,6001 + 2,4774 * x$
Alkalinity types (L - low; M – moderate; H - high)					
L	518	0,256	0,506	$p = 0,000$	$y = 2,5912 + 1,2745 * x$
M	356	0,400	0,632	$p = 0,000$	$y = 2,9305 + 1,7035 * x$
H	515	0,393	0,627	$p = 0,000$	$y = 4,4066 + 1,2703 * x$

When considering the ICM_LM distribution in IC lake types a distinct differences in the mean values were found (Fig. 3.4). In Nordic lakes ICM_LM was lower (<5, in general) than in Central-Baltic lake types (>5).

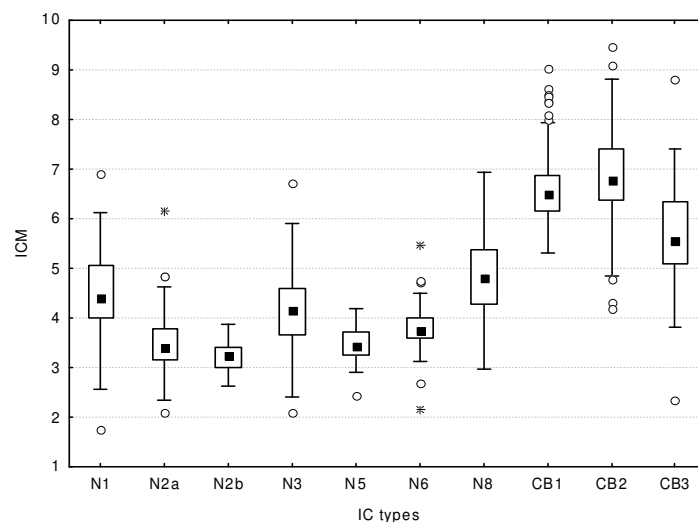


Fig. 3.4. The distribution of ICM_LM values in IC lake types from Nordic and Central-Baltic GIG. Number of lakes in types as in Table 3.1

In different common IC lake types the response of ICM_LM varied considerably (Table 3.7). The metric performed best in two Nordic types, both lowland, shallow and of moderate alkalinity: N1 (clear water type) and N8 (humic water type), followed by three Central-Baltic

lake types. In Nordic low alkalinity lake types the relationships were much weaker (N3, N6) or non-significant (N2a, N2b, N5). The results indicate that the ICM_LM works better in moderate- and high alkalinity lakes and its use in ecosystems of the alkalinity <0.2 meq/L is limited.

Table 3.7. The relationships between Intercalibration Common Metric for lake macrophytes and TP concentration (ug/L) in different European lake types (in order of strength of relationship); determination coefficient $R^2 > 0.30$ and correlation coefficient $R > 0.55$ in linear regression model marked in bold; ns – non-significant at $p > 0.05$

Type	n	R^2	R		Regression equation
ALL	864	0,564	0,751	$p = 0,000$	$y = 1,999 + 2,3424 * x$
N1	49	0,505	0,711	$p = 0,000$	$y = 2,5945 + 1,8711 * x$
N8	92	0,412	0,642	$p = 0,000$	$y = 2,6788 + 1,5539 * x$
CB3	50	0,316	0,563	$p = 0,000$	$y = 2,6999 + 2,0183 * x$
CB1	207	0,307	0,554	$p = 0,000$	$y = 4,9639 + 0,9836 * x$
CB2	186	0,298	0,546	$p = 0,000$	$y = 4,819 + 1,0586 * x$
N3	155	0,274	0,523	$p = 0,000$	$y = 2,4579 + 1,3549 * x$
N6	33	0,250	0,500	$p = 0,004$	$y = 2,4116 + 1,1017 * x$
N2a	67	0,057	0,238	ns	ns
N5	14	0,019	0,137	ns	ns
N2b	11	0,008	0,086	ns	ns

In the lake types where ICM_LM performed best distinct differences in a range of ICM_LM values between Nordic and Central-Baltic types were observed, although the slopes of the regression lines in a linear model were very similar (Fig. 3.5).

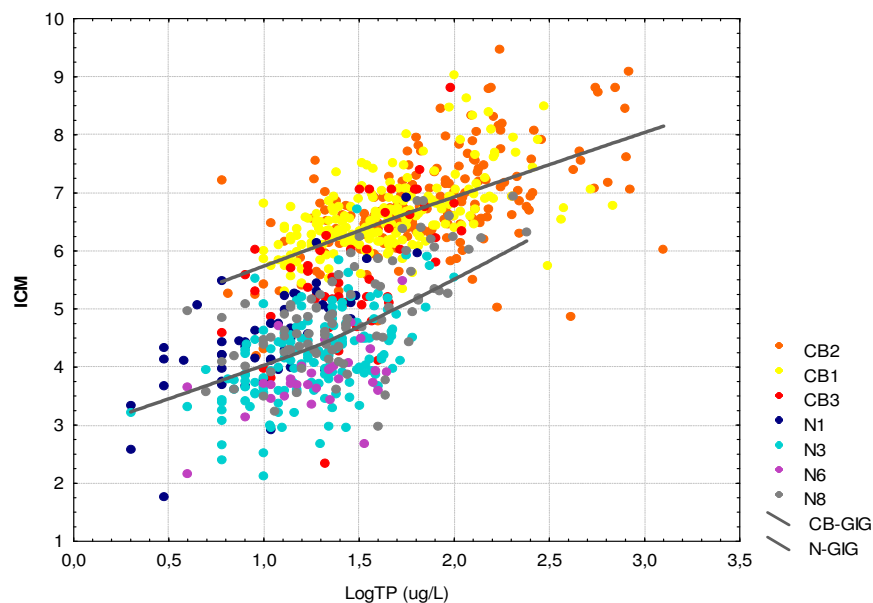


Fig. 3.5. The relationship between ICM_LM and TP in seven N and CB lake types

The median values of ICM_LM in reference compared to all the other lakes showed a statistically significant differences in all the lakes ($p=0.0000$), as well as in three alkalinity lake types (Fig. 3.6).

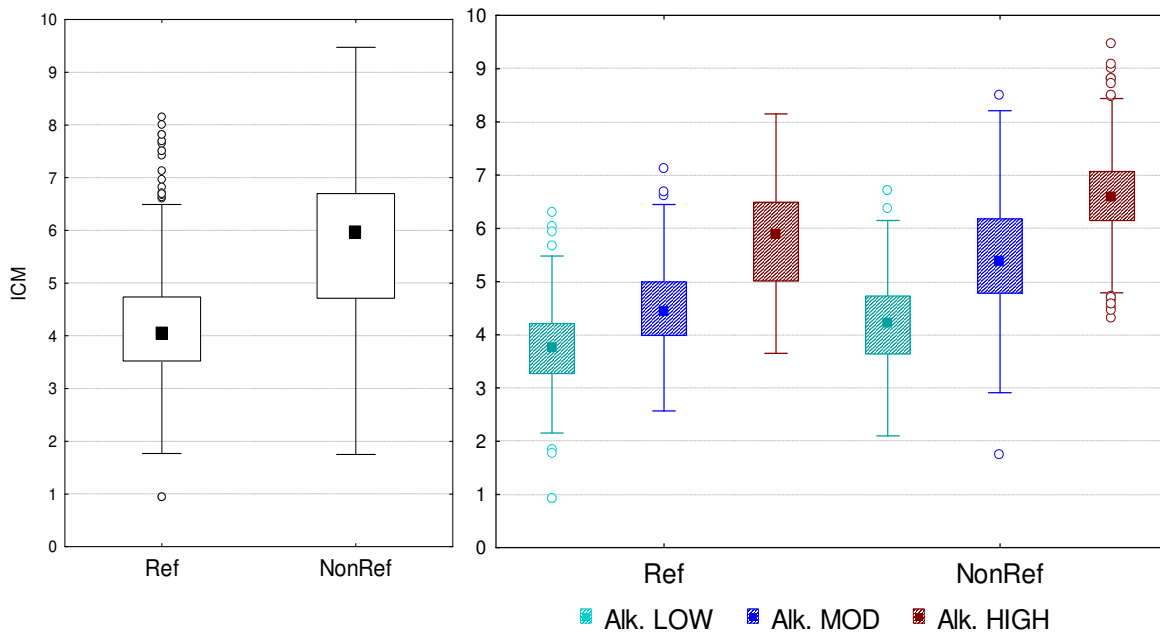


Fig. 3.6. The distribution of ICM_LM values in reference and non-reference lakes in a pool of all the lakes (left) and in three alkalinity lake types (right)

In all the lakes indicated as reference the ICM_LM median value was 4.0 compared to almost 6.0 in all the other lakes (Table 3.8).

Table 3.8. The comparison of the main statistics of ICM_LM values in lakes indicated as reference and all the other lakes in different IC lake types; ns - median values statistically not different (types where ICM_LM:TP relationship insignificant not included); differences significant at $p>0.05$

Type		Reference lakes				Non-reference lakes			
		n	range	median	75perc.	n	range	median	75perc.
ALL	$p=0,000$	487	0,93-8,15	4,05	4,74	1021	1,75-9,47	5,96	6,70
N1	$p=0,000$	21	2,57-5,48	4,11	4,42	28	1,75-6,92	4,99	5,25
N3	$p=0,004$	59	2,40-5,27	3,97	4,31	96	2,11-6,71	4,39	4,75
N5	ns	10	2,44-4,18	3,37	3,58	4	3,38-4,11	3,70	3,94
N6	ns	25	2,15-5,48	3,72	4,01	7	3,62-4,71	3,89	3,97
N8	$p=0,003$	30	3,23-5,46	4,48	5,02	62	2,97-6,94	4,90	5,86
CB1	ns	18	5,44-8,00	6,36	6,70	188	5,30-9,02	6,54	6,91
CB2	$p=0,009$	11	4,18-7,81	6,28	6,55	174	4,31-9,47	6,75	7,24
CB3	$p=0,039$	7	2,34-6,01	5,10	5,70	43	3,81-8,81	5,65	6,62

In low alkalinity lakes reference median value was 3.75 compared to 4.21 in non-reference lakes, in moderate alkalinity 4.42 vs 5.38 and in high alkalinity lakes 5.89 vs 6.58. In Table 3.8 the main statistics for ICM_LM in reference and non-reference lakes representing different IC types are presented. Only in five of the analysed lake types the reference values differed significantly from these in non-reference lakes.

3.3.2 Ellenberg Index (EI)

Both Ellenberg N-score and LTR-score for 98 taxa in the common database were available. The correlation between them was significant and relatively high ($R^2=0.64$, $R=0.80$, $p=0.000$; Fig. 3.7). Therefore, the regression equation was used to calculate missing Ellenberg scores for 37 important aquatic taxa (Appendix 3.1).

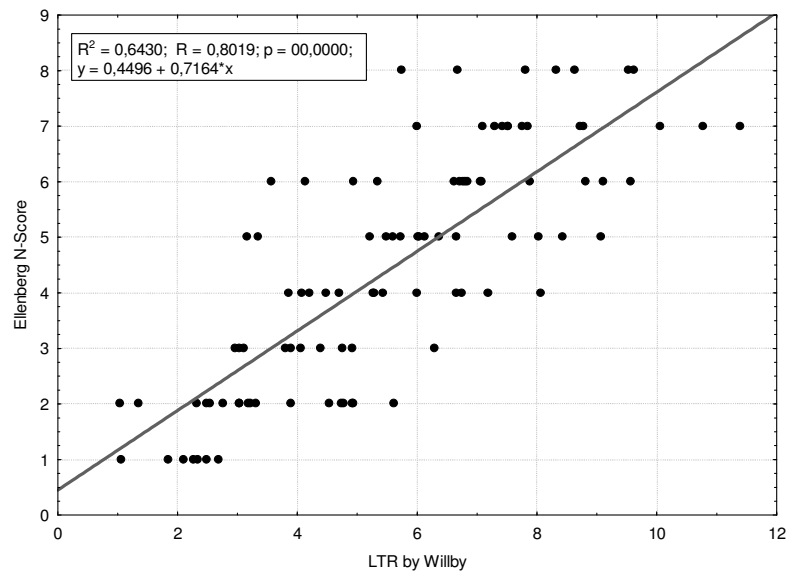


Fig. 3.7. The relationship between lake trophic score (LTR) elaborated by Willby and Ellenberg N-score for 98 macrophyte taxa

The Ellenberg Index values in all analysed lakes ranged between 1,0 and 8,0. Although the overall relationship between Ellenberg Index calculated for all taxa identified (EI_TT) and TP was slightly lower ($R^2=0.47$, $R=0.68$, $p=0.000$) than this found between ICM_LM and TP, it was still relatively high and sufficiently strong to accept EI as a well performing metric (Fig. 3.8). Therefore, the metric was further explored for its usefulness to detect pressure in different countries and lake types.

Since the Ellenberg Index includes trophic scores for both submerged and emergent plants, the influence of including or excluding helophytes on the strength of metric response to pressure was explored.

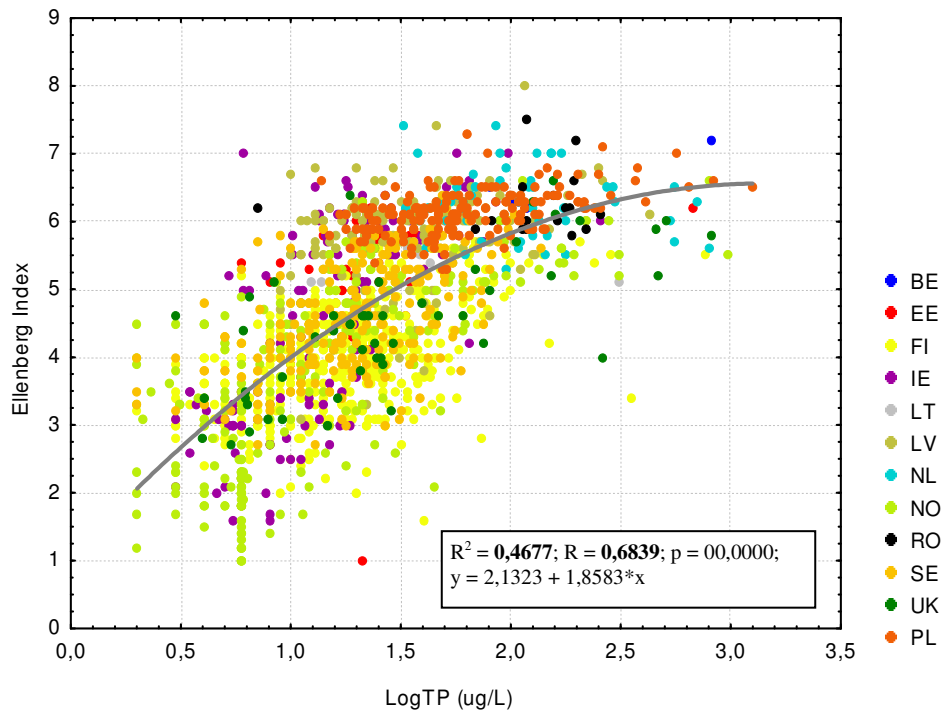


Fig. 3.8. The relationship between Ellenberg Index and total phosphorus concentration in lakes from 12 countries collected in the common WISER macrophyte database (n=1501); gray line represents multinominal model fit

The EI:TP relationships were statistically significant in nine of 12 countries, only in NL, LT and RO were non-significant, both in the case of EI_{TT} and EI_{ST} (Fig. 3.9). In eight countries Ellenberg Index calculated on a basis of submerged taxa only (EI_{ST}) performed worse than the one calculated on total number of taxa (EI_{TT}). The biggest differences in metrics response in BE, SE, FI and PL lakes were recognised, whereas in NO and IE lakes they were almost negligible. The only country where including helophytes weakened the metric diagnostic value was UK. The results indicate that including helophytes improves the strength of metric:TP relationship, in general. Since EI_{TT} performed better than EI_{ST}, it was used for the further exploration.

The highest applicability of EI_{TT} in detecting eutrophication in NO, UK and IE lakes was found (Table 3.9). In Belgium, although the relationship was statistically significant, the number of lakes was too scarce (n=7) to provide a reliable result. In remaining countries the diagnostic value of the index was considerably lower or insignificant.

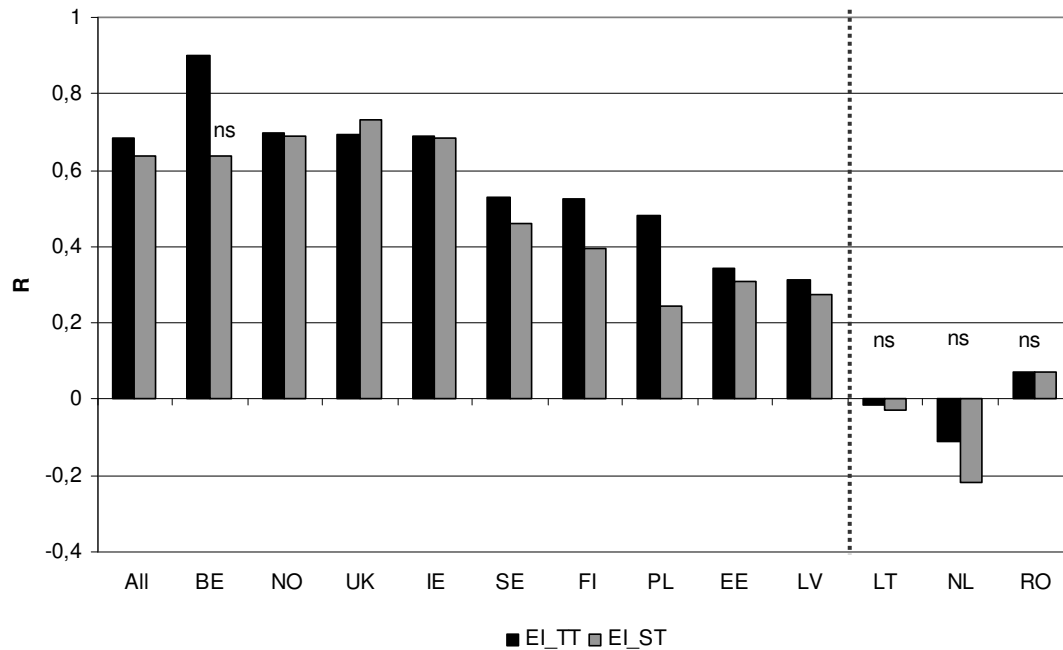


Fig. 3.9. The differences in strength of relationship (Pearson's correlation coefficient R value) between TP and the Ellenberg Index calculated using total number of taxa (EI_TT) or submerged taxa only (EI_ST); ns –relationship non-significant

Table 3.9. The correlations between Ellenberg Index calculated for all taxa (EI_TT) and TP concentration (ug/L) in lakes in different European countries (in order of strength of relationship); determination coefficient $R^2 > 0.30$ and Pearson's correlation coefficient $R > 0.55$ in linear regression model marked in bold; ns – non-significant at $p > 0.05$; *number of lakes too scarce

Country	n	R^2	R		Regression equation
BE	7*	0,808	0,899	$p = 0,006$	$y = 4,7609 + 0,8063 * x$
NO	230	0,486	0,697	$p = 0,000$	$y = 1,6485 + 1,839 * x$
UK	54	0,481	0,694	$p = 0,000$	$y = 2,781 + 1,1573 * x$
IE	126	0,476	0,690	$p = 0,000$	$y = 1,2252 + 2,8729 * x$
SE	250	0,282	0,531	$p = 0,000$	$y = 2,9431 + 1,2678 * x$
FI	403	0,276	0,526	$p = 0,000$	$y = 2,5996 + 1,1012 * x$
PL	175	0,233	0,483	$p = 0,000$	$y = 5,3479 + 0,4439 * x$
EE	35	0,117	0,343	$p = 0,044$	$y = 3,9827 + 0,9058 * x$
LV	150	0,097	0,311	$p = 0,000$	$y = 4,8817 + 0,5995 * x$
NL	54	0,012	0,110	ns	ns
RO	17	0,005	0,071	ns	ns
LT	7	0,000	-0,016	ns	ns

In lakes in different depth and alkalinity classes the EI_TT performed similarly (Fig. 3.10). In a range of TP 0-1000 ug/L in very shallow lakes and also low- and moderate

alkalinity lakes the relationship was linear. In shallow and high alkalinity lakes the index tended to have more logarithmic and in deep more exponential distribution.

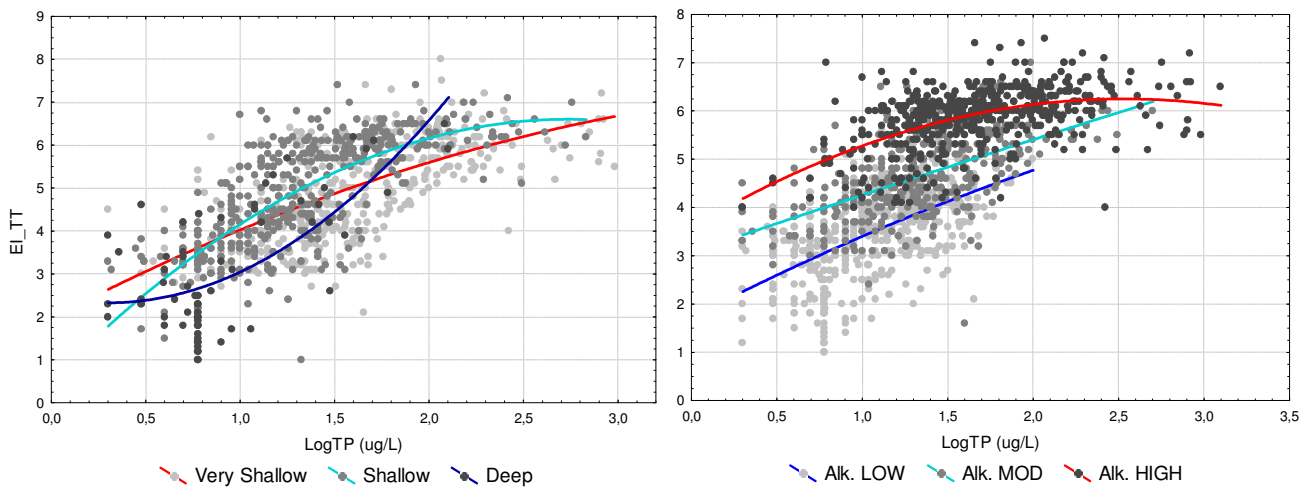


Fig. 3.10. The relationship between EI_{TT} and TP concentration in lakes in different depth types ($n=906$) and alkalinity types ($n=1389$)

The best relationships in shallow and deep lakes, and slightly worse in very shallow lakes were found (Table 3.10). In three alkalinity types the values of correlation coefficient were lower than in depth types, the lowest in high alkalinity lakes (the only below the threshold value $R=0.55$).

Table 3.10. The relationships between Ellenberg Index (EI_{TT}) and TP concentration ($\mu\text{g/L}$) in lakes of different depth and alkalinity type (in order of strength of relationship); determination coefficient $R^2 > 0.30$ and Pearson's correlation coefficient $R > 0.55$ in linear regression model marked in bold

Type	n	R^2	R		Regression equation
Depth types (VS – very shallow <3 m, S – shallow 3-15 m; D – deep >15 m of mean depth)					
VS	415	0,395	0,628	$p = 0,000$	$y = 2,596 + 1,4854 * x$
S	423	0,520	0,721	$p = 0,000$	$y = 2,0635 + 2,0828 * x$
D	68	0,461	0,679	$p = 0,000$	$y = 0,8566 + 2,465 * x$
Alkalinity types (L - low; M – moderate; H - high)					
L	518	0,306	0,553	$p = 0,000$	$y = 1,8825 + 1,5013 * x$
M	356	0,312	0,559	$p = 0,000$	$y = 3,1015 + 1,1547 * x$
H	515	0,225	0,474	$p = 0,000$	$y = 4,768 + 0,6528 * x$

When considering the IC lake types separately, the correlations were best in four Nordic low and moderate alkalinity lake types, and slightly lower in three Central-Baltic lake types. Only in three Nordic types the correlation coefficient exceeded the threshold value for well performing metric of $R=0.55$ (Table 3.11).

Table 3.11. The relationships between Ellenberg Index (EI_{TT}) and TP concentration (ug/L) in different European lake types (in order of strength of relationship); determination coefficient $R^2 > 0.30$ and correlation coefficient $R > 0.55$ in linear regression model marked in bold; ns – non-significant at $p > 0.05$

Type	n	R ²	R	p	Regression equation
N-GIG	894	0,280	0,529	p = 0,000	y = 2,4591 + 1,2437*x
CB-GIG	443	0,163	0,403	p = 0,000	y = 4,8249 + 0,6427*x
N1	49	0,374	0,611	p = 0,000	y = 2,8404 + 1,2183*x
N8	92	0,310	0,556	p = 0,000	y = 2,9578 + 1,088*x
N6	33	0,301	0,549	p = 0,001	y = 2,2228 + 1,0342*x
N3	155	0,279	0,528	p = 0,000	y = 1,9834 + 1,4828*x
CB3	50	0,200	0,447	p = 0,001	y = 2,8827 + 1,4589*x
CB1	207	0,154	0,393	p = 0,000	y = 5,3218 + 0,4558*x
CB2	186	0,145	0,381	p = 0,000	y = 5,0942 + 0,494*x
N2a	67	0,031	0,175	ns	ns
N5	14	0,005	0,069	ns	ns
N2b	11	0,004	0,064	ns	ns

The median values of EI_{TT} in reference compared to all the other lakes showed a statistically significant differences in all the lakes ($p=0.0000$), as well as in three alkalinity lake types (Fig. 3.11). In all the lakes indicated as reference the EI_{TT} median value was 3.8 compared to 5.3 in all the other lakes (Table 3.12) Only in three lake types the reference values differed significantly from these in non-reference lakes.

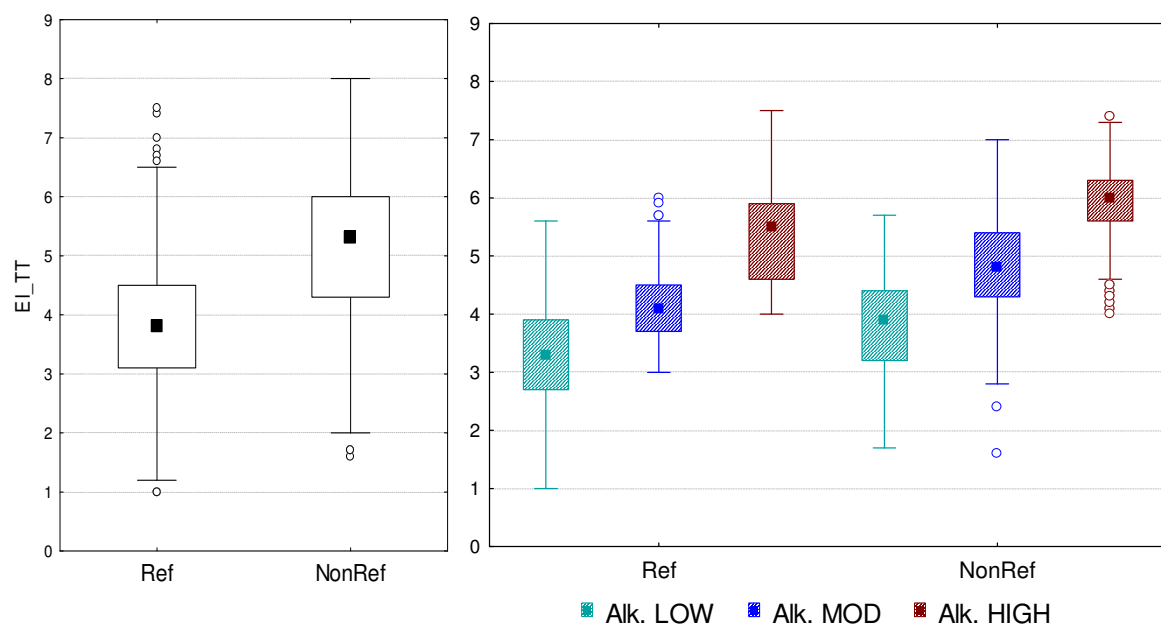


Fig. 3.11. The distribution of EI_{TT} values in reference and non-reference lakes in a poll of all the lakes (left panel) and in three alkalinity lake types (right panel)

Table 3.12. The comparison of the main statistics of EI_TT values in lakes indicated as reference and all the other lakes in IC lake types (types where EI_TT:TP relationship insignificant not included); differences significant at $p > 0,05$

Type		Reference lakes				Non-reference lakes			
		n	range	median	75perc.	n	range	median	75perc.
ALL	p=0,000	487	1,0-7,5	3,8	4,5	1021	1,6-8,0	5,3	6,0
N1	p=0,000	21	3,1-5,2	3,7	4,0	28	2,4-5,6	4,4	4,8
N3	ns	59	1,2-5,0	3,7	4,2	96	2,0-5,7	4,0	4,5
N5	ns	10	2,2-4,0	3,2	3,4	4	3,1-3,5	3,2	3,4
N6	ns	25	2,0-4,4	3,6	3,8	7	3,3-4,2	3,8	3,9
N8	p=0,003	30	3,1-5,0	4,2	4,6	62	1,6-5,7	4,6	5,3
CB1	ns	18	5,1-7,4	5,9	6,5	189	5,0-8,0	6,1	6,3
CB2	p=0,001	11	3,7-6,4	5,5	6,0	174	4,0-7,4	6,1	6,3
CB3	ns	7	1,0-5,5	5,0	5,3	43	3,1-6,4	5,0	6,0

The Ellenberg Index was recognised as relatively well performing one, however its usefulness for detecting eutrophication in different countries and lake types appeared to be lower than as it was proved in the case of ICM_LM. Although the EI was related purely to nitrogen and the ICM_LM to fosforus the relationship between these two indices was high ($R^2=0.85$, $R=0.92$, $p=0.000$; Fig. 3.12).

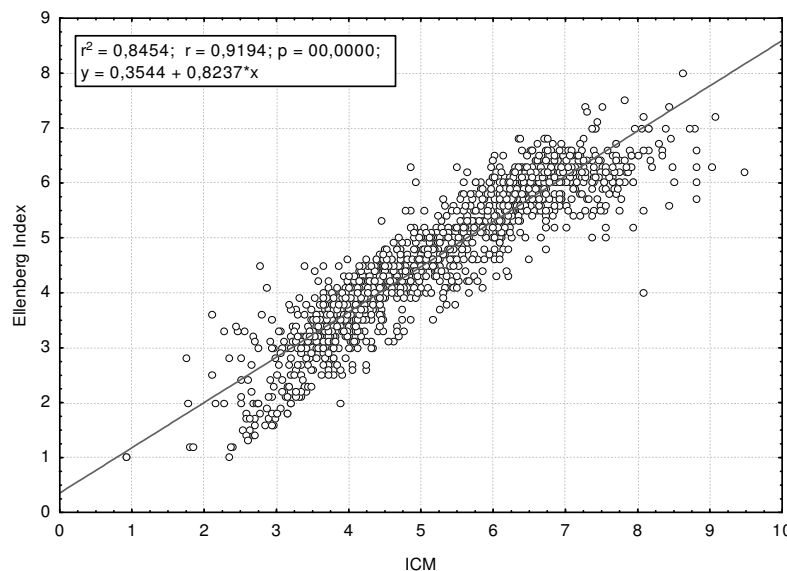


Fig. 3.12. The relationship between Intercalibration Common Metric (ICM calculated on LTR elaborated by Willby) and Ellenberg Index (EI calculated on N-score)

In countries where macrophyte-based assessment methods have not been developed yet and no trophic scores for local flora is available, the well known and widely applicable Ellenberg Index can be considered as taxonomic composition component at first.

3.4 Metrics based on species richness

3.4.1 Total number of taxa (N_{TT}) and number of submerged taxa (N_{ST})

The number of taxa in analysed lakes ranged between 1 and over 60. The lowest mean number of all taxa was present in IE, BE, NL, LV and NO lakes (<15, in general), and the highest in EE, FI, SE and UK lakes (>20). Additionally, the comparison of the number of all taxa (N_{TT}) and taxa submerged only (N_{ST}) proved that in lakes from EE, PL, FI, SE and UK there are distinct differences in these two metric values (Fig. 3.13). In these countries helophytes are considered as an important part of aquatic vegetation and they are included in the sampling procedure. In IE, NL, NO, RO, LV and BE no major differences between N_{TT} and N_{ST} were found. In these countries most probably helophytes are either very sparse due to natural conditions or not included in the sampling procedure.

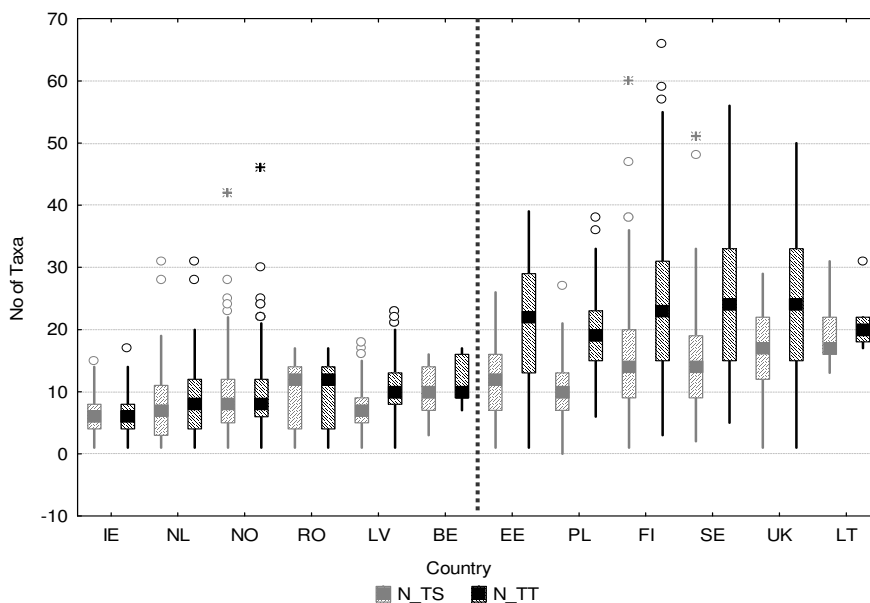


Fig. 3.13. The distribution of number of all taxa (N_{TT}) and number of taxa submerged (N_{TS}) in lakes from 12 European countries

In all the lakes analysed the relationships between TP and total number of taxa, and number of submerged taxa were unimodal (Fig. 3.14), thus Spearman test was used in analyses. In the pool of all lakes the Spearman correlation coefficient was significant, very low however, only in the case of N_{TT} ($R_{Sp}=0.05$, $p=0.04$) and non-significant in the case of N_{ST}.

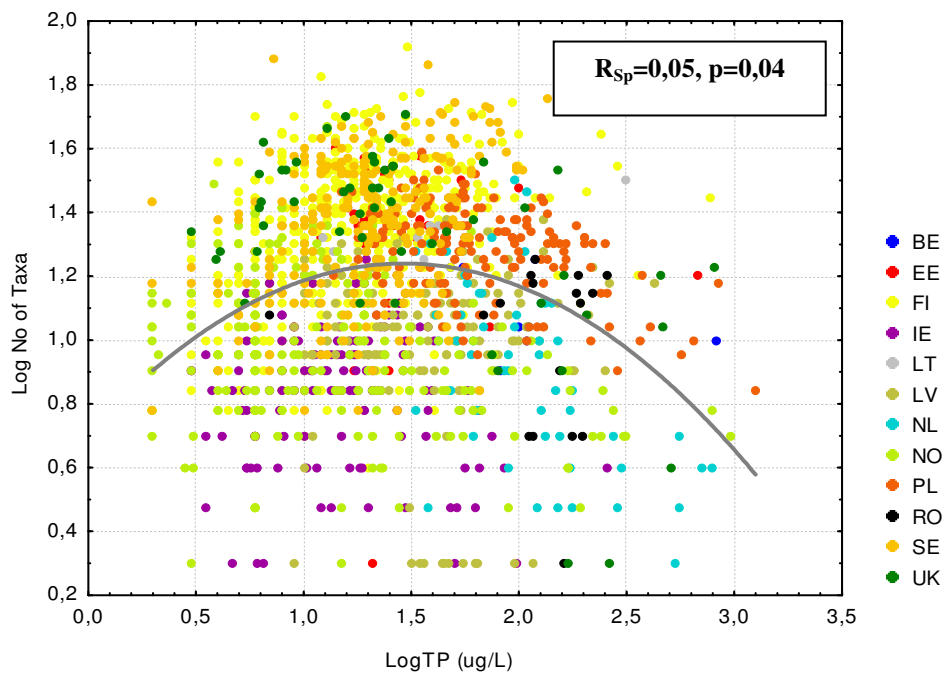


Fig. 3.14. The relationship between number of all taxa (N_{TT}) and TP concentration in all the lakes from all the countries analysed ($n=1501$)

When testing the relationships country by country, both N_{TT} and N_{ST} were significantly correlated with TP in six countries, in FI and SE positively and in NL, UK, PL and NO negatively (Fig. 3.15, Table 3.13). In LV only the metric based on submerged taxa was significantly correlated with TP gradient. Based on the analyses of the directions and strength of relationships in six countries where metrics responded significantly, the shift in metric response was observed at the TP value about 15-20 ug/L.

The comparison of the strength of metric response to TP in two variants: when including or excluding helophytes, demonstrated clear differences. In countries where metrics were positively correlated with pressure, the exclusion of helophytes reduced the strength of the metric:TP relationship, whereas in most countries with negative correlation (except for UK) a metric based on submerged taxa only had a stronger diagnostic value than this based on total number of taxa.

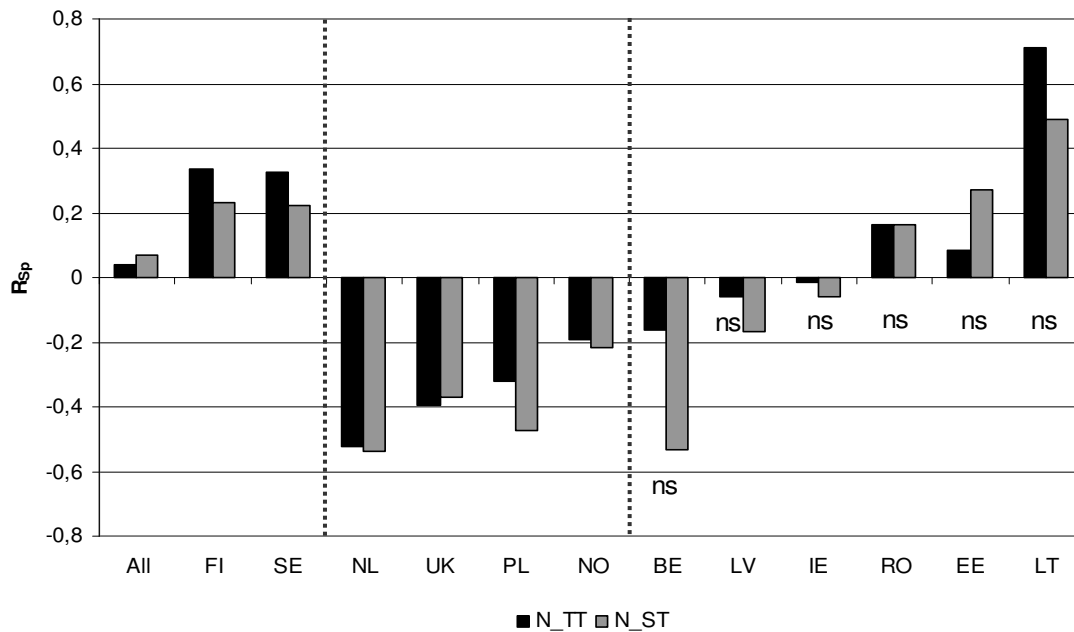


Fig. 3.15. The differences in strength of relationship (Spearman's correlation coefficient R value) between TP and the total number of taxa (N_{TT}) and submerged taxa only (N_{ST})

Table 3.13. The relationship between total number of macrophyte taxa (N_{TT}) and number of submerged taxa (N_{ST}), and TP concentration ($\mu\text{g/L}$) in lakes in different European countries; statistically significant Spearman's correlation coefficient values R_{Sp} , marked in bold; ns – non-significant at $p > 0,05$; \uparrow - strengthening; \downarrow - weakening of the metric:TP relationship when excluding helophytes

Country	n	N_TT		N_St		Direction of change
		R_{Sp}	p	R_{Sp}	p	
Positive relationship with TP						
FI	403	0,337	0,000	0,231	0,000	\downarrow
SE	250	0,324	0,000	0,222	0,000	\downarrow
Negative relationship with TP						
NL	54	-0,524	0,000	-0,539	0,000	\uparrow
UK	52	-0,396	0,000	-0,368	0,007	\downarrow
PL	170	-0,320	0,000	-0,476	0,000	\uparrow
NO	229	-0,192	0,004	-0,217	0,001	\uparrow
LV	150	-0,061	ns	-0,168	0,039	\uparrow
No statistically significant relationship with TP						
BE	7	-0,164	ns	-0,536	ns	-
IE	126	-0,017	ns	-0,057	ns	-
EE	35	0,086	ns	0,274	ns	-
RO	17	0,164	ns	0,164	ns	-
LT	7	0,709	ns	0,487	ns	-

Interestingly, including helophytes affected the TP:metric relationship in countries where sampling procedure seems not to be focused on emergent vegetation (see Fig. 3.13). One of the reasons may be a lack of clear definition of what is a real helophyte. Thus, some taxa considered as helophyte according to the common taxa list used in this analysis may be not considered as helophyte in a given country). This issue has been discussed for long within IC process and also REBECCA and WISER project.

When comparing the N_TT and N_ST response to eutrophication in different lake types, excluding helophytes significantly improved the relationships in all Central-Baltic lake types and reduced the strength of relationships in almost all Nordic lake types (Table 3.14).

Based on these results it may be assumed that in eutrophic ecosystems the increase of the number of helophyte taxa along with increasing a TP level compensates the decrease the number of submerged taxa, and this diminishes the metric diagnostic value. Such as phenomenon was not observed in Nordic ecosystems where increase of trophy results in increasing the number of both hydrophyte and helophyte taxa. In general, species richness is higher in habitats ranging from mesotrophic to eutrophic and lower in nutrient-poor oligotrophic and dystrophic and nutrient-rich hypereutrophic conditions. Such a phenomenon is well known and has been described by many authors (e.g Rørslett 1991, Toivonen and Huttunen 1995, Murphy 2002). Nevertheless, the use of species richness for assessing eutrophication process due to a relatively poor metric response is very limited. To some extent the number of all taxa may be used in some Nordic and number of submerged taxa in some Central-Baltic countries or lake types. However, the potential use of these metrics for IC purposes is doubtful.

Table 3.14. The relationship between total number of macrophyte taxa (N_TT) and number of submerged taxa (N_ST), and TP concentration (ug/L) in lakes in different GIGs and IC lake types; all statistically significant Spearman's correlation coefficient values R_{Sp} marked in bold; ns – non-significant at $p > 0,05$; ↑ - strengthening; ↓ - weakening of the metric:TP relationship when excluding helophytes

GIG/type	n	N_TT		N_TS		Direction
		R_{Sp}	p	R_{Sp}	p	
ATL-GIG	119	-0,036	ns	-0,081	ns	-
CB-GIG	443	-0,163	0,001	-0,236	0,000	↑
CB1	207	-0,080	ns	-0,181	0,009	↑
CB2	186	-0,299	0,000	-0,348	0,000	↑
CB3	50	-0,254	ns	-0,303	0,032	↑
N-GIG	894	0,281	0,000	0,171	0,000	↓
N1	49	0,289	0,044	0,149	ns	↓
N2a	67	0,196	ns	0,143	ns	-
N2b	11	-0,402	ns	-0,452	ns	-
N3	155	0,305	0,000	0,213	0,008	↓
N5	14	0,565	0,035	0,607	0,021	↑
N6	32	-0,087	ns	-0,225	ns	-
N8	92	0,265	0,011	0,075	ns	↓

Based on the analyses of the median values of N_{TT} in reference and non-reference lakes the only statistically significant differences ($p < 0.05$) in lakes from two countries (NO and SE), one alkalinity type (low alkalinity lakes) and one lake type (LCB2) were found. In remaining countries and IC types the number of taxa in reference lakes didn't differ from this identified in impacted lakes.

3.4.2 Number of taxa form functional groups: charids (N_{char}) and isoetids (N_{iso})

Stoneworts are known to play a distinctive role of in non-impacted clear-water lakes of a high calcium concentration (Krause 1981, Schwarz et al. 1999, van den Berg 1999). A negative influence of anthropogenic pressure and accompanied increase of water trophy and decrease of water transparency on occurrence, abundance, biomass and colonisation depth of *Chara*-species is well recognised (van den Berg et al. 1999, Blindow et al. 2002). In soft-water lakes isoetids adapted to successful growth in carbon-limited oligotrophic waters are considered to be reliable indicators of environmental changes caused by human activity (Murphy 2002 and extensive literature quoted there). Therefore, the possibility of using these two groups of aquatic plants in assessing eutrophication process in lakes of various alkalinity levels was explored.

The overall relationships between number of characeans (N_{char}) and number of isoetids (N_{iso}) in all the lakes were statistically significant, very poor in the case of N_{char} ($R_{Sp} = -0.07$, $p = 0.008$) and insufficiently strong in the case of N_{iso} ($R_{Sp} = -0.46$, $p = 0.000$) (Fig. 3.16).

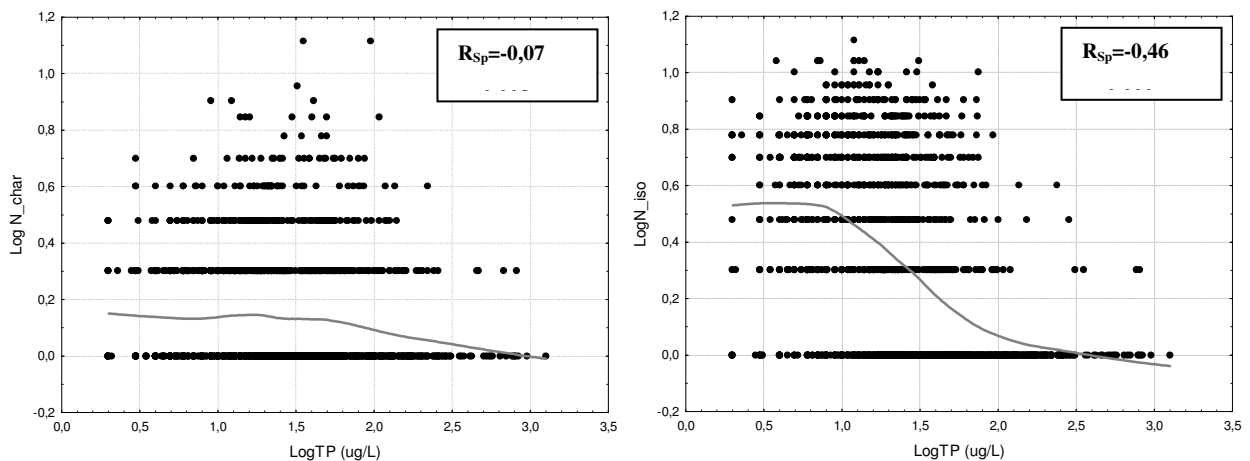


Fig. 3.16. The relationship between total number of characeans (N_{char}) and total number of isoetids (N_{iso}), and TP concentration (ug/L) in all the lakes analysed (n=1501); gray line represents a lowess model fit

In lakes of different alkalinity level N_{char} correlated best with TP in moderate and high alkalinity lakes, and N_{iso} in moderate alkalinity lakes (Table 3.15). None of the relationships achieved a threshold value of correlation coefficient R_{Sp} set for well responding metrics.

Table 3.15. The relationship between total number of characeans (N_{char}) and number of isoetids (N_{iso}), and TP concentration ($\mu\text{g/L}$) in lakes in different IC lake types; all statistically significant Spearman's correlation coefficient values R_{Sp} marked in bold; ns – non-significant at $p > 0,05$

Alkalinity type	n	N_char		N_iso	
		R_{Sp}	p	R_{Sp}	p
L	518	-0,088	0,046	0,010	ns
M	356	-0,326	0,000	-0,276	0,000
H	515	-0,324	0,000	-0,106	0,016

The only statistically significant relationships were found between TP and N_{char} in two highly alkaline Central-Baltic lake types (CB1 and CB2), and between TP and N_{iso} in one low alkalinity Nordic (N5) and all three Central-Baltic lake types (Table 3.16). A surprisingly high correlation of TP: N_{iso} in lake type CB1 was caused by the presence of one species considered as isoetid (*Eleocharis acicularis*) in Latvian and Lithuanian highly alkaline lakes.

Table 3.16. The relationship between total number of characeans (N_{char}) and number of isoetids (N_{iso}), and TP concentration ($\mu\text{g/L}$) in lakes in different IC lake types; all statistically significant Spearman's correlation coefficient values R_{Sp} marked in bold; ns – non-significant at $p > 0,05$

IC lake type	n	N_char		N_iso	
		R_{Sp}	p	R_{Sp}	p
All lakes	1501	-0,068	0,008	-0,461	0,000
CB1	207	-0,260	0,000	-0,511	0,000
CB2	186	-0,425	0,000	-0,163	0,027
CB3	50	-0,273	ns	-0,358	0,011
N1	49	-0,183	ns	0,157	ns
N2a	67	-0,148	ns	0,053	ns
N2b	11	-0,075	ns	0,422	ns
N3	155	0,100	ns	-0,094	ns
N5	14	-0,019	ns	0,547	0,043
N6	32	0,089	ns	-0,324	ns
N8	92	-0,159	ns	-0,129	ns

Since the metrics response in most cases was insignificant or very weak only (in all the cases $R_{Sp} < 0.60$) they cannot be recommended as promising and useful for detecting the impact of eutrophication.

3.4.3 Proportion of characeans (% char) and isoetids (% iso) in number of submerged taxa

Since neither number of taxa (N_{TT}) nor number of taxa from functional groups (N_{char}, N_{iso}) appeared to be a reliable and universally applicable metrics for assessment of impact of eutrophication process in European lakes the possibility of using metrics based on the proportion of functional groups (characeans and isoetids) in total number of submerged taxa have been explored. The overall relationships between proportion of characeans (%_{char}) and isoetids (%_{iso}) in submerged taxa in all the lakes were statistically significant, very poor in the case of %_{char} ($R_{Sp}=-0.08$, $p=0.002$) but relatively strong in the case of %_{iso} ($R_{Sp}=-0.53$, $p=0.000$) (Fig. 3.17).

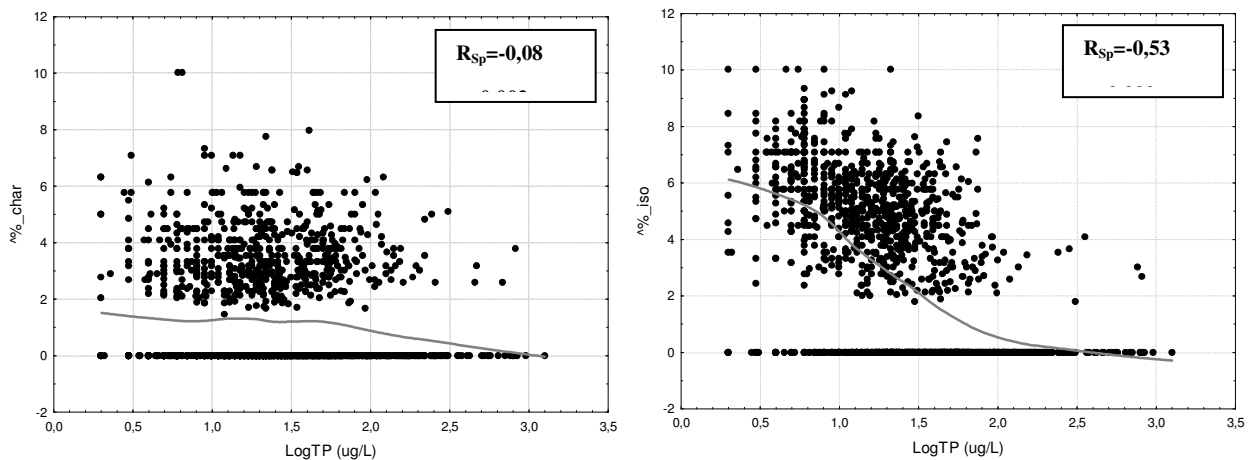


Fig. 3.17. The relationship between total number of characeans (N_{char}) and total number of isoetids (N_{iso}), and TP concentration (ug/L) in all the lakes analysed (n=1501); grey line represents a lowess model fit

In lakes representing different alkalinity level %_{char} correlated best with TP in moderate and high alkalinity lakes, and %_{iso} in low and moderate alkalinity lakes (Table 3.17) in none of the alkalinity types reaching a determined threshold value of correlation coefficient $R_{Sp} \geq 0.60$.

Table 3.17. The relationship between proportion of characeans (%_{char}) and isoetids (%_{iso}) in total number of submerged taxa, and TP concentration (ug/L) in lakes in different IC lake types; all statistically significant Spearman's correlation coefficient values R_{Sp} marked in bold; ns – non-significant at $p > 0,05$

Alkalinity type	n	% _{char}		% _{iso}	
		R_{Sp}	p	R_{Sp}	p
L	518	-0,115	0,009	-0,355	0,000
M	356	-0,334	0,000	-0,301	0,000
H	515	-0,355	0,000	-0,104	0,019

The only statistically significant relationships were found between TP and %_{char} in all three Central-Baltic lake types (the highest in LCB2 and the lowest in LCB3), and between TP and %_{iso} in two Central-Baltic and two Nordic lake types (Table 3.18). In none of the lake type the Spearman correlation coefficient reached threshold value $R_{Sp} \geq 0,60$.

Table 3.18. The relationship between proportion of characeans (%_char) and proportion of isoetids (%_iso) in total number of submerged taxa, and TP concentration (ug/L) in lakes in different IC lake types; all statistically significant Spearman's correlation coefficient values R_{Sp} marked in bold; ns – non-significant at $p>0,05$

IC lake type	n	%_char		%_iso	
		R_{Sp}	p	R_{Sp}	p
All lakes	1501	-0,079	0,002	-0,531	0,000
CB1	207	-0,332	0,000	-0,077	ns
CB2	186	-0,418	0,000	-0,156	0,034
CB3	50	-0,263	ns	-0,366	0,009
N1	48	-0,196	ns	-0,026	ns
N2a	67	-0,193	ns	-0,111	ns
N2b	11	-0,109	ns	0,679	0,022
N3	155	0,103	ns	-0,262	0,001
N5	14	-0,100	ns	0,034	ns
N6	32	0,083	ns	-0,346	ns
N8	92	-0,161	ns	-0,184	ns

3.5 Conclusions:

- The best performing metrics were these based on trophic scores – **Intercalibration Common Metric (ICM_LM) and Ellenberg Index (EI)**. They can be recommended in many countries and lakes types as common metrics for IC purposes.
- The ICM_LM performed better in moderate- and high alkalinity lakes and its use in ecosystems of the alkalinity $<0,2$ meq/L may be limited.
- The Ellenberg Index was a relatively well performing metric, however its usefulness for detecting eutrophication in different countries and lake types appeared to be lower than as it was proved in the case of ICM_LM. In countries where macrophyte-based assessment methods have not been developed yet and no trophic scores for local flora is available, the well known and widely applicable Ellenberg Index can be considered as taxonomic composition component at first.
- When using EI it is recommended to include helophytes when recorded since the assessment of eutrophication seems to be more reliable when more scored taxa are considered. The higher the number of the species with an indicative value the more reliable the assessment.
- In Nordic lakes the overall number of taxa responded better to pressure gradient than the number of submerged taxa only, whereas in Central Baltic lakes the number of submerged taxa had a higher diagnostic value. This indicates that in eutrophic ecosystems the increase of the number of helophyte taxa along with increasing a TP level compensates the decrease the number of submerged taxa weakening the metric diagnostic value. This was not observed in ecosystems of lower trophy where increase of trophy results in increasing the number of both hydrophyte and helophyte taxa.
- The metrics based on taxa richness responded much weaker than those based on trophic scores and they cannot be recommended as useful indicators for assessment of eutrophication process.

3.6 References

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Appendix 3.1

The list of macrophyte taxa used for calculating ICM_LM (based on the LTR score elaborated by Willby) and Ellenberg index based on the N-scores by Ellenberg 1991); LTR – Lake Trophic Rank, N-scores derived from the regression of LTR:Ellenber relationship marked with an asterisks

TaxonName	TaxonCode	n	LTR by Willby	N score by Ellenberg
Acorus calamus	ACO1CAL1	189		7
Filamentous algae	ALG1ZZZ1	326	8,78	7
Alisma gramineum	ALI1GRA1	2		4
Alisma lanceolatum	ALI1LAN1	2		5
Alisma plantago-aquatica	ALI1PLA1	343		8
Alopecurus aequalis	ALO1AEQ1	129		9
Apium inundatum	API1INU1	6	5,62	2
Baldellia ranunculoides	BAL1RAN1	9	4,92	2
Berula erecta	BER1ERE1	8		6
Bidens cernua	BID1CER1	17		9
Bidens tripartita	BID1TRI1	47		8
Bolboschoenus maritimus	BOL1MAR1	8		7
Butomus umbellatus	BUT1UMB1	141	8,73	7
Callitriche brutia var. hamulata	CAL1BRU2	109		4
Callitriche cophocarpa	CAL1COP1	32	5,06	4*
Callitriche hermaphroditica	CAL1HER1	117	6,29	3
Callitriche palustris	CAL1PAL1	268	3,59	3*
Callitriche platycarpa	CAL1PLA1	5	10,05	7
Callitriche stagnalis	CAL1STA1	22	5,99	4
Callitriche truncata	CAL1TRU1	4	10,77	7
Callitriche sp.	CAL1ZZZ1	100	6,85	6
Caltha palustris	CAL3PAL1	211		6
Calystegia sepium	CAL7SEP1	3		9
Cardamine amara	CAR1AMA1	1		4
Cardamine pratensis	CAR1PRA1	5		4
Carex acuta	CAR2ACU1	238		4
Carex acutiformis	CAR2ACU2	38		5
Carex appropinquata	CAR2APP1	1		4
Carex aquatilis	CAR2AQU1	321		4
Carex diandra	CAR2DIA1	25		3
Carex disticha	CAR2DIS1	2		5
Carex elata	CAR2ELA1	108		5
Carex flacca	CAR2FLA1	2		4
Carex hirta	CAR2HIR1	3		5
Carex lasiocarpa	CAR2LAS1	418		3
Carex nigra	CAR2NIG1	27		2
Carex panicea	CAR2PAN1	3		4
Carex paniculata	CAR2PAN2	51		4
Carex pseudocyperus	CAR2PSE1	95		5
Carex riparia	CAR2RIP1	96		4
Carex rostrata	CAR2ROS1	916		3
Carex vesicaria	CAR2VES1	135		5
Carex viridula ssp. oedocarpa	CAR2VIR1	6		2
Carex sp.	CAR2ZZZ1	44		4
Catabrosa aquatica	CAT1AQU1	1		8
Ceratophyllum demersum	CER1DEM1	1276	7,82	8
Ceratophyllum submersum	CER1SUB1	38	7,85	7
Chara aspera	CHA1ASP1	153	4,70	4

TaxonName	TaxonCode	n	LTR by Willby	N score by Ellenberg
Chara contraria	CHA1CON2	88	5,60	5
Chara curta	CHA1CUR1	1	4,07	3*
Chara filiformis	CHA1FIL1	16	2,82	2*
Chara globularis	CHA1GLO1	338	6,80	6
Chara hispida	CHA1HIS1	37	4,48	4*
Chara rudis	CHA1RUD1	47	4,08	3*
Chara strigosa	CHA1STR1	8	5,02	4*
Chara tomentosa	CHA1TOM1	317	5,27	4
Chara virgata	CHA1VIR1	9	5,96	5*
Chara virgata var. annulata	CHA1VIR2	5	4,30	4*
Chara vulgaris	CHA1VUL1	17	6,53	5*
Chara sp.	CHA1ZZZ1	175	6,03	5
Charophyta	CHA2ZZZ1	120	5,41	4*
Cicuta virosa	CIC1VIR1	251		5
Cladium mariscus	CLA2MAR1	6		3
Comarum palustre	COM1PAL1	536		2
Crassula aquatica	CRA2AQU1	24	3,04	2
Crassula helmsii	CRA2HEL1	116		7
Elatine hexandra	ELA1HEX1	28	4,95	2
Elatine hydropiper	ELA1HYD1	148	4,92	3
Elatine orthosperma	ELA1ORT1	3	-0,28	0
Elatine triandra	ELA1TRI1	64	5,21	4*
Eleocharis acicularis	ELE1ACI1	493	4,78	2
Eleocharis multicaulis	ELE1MUL1	10	3,18	2
Eleocharis palustris	ELE1PAL1	512		4
Eleocharis uniglumis	ELE1UNI1	2		5
Eleogiton fluitans	ELE2FLU1	4	1,35	2
Elodea canadensis	ELO1CAN1	559	7,42	7
Elodea nuttallii	ELO1NUT1	154	7,52	7
Enteromorpha sp.	ENT1ZZZ1	18	9,34	7*
Epilobium hirsutum	EPI1HIR1	5		8
Epilobium montanum	EPI1MON1	2		6
Epilobium palustre	EPI1PAL1	3		2
Equisetum arvense	EQU1ARV1	1		3
Equisetum fluviatile	EQU1FLU1	1116		5
Equisetum palustre	EQU1PAL1	12		5
Eriophorum angustifolium	ERI1ANG1	13		2
Eriocaulon aquaticum	ERI2AQU1	22		1
Eupatorium cannabinum	EUP2CAN1	1		8
Filipendula ulmaria	FIL1ULM1	8		5
Fontinalis antipyretica	FON1ANT1	681	5,48	5
Galium palustre	GAL1PAL1	21		4
Glaux maritima	GLA1MAR1	1		5
Glyceria fluitans	GLY1FLU1	137		7
Glyceria maxima	GLY1MAX1	198		9
Hippuris vulgaris	HIP1VUL1	313	4,49	4
Hottonia palustris	HOT1PAL1	1	6,67	4
Hydrilla verticillata	HYD1VER1	7	4,76	3
Hydrocharis morsus-ranae	HYD2MOR1	243	7,09	6
Hydrocotyle vulgaris	HYD3VUL1	17		2
Hypochoeris radiata	HYP1RAD1	1		3
Hypericum elodes	HYP2ELO1	4		1
Iris pseudacorus	IRI1PSE1	227		7
Isoetes echinospora	ISO1ECH1	486	2,48	1

TaxonName	TaxonCode	n	LTR by Willby	N score by Ellenberg
Isoetes lacustris	ISO1LAC1	913	2,35	1
Isoetes sp	ISO1ZZZ1	19	2,59	2*
Juncus acutiflorus	JUN1ACU1	9		3
Juncus articulatus	JUN1ART1	31		2
Juncus bulbosus	JUN1BUL1	265	2,77	2
Juncus conglomeratus	JUN1CON1	3		3
Juncus effusus	JUN1EFF1	31		4
Lagarosiphon major	LAG1MAJ1	176	3,57	6
Lemna gibba	LEM1GIB1	8	9,63	8
Lemna minor	LEM1MIN1	413	8,82	6
Lemna minuta	LEM1MIN2	10	11,40	7
Lemna trisulca	LEM1TRI1	237	8,44	5
Leptodictyum riparium	LEP1RIP1	1	10,00	8*
Limosella aquatica	LIM1AQU1	25	3,90	3
Littorella uniflora	LIT1UNI1	372	3,90	2
Lobelia dortmanna	LOB1DOR1	501	1,86	1
Ludwigia palustris	LUD1PAL1	1		4
Ludwigia peploides	LUD1PEP1	9		4
Luronium natans.	LUR1NAT1	3	4,39	3
Lycopus europaeus	LYC2EUR1	61		7
Lysimachia thysiflora	LYS1THY1	683		4
Lysimachia vulgaris	LYS1VUL1	31		5
Lythrum portula	LYT1POR1	14	4,53	2
Lythrum salicaria	LYT1SAL1	112		5
Mentha aquatica	MEN1AQU1	45		5
Menyanthes trifoliata	MEN2TRI1	529		3
Montia fontana	MON1FON1	9		4
Myosotis scorpioides	MYO1SCO1	34		5
Myriophyllum alterniflorum	MYR1ALT1	776	3,05	3
Myriophyllum sibiricum	MYR1SIB1	86	5,02	4*
Myriophyllum spicatum	MYR1SPI1	815	7,30	7
Myriophyllum verticillatum	MYR1VER2	141	5,74	8
Najas flexilis	NAJ1FLE1	16	3,35	5
Najas marina	NAJ1MAR1	239	6,78	6
Najas minor	NAJ1MIN1	24		5
Najas tenuissima	NAJ1TEN1	5	3,13	3*
Nitella confervacea	NIT1CON1	1	3,65	3*
Nitella flexilis	NIT1FLE1	96	5,28	4
Nitella gracilis	NIT1GRA1	4	4,17	3*
Nitella mucronata	NIT1MUC1	16	6,46	5*
Nitella opaca	NIT1OPA1	195	2,55	2
Nitella translucens	NIT1TRA1	16	4,21	4
Nitella sp.	NIT1ZZZ1	153	3,81	3
Nitellopsis obtusa	NIT2OBT1	207	6,13	5
Nuphar lutea	NUP1LUT1	1792	7,05	6
Nuphar pumila	NUP1PUM1	304	4,73	2
Nuphar x spenneriana	NUP1SPE1	268	3,75	3*
Nymphaea alba	NYM1ALB1	596	6,02	5
Nymphaea candida	NYM1CAN1	170	6,66	5
Nymphaea tetragona	NYM1TET1	28	4,57	4*
Nymphaea alba x candida	NYM1XAL1	10	3,96	3*
Nymphaea sp.	NYM1ZZZ1	12		5
Nymphoides peltata	NYM2PEL1	37	7,76	7
Oenanthe aquatica	OEN1AQU1	6		6

TaxonName	TaxonCode	n	LTR by Wilby	N score by Ellenberg
Oenanthe crocata	OEN1CRO1	1		7
Persicaria amphibia	PER1AMP1	454	8,07	4
Persicaria hydropiper	PER1HYD1	2		5
Persicaria minor	PER1MIN1	1		8
Peucedanum palustre	PEU1PAL1	8		4
Phalaris arundinacea	PHA1ARU1	146		7
Phragmites australis	PHR1AUS1	1776		7
Pilularia globulifera	PIL1GLO1	6	3,31	2
Potamogeton acutifolius	POT1ACU1	3		6
Potamogeton alpinus	POT1ALP1	413	4,13	6
Potamogeton berchtoldii	POT1BER1	446	5,73	5
Potamogeton compressus	POT1COM1	167	5,43	4
Potamogeton crispus	POT1CRI1	200	8,02	5
Potamogeton filiformis	POT1FIL1	94	2,96	3
Potamogeton friesii	POT1FRI1	130	5,35	6
Potamogeton gramineus	POT1GRA1	400	3,17	5
Potamogeton lucens	POT1LUC1	406	6,01	7
Potamogeton natans	POT1NAT1	1053	5,21	5
Potamogeton nodosus	POT1NOD1	3		5
Potamogeton obtusifolius	POT1OBT1	239	7,89	6
Potamogeton pectinatus	POT1PEC1	370	8,64	8
Potamogeton perfoliatus	POT1PER1	1101	4,95	6
Potamogeton polygonifolius	POT1POL1	28	2,49	2
Potamogeton praelongus	POT1PRA1	317	4,08	4
Potamogeton pusillus	POT1PUS1	119	9,10	6
Potamogeton rutilus	POT1RUT1	29	6,37	5
Potamogeton trichoides	POT1TRI1	26	7,19	4
Potamogeton x nitens	POT1XGR2	39	3,69	3*
Potamogeton x sparganiifolius	POT1XSP1	4	2,45	2*
Potamogeton sp.	POT1ZZZ1	42	6,03	5*
Pteridium aquilinum	PTE1AQU1	1		3
Ranunculus aquatilis var. aquatilis	RAN1AQU2	67		5
Ranunculus aquatilis var. diffusus	RAN1AQU3	15		5
Ranunculus circinatus	RAN1CIR1	229	6,68	8
Ranunculus confervoides	RAN1CON1	48	1,54	2*
Ranunculus flammula	RAN1FLA1	30		2
Ranunculus hederaceus	RAN1HED1	6	9,07	5
Ranunculus lingua	RAN1LIN1	82	7,51	7
Ranunculus omiophyllus	RAN1OMI1	2	6,75	4
Ranunculus peltatus ssp. peltatus	RAN1PEL3	335		6
Ranunculus peltatus ssp. baudottii	RAN1PEL4	2		6
Ranunculus penicillatus ssp. penicillatus	RAN1PEN2	2		5
Ranunculus reptans	RAN1REP2	604	3,04	2
Ranunculus sceleratus	RAN1SCE1	3		9
Ranunculus sp.	RAN1ZZZ1	7	3,81	3*
Rorippa amphibia	ROR1AMP1	3		8
Rorippa palustris	ROR1PAL1	9		8
Rumex aquaticus	RUM1AQU1	44		8
Rumex hydrolapathum	RUM1HYD1	86		7
Rumex obtusifolius	RUM1OBT1	2		9
Ruppia cirrhosa	RUP1CIR1	1	7,58	5
Ruppia maritima	RUP1MAR1	3	8,33	8
Sagittaria sagittifolia	SAG1SAG1	225	6,71	6
Sagittaria x lunata	SAG1XSA1	13	6,90	5*

TaxonName	TaxonCode	n	LTR by Willby	N score by Ellenberg
Sagittaria sp.	SAG1ZZZ1	7	5,74	5*
Salix aurita	SAL2AUR1	5		3
Schoenoplectus lacustris	SCH1LAC1	856		6
Schoenoplectus tabernaemontani	SCH1TAB1	25		6
Scirpus sylvaticus	SCI1SYL1	13		4
Scutellaria galericulata	SCU1GAL1	2		6
Senecio aquaticus	SEN1AQU1	3		5
Sium latifolium	SIU1LAT1	14		7
Solanum dulcamara	SOL1DUL1	12		8
Sparganium angustifolium	SPA1ANG1	528	2,69	1
Sparganium emersum	SPA1EME1	488	7,10	7
Sparganium erectum	SPA1ERE1	291		7
Sparganium gramineum	SPA1GRA1	243	3,76	3*
Sparganium hyperboreum	SPA1HYP1	34	-0,48	0
Sparganium natans	SPA1NAT1	137	4,07	3
Sparganium angustifolium x gramineum	SPA1XAN1	22	3,88	3*
Sphagnum sp.	SPH1ZZZ1	29	3,09	3*
Spirodela polyrhiza	SPI1POL1	133	9,57	6
Stellaria palustris	STE1PAL1	1		2
Stratiotes aloides	STR1ALO1	240	6,63	6
Subularia aquatica	SUB1AQU1	385	2,27	1
Thelypteris palustris	THE1PAL1	89		6
Tolypella canadensis	TOL1CAN1	5	-2,22	-1
Tolypella glomerata	TOL1GLO1	2	5,70	5*
Triglochin maritimum	TRI1MAR1	1		5
Typha angustifolia	TYP1ANG1	456		7
Typha latifolia	TYP1LAT1	407		8
Utricularia australis	UTR1AUS1	14	3,11	3
Utricularia intermedia	UTR1INT1	171	2,11	1
Utricularia minor	UTR1MIN1	122	2,32	2
Utricularia ochroleuca	UTR1OCH1	49	1,06	1
Utricularia stygia	UTR1STY1	1	1,04	2
Utricularia vulgaris	UTR1VUL1	575	3,86	4
Utricularia sp.	UTR1ZZZ1	87	3,22	2
Veronica anagallis-aquatica	VER1ANA1	3		6
Veronica beccabunga	VER1BEC1	5		6
Veronica scutellata	VER1SCU1	1		3
Zannichellia palustris	ZAN1PAL1	87	9,53	8

4. Abundance macrophyte metrics for eutrophication

Responsible: Martin Søndergaard Contributors : Seppo Hellsten, Marit Mjelde

4.1 Background

The abundance of submerged macrophytes depends on light availability and, generally, macrophytes can only survive down to a depth where about 20% of surface light is still present (Chambers & Kaiff, 1985) or 4.5% of incident red light (Hellsten, 1997). The depth limit is, however, also species dependent. In a study on 45 Danish lakes Middelboe & Markager (1997) found a zonation with caulescent angiosperms and charophytes growing deepest followed by bryophytes and *Isoetes* spp. in lakes with relatively low Secchi depth (< 7m), while in more transparent lakes bryophytes grew deepest followed by charophytes, caulescent angiosperms and *Isoetes* spp.

Many lakes all over the world has experienced a decrease in the abundance of macrophytes during the past century because increased nutrient availability has increased phytoplankton in lakes, which led to increased turbidity and impoverished light conditions (Blindow et al., 2006; Hilt et al., 2006). In some lakes, as for example many Danish lakes, submerged macrophytes have disappeared completely or been dramatically reduced in abundance (Sand-Jensen et al., 2000; Søndergaard et al., 2010). Thus, submerged macrophyte abundance potentially seems a good candidate metric for describing the pressure from eutrophication. Furthermore, macrophytes play a highly central role in structuring the function of lakes, and their abundance has important implications for the overall lake water quality and also for other biological quality elements used in the ecological classification of lakes (Carpenter & Lodge, 1986; Jeppesen et al., 1997).

Changes in the abundance of submerged macrophytes may be expressed both by coverage and maximum depth of colonization. In this chapter we will explore these two metrics in relation to eutrophication as a pressure. Our focus will be on maximum colonization depth, which has been widely used in the monitoring of lakes and therefore provides the best opportunity for data analysis. Quantitative data on coverage are scarcer and have been analysed in less detail, and mostly derive from Danish lakes.

4.2 Methods, definitions and shortcuts

Lake data were collected from different sources, mainly from the geographical intercalibration groups and the WISER partners. This includes data mainly from the Central-Baltic GIG and the Northern GIG, but these data has been supplemented with country specific data from Polen, UK, Norway, Sweden, Finland and Italy. Many different methods have been applied (underwater samples, rake, aquascope, etc.) and the amount of resources that has been spent probably differs widely for the individual lakes and between countries. This introduces considerable variability

to the dataset which must be considered when interpreting the data and when defining boundaries.

On some lakes data are available for more than one year, but to make the dataset a large possible these are regarded as independent observations although there might be impact from the macrophyte distribution from one year to another.

Only lakes where maximum colonization depth (C_max) has been recorded were included in the analyses. In the general analyses we used maximum depth of colonization recorded in the dataset. This is probably the maximum depth recorded for any submerged macrophyte present, i.e. we have not discriminated between or excluded any macrophyte types, including mosses or poorly rooted macrophytes, and this introduces an unknown source of error, but clear definitions of C_max are not available in the data sets. Correspondingly, the definition of C_max may differ between countries which adds further to the uncertainty.

The categorisation of ecological class into high (H), good (G), moderate (M), poor (P) or bad (B) is solely based on the class reported from the individual data sources. These definitions are not necessarily comparable between countries. To distinguish between different lake types we used data on lake depth and alkalinity. Shallow lakes were defined as lakes with a mean depth < 3 m and deep lakes as lakes with mean depth > 3 m. Siliceous lakes were defined as lakes with a total alkalinity (TA) < 1 meq/l and calcareous lakes as lakes with TA > 1 meq/l. Future work should include data from more lakes types, as for example low alkaline lakes, which probably have other abundance characteristics due to their dominance of isoetids. Data on lake colour were only available from a few lakes, but we assumed (probably not always correctly, however) that if no colour data was reported then the colour was < 100 mgPt/l. A special section on the use of C_max in coloured lakes is included based on experience from Finland.

Coverage of macrophytes represents mean macrophyte coverage relative to the whole lake area (% of total lake area). The use of coverage is only relevant for shallow lakes where most the lake bottom potentially can be colonized by macrophytes and therefore only analysed for lakes with mean depth < 3 m.

4.3 Description of data availability and data

The total dataset includes data from 1002 lake-years representing about 700 lakes. Data on C_max are dominated by lakes from Poland (18%), Germany (17%), Denmark (17%), Norway (14%) and the UK (11%), constituting 77% of lakes with C_max data (Fig 4.1). The C_max analysis is thus based mainly on data from Northern Europe (the CB and Nordic GIGs) and the results may not necessarily be transferred to other GIGs.

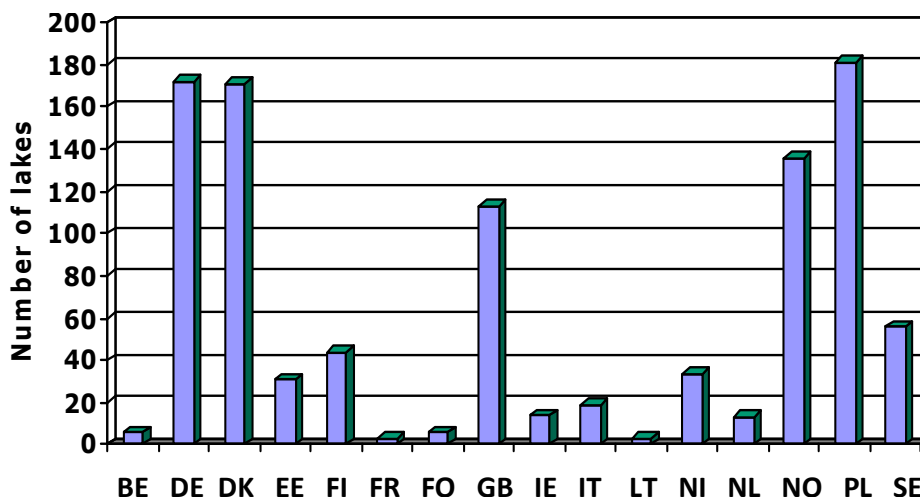


Fig 4.1 Number of lakes (lake years) from each country included in the analysis of maximum colonization depth

A majority of the lakes are relatively small; most lakes in the C_max dataset have an area <500 ha and often <100 ha (Fig. 4.2), and the dataset includes only five lakes with an area >100 km² (from Italy, Norway and Northern Ireland). Apart from a few lakes in Norway all lakes are lowland lakes situated at an altitude <200 m. Maximum and mean depths vary considerably, four lakes have a maximum depth >100 m and two lakes have a mean depth >50 m. However, the majority of the lakes are relatively shallow with a maximum depth <30 m and a mean depth <10 m (Fig. 4.2).

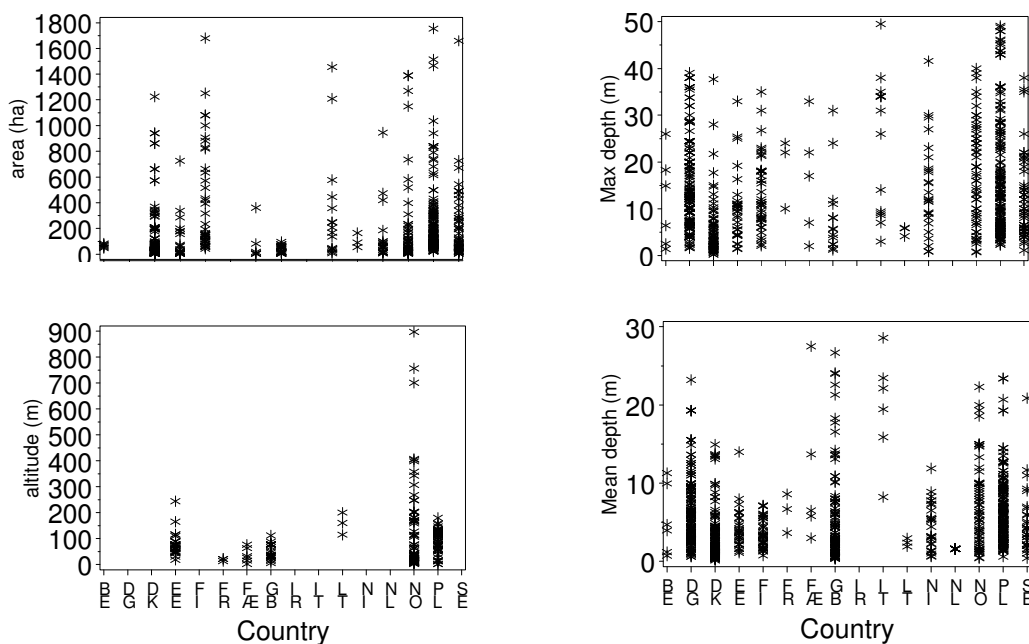


Fig 4.2. Characteristics of the lakes included from the different countries. In the figures 15 lakes with an area above 2,000 ha are excluded, as are 7 lakes with a maximum depth > 50 m and 4 lakes with a mean depth > 30 m

Coloured lakes are primarily found in the datasets from Finland and Sweden (Fig. 4.3), but from most countries there are no data on colour. Secchi depth varies considerably between lakes with Secchi depths up to 16 m, but in most lakes it is below 6. Nutrient concentrations are particularly high in the Danish and Polish lakes, exceeding 100 $\mu\text{gP/l}$ and 1 mgN/l in most of the lakes from these countries (Fig. 4.3).

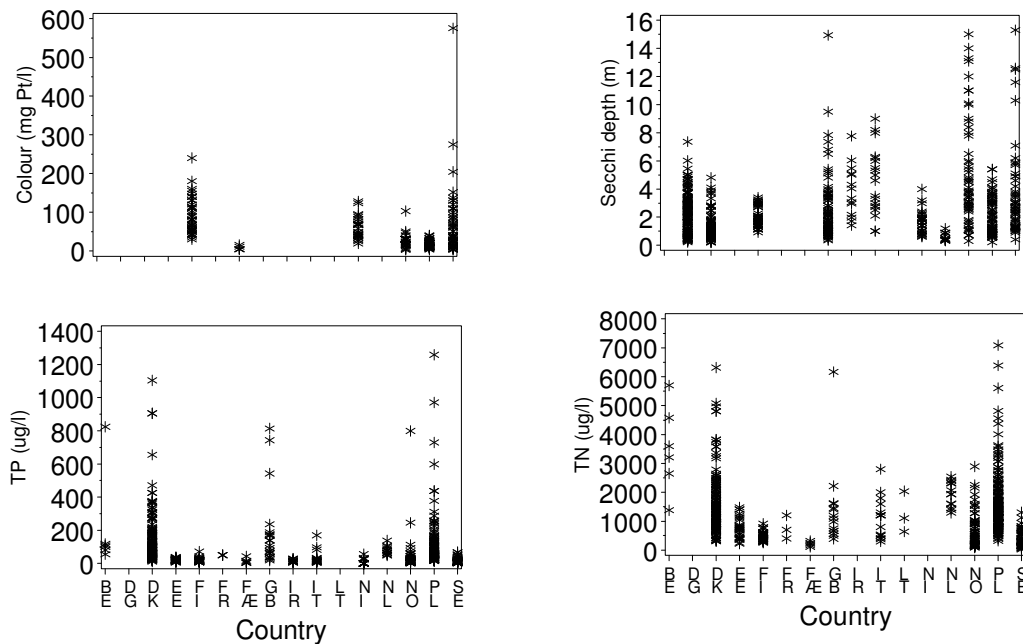


Fig. 4.3 Colour, Secchi depth, total phosphorus (TP) and total nitrogen (TN) concentrations in the lakes with C_max data

Chlorophyll *a* concentrations in the lakes varies from less than 1 $\mu\text{g/l}$ to over 200 $\mu\text{g/l}$, but most lakes exhibit chlorophyll *a* concentrations between 2 and 20 $\mu\text{g/l}$, although higher concentrations are often seen in lakes from Germany, Denmark and Poland (Fig. 4.4). Only few of the lakes have been assigned to a specific ecological class, but the chlorophyll levels in those where it has been assigned vary considerably. For the lakes classified as reference, chlorophyll *a* concentrations range between 1 and 10 $\mu\text{g/l}$, with particularly high chlorophyll *a* concentrations occurring in the Finish reference lakes. Chlorophyll *a* concentrations in the H-classified lakes are relatively low, most lakes having concentrations below 10 $\mu\text{g/l}$, although some UK lakes exhibit considerably higher concentrations. More lakes are classified as G, and in the lakes classified as either H or G most countries are represented. Again, here relatively strong variations are seen, with chlorophyll *a* concentrations ranging from 1 to over 100 $\mu\text{g/l}$. Also, within the individual countries the values regarded as either H or G vary considerably, especially in the UK and Poland (Fig. 4.4).

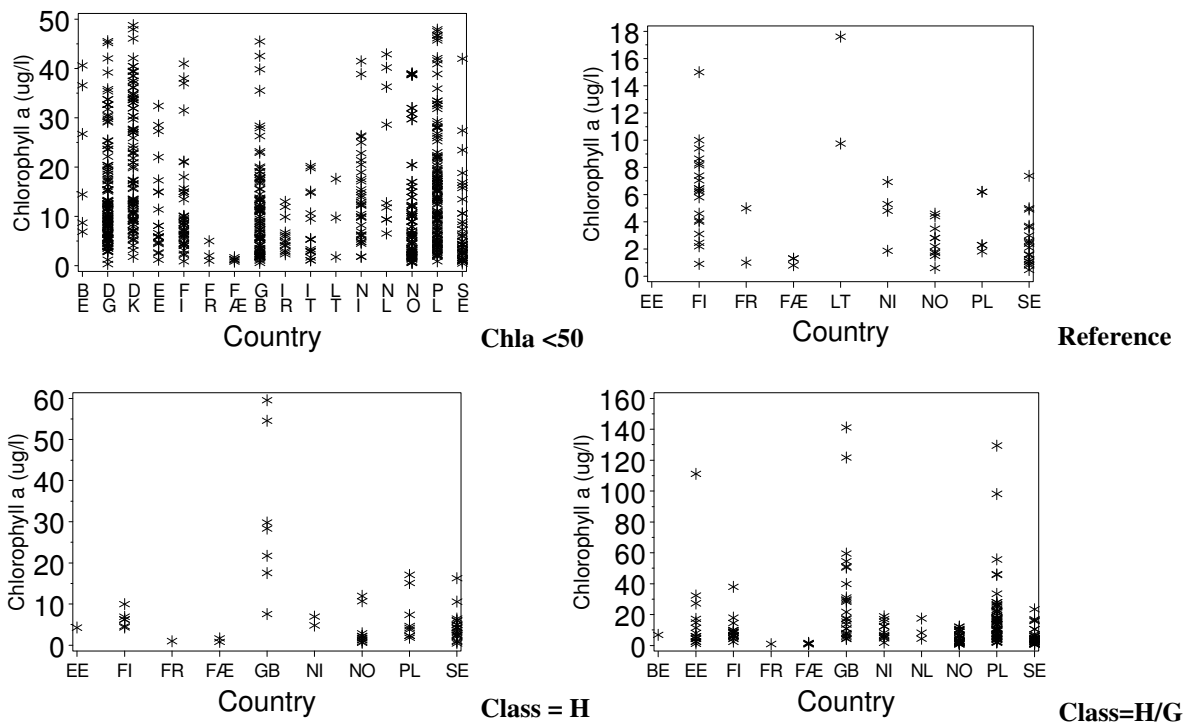


Fig. 4.4 Chlorophyll a concentrations in the lakes and in lakes with different ecological class

4.4 Maximum depth of colonization (C_max)

4.4.1 C_max at reference conditions or at high or good ecological quality

Maximum depth of colonization varies from 0.3 to 24.0 m, but in most countries C_max is below 7.0 m (Fig. 4.5). Lakes without submerged macrophytes (C_max = 0) are probably underrepresented as they are not reported and therefore not included in the dataset on maximum colonization depth. This will make a small (but unknown) bias in the regression analyses and relationships involving C_max. From Danish lakes it is well established that many eutrophic lakes are completely without submerged macrophytes (Søndergaard et al., 2010) and this is probably the case for most highly eutrophic lakes.

Similarly to the variations seen in chlorophyll a, C_max varies greatly in lakes classified as reference, H or H/G lakes (Fig. 4.5). Although the number of lakes classified as reference lakes is only 76, and some countries only have very few reference lakes, there is high variability in the measured C_max (Table 4.1). The mean C_max in the four countries with data from at least 7 lakes ranges from 2.3 to 4.9 m.

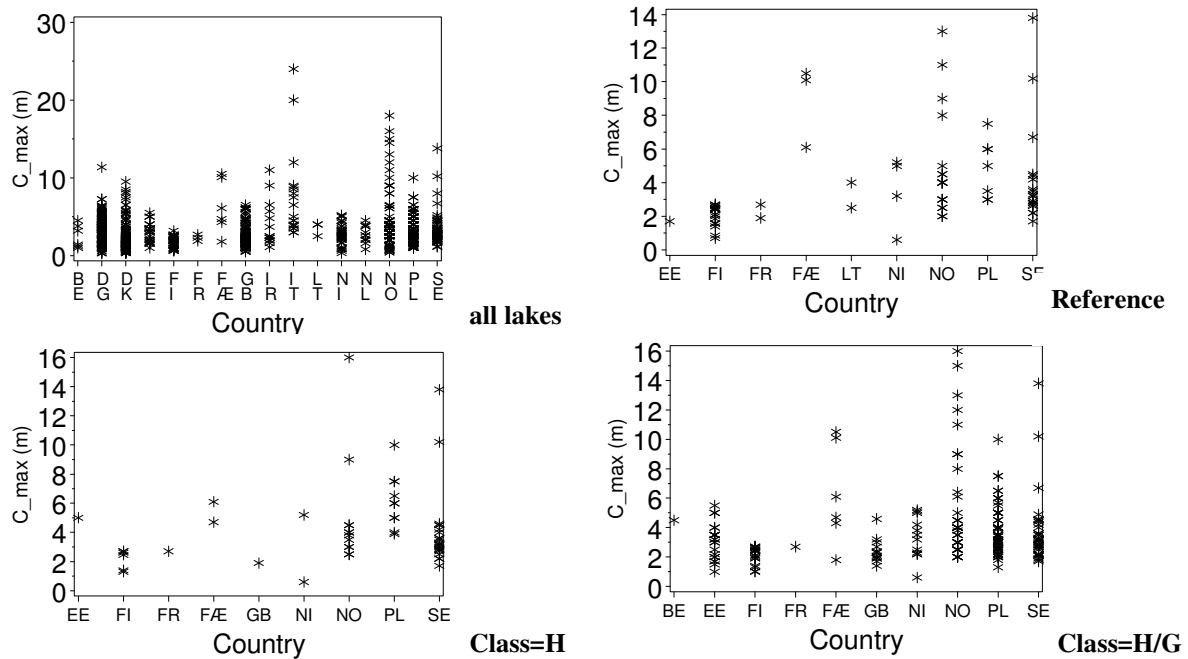


Fig. 4.5 C_{max} in different countries and at different conditions (lakes are defined as reference lakes or lakes with high (Class = H) or high or good (Class = H/G) ecological class)

The number of lakes classified as either high or good is considerably higher than that of reference lakes, but C_{max} still varies considerably between and within countries (Fig. 4.5, Table 4.2). As for the reference lakes, Finnish lakes classified as H or G seemingly have a lower C_{max} than those of the remaining countries, both as mean values and as fractiles. Although lakes with colour levels >100 mgPt/l were excluded, this might reflect that relatively coloured lakes (30-100 mg Pt/l, see also fig. 4.11) probably constitutes a large part of the Finnish lakes which impacts the results, or it could reflect the use of other criteria in the definition of reference conditions and ecological classes.

Table 4.1 Measured C_{max} in lakes classified as reference lakes. Only lakes with maximum depth >6 m, colour <100 mgPt/l and TA <1 meq/l are included (almost all lakes classified as reference lakes have TA >1 meq/l) and only countries with minimum 7 lakes classified as reference lakes. N - number of lakes.

Country	N	Mean	Min	25%	Median	75%	Max
Finland	13	2.3	0.9	2.1	2.5	2.6	2.7
Norway	14	4.3	2.0	3.0	4.0	4.5	9.0
Poland	7	4.9	3.0	3.0	5.0	6.0	7.5
Sweden	18	4.4	1.7	2.8	3.3	4.4	13.8

Table 4.2 Measured C_{max} in lakes classified as with high or good (divided into lakes with high or low alkalinity). Only lakes with maximum depth >6 m and colour <100 mg Pt/l are included and only countries with minimum 7 lakes classified as H or G. N - number of lakes.

Country	N	Mean	Min	25%	Median	75%	Max
TA > 1							
Northern Ireland	7	3.6	2.2	2.3	3.8	5.0	5.1
Norway	10	3.0	2.0	2.5	3.0	3.5	4.5
TA < 1							
Estonia	15	3.3	1.0	2.5	3.5	4.0	5.5
Finland	17	2.2	1.0	2.0	2.5	2.6	2.7
Norway	27	4.9	2.0	3.0	4.0	5.0	16.0
Poland	61	3.9	1.3	2.6	3.5	5.0	10.0
Sweden	23	4.2	1.7	2.8	3.2	4.5	13.8

The relationship between C_{max} and maximum lake water depth is shown in Fig. 4.6 and clearly demonstrates that C_{max} may preferably be used in deep lake as C_{max} often corresponds to max depth in shallow lakes. However, provided that the maximum lake depth is above 5-6 m, C_{max} is a relevant variable to measure.

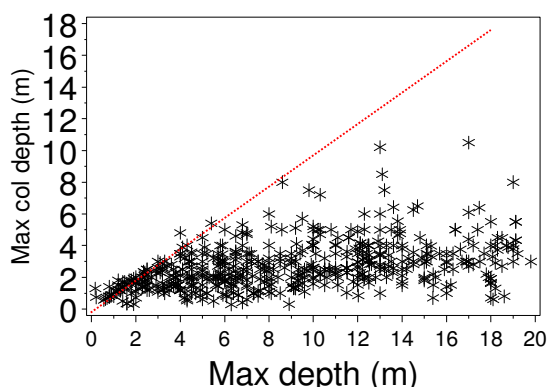


Fig. 4.6 C_{max} relative to lake maximum depth. 1:1 line is inserted

4.4.2 C_{max} along a eutrophication gradient

The maximum colonization depth relates negatively to chlorophyll a , but the variability within a given chlorophyll a range is high – particularly at chlorophyll a concentrations below 10 $\mu\text{g/l}$ (Fig. 4.7).

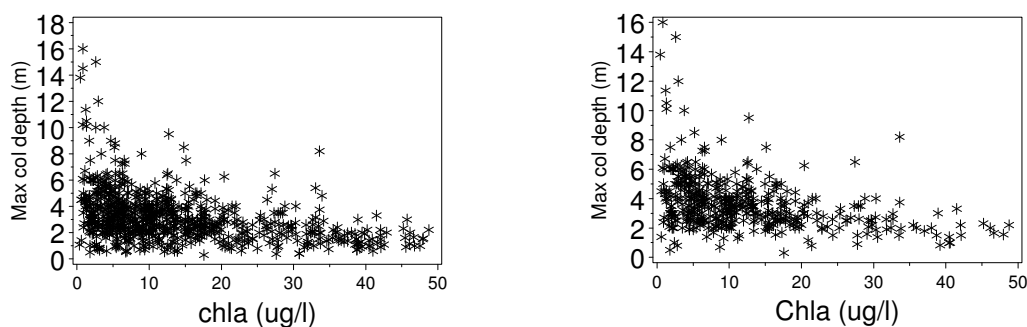


Fig. 4.7 C_{max} plotted against chlorophyll a in lakes with chlorophyll a below 50 $\mu\text{g/l}$ and C_{max} <20 m (left). Right: the same, but including only lakes with mean depth > 3 m and colour <100 mgPt/l

Similarly, there is a strong positive relationship between C_{max} and Secchi depth, although variability in the recorded C_{max} at a given Secchi depth remains high (Fig. 4.8).

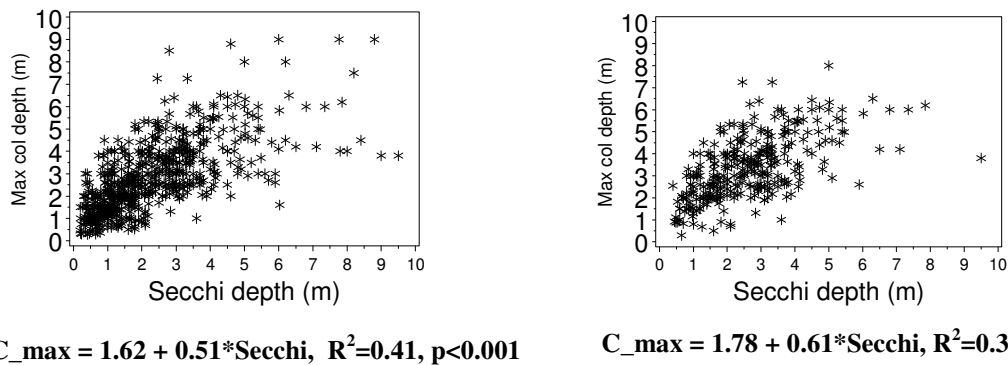


Fig. 4.8 C_{max} plotted against Secchi depth in lakes with C_{max} <10 m (left). Right: the same, but including only lakes with mean depth >3 m and colour <100 mgPt/l. The model for linear regression is shown below each figure

Use of log-log regression analyses between C_{max} and different variables gives the following correlations presented for all lakes (with mean depth >3 m) and for lakes with TA > or <1 meq/l:

All lakes (mean depth > 3 m):

Log-log regression between C_{max} and Secchi depth, nutrients, colour and chlorophyll *a*:

$$\begin{aligned} \text{Log } C_{\max} &= 0.27 + 0.59 * \text{log Secchi}, n= 331, p<0.001, R^2=0.45 \\ \text{Log } C_{\max} &= 0.56 - 0.05 * \text{log TP}, n= 325, p=0.002, R^2 =0.03 \\ \text{Log } C_{\max} &= 1.08 - 0.21 * \text{log TN}, n= 305, p<0.001, R^2=0.10 \\ \text{Log } C_{\max} &= 0.94 - 0.32 * \text{log colour}, n= 169, p<0.001, R^2=0.24 \\ \text{Log } C_{\max} &= 0.74 - 0.25 * \text{log chla}, n= 481, p<0.001, R^2=0.24 \end{aligned}$$

Log-log multiple regression between C_{max} and chlorophyll *a* and lake colour:

$$\begin{aligned} \text{Log } C_{\max} &= 1.04 - 0.19 * \text{log chla} - 0.28 \text{ log colour}, n= 157, p<0.001, R^2=0.42 \\ \text{Log } C_{\max} &= 0.62 + 0.43 * \text{log Secchi} - 0.23 \text{ log colour}, n= 121, p<0.001, R^2 =0.49 \end{aligned}$$

Lakes with TA >= 1 meq/l (mean depth >3 m):

Log-log regression between C_{max} and Secchi depth, nutrients, colour and chlorophyll *a*:

$$\begin{aligned} \text{Log } C_{\max} &= 0.30 + 0.70 * \text{log Secchi}, n= 76, p<0.001, R^2 =0.58 \\ \text{Log } C_{\max} &= \text{log TP}, n= 92, \text{ not significant} \\ \text{Log } C_{\max} &= 1.46 - 0.32 * \text{log TN}, n= 76, p<0.001, R^2=0.22 \\ \text{Log } C_{\max} &= 0.78 - 0.24 * \text{log colour}, n= 84, p=0.002, R^2=0.11 \\ \text{Log } C_{\max} &= 0.84 - 0.32 * \text{log chla}, n= 86, p<0.001, R^2=0.35 \end{aligned}$$

Log-log multiple regression between C_{max} and chlorophyll *a* and lake colour:

$$\begin{aligned} \text{Log } C_{\max} &= 0.87 - 0.19 * \text{log chla} - 0.15 \text{ log colour}, n= 82, p<0.001, R^2=0.27 \\ \text{Log } C_{\max} &= \text{log Secchi} * \text{log colour}, n= 69, \text{ only significant to Secchi.} \end{aligned}$$

Lakes with TA < 1 meq/l (mean depth >3 m):

Log-log regression between C_max and Secchi depth, nutrients, colour and chlorophyll a:

- Log C_max = 0.26 + 0.57*log Secchi, n= 255, p<0.001, R²=0.43
- Log C_max = 0.62 - 0.10*log TP, n= 234, p<0.001, R²=0.08
- Log C_max = 1.05 - 0.20*log TN, n= 229, p<0.001, R²=0.10
- Log C_max = 1.27 - 0.50*log colour, n= 85, p<0.001, R²=0.48
- Log C_max = 0.73 - 0.24*log chla, n= 395, p<0.001, R²=0.22

Log-log multiple regression between C_max and chlorophyll a and lake colour:

- Log C_max = 1.18 - 0.18*log chla - 0.37 log colour, n= 76, p<0.001, R²=0.56
- Log C_max = 1.03 + 0.21*log Secchi - 0.41 log colour, n= 53, p<0.001, R² =0.55

The correlation coefficient in the regression using single factors to explain the variability in C_max is highest for Secchi depth (R² = 0.43-0.58) and relatively low for both TN and TP (R² = 0-0.22). In the multiple regression, Secchi and colour explain 49-55% of the variability seen in C_max. The regression between C_max and Secchi depth differ between the growth form groups; charophytes, angiosperms (isotids, elodeids), mosses (i.e. Chambers & Kaiff 1985, Middelboe & Markager 1997).

4.4.3 C_max:Secchi ratio

The ratio between C_max and Secchi depth is shown in Fig. 4.9. The ratio is not constant and varies between less than 1 to more than 3 within and between countries. In Finland the ratio is below 1 in most lakes (but around 1 when excluding the most coloured lakes). In contrast, in The Netherland it is above 4 in most lakes, which probably reflects the very low Secchi depth in most Dutch lakes. Seen along a chlorophyll gradient the ratio decreases at chlorophyll a concentrations below 10 µg/l (Fig. 4.10). The C_max:Secchi ratio decreases at increasing Secchi depth and approaches 1 at Secchi depths above 2-3 m.

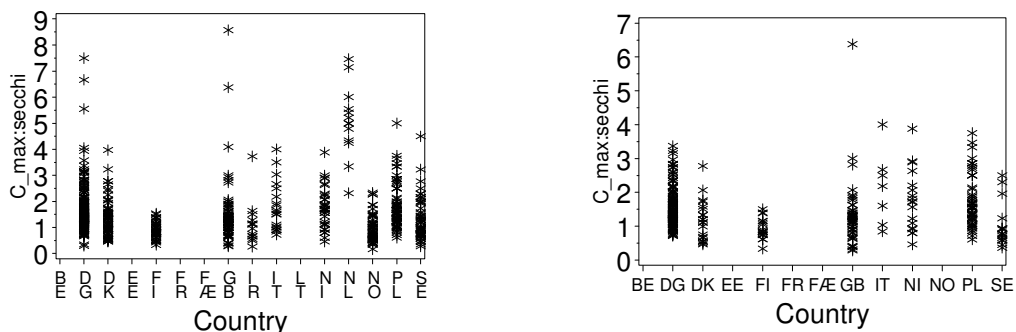


Fig. 4.9 The ratio between C_max and Secchi depth in the different countries. Left: all lakes. Right: lakes with mean depth >3 m and colour <100 mg Pt/l

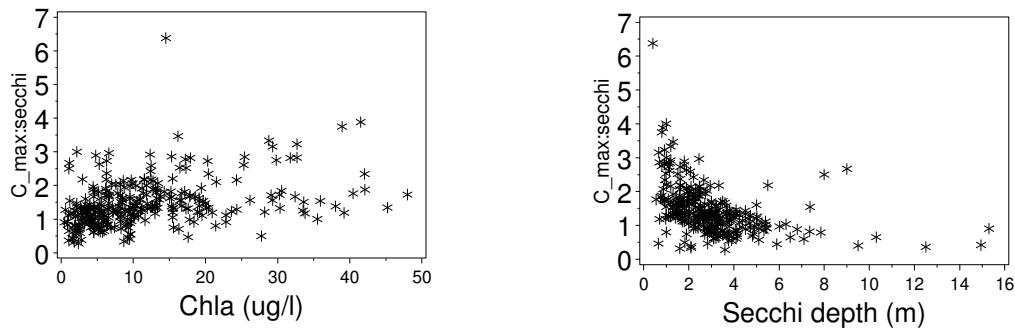


Fig. 4.10 The ratio between C_{max} and Secchi depth along a chl_a -gradient and a Secchi gradient in lakes with mean depth >3 m, colour <100 mg Pt/l and chlorophyll a <50 ug/l

4.4.4 C_{max} in coloured lakes

Specific properties of light climate in humic lakes

Light climate in humic lakes differs from clear water lakes significantly. Humic substances reduce light penetration efficiently with similar effect of increased chlorophyll content caused by eutrophication. Eloranta (1978) investigated 30 Finnish lakes and found marked difference in depth of euphotic (1% of incident light) productive zone by increase of humic content of water. Depth of this zone decreased rapidly from 10 meters to 4-5 meters when the colour of water increased to 20 mgPt/l. The depth of euphotic zone was about 1.5 meters when the colour was 60-70 mgPt/l, but decreased only slowly by increasing colour (Fig. 4.11).

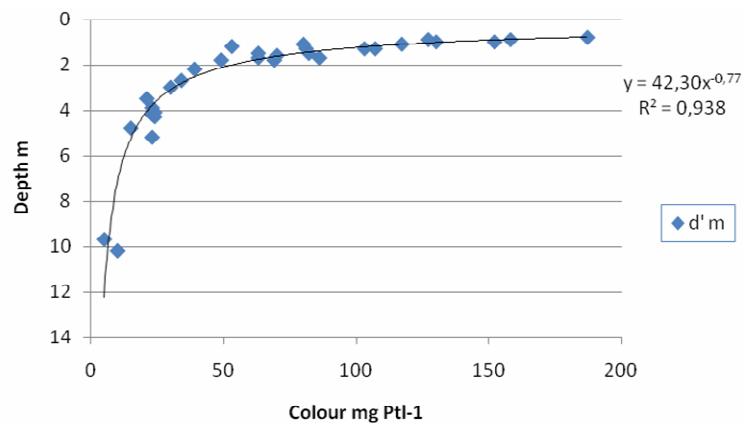


Fig. 4.11. Relationship between euphotic zone (d') and water colour (Eloranta 1978)

Euphotic zone describes more production of phytoplankton whereas penetration of red light describes better maximum growing depth of aquatic macrophytes. Based on surveys of Eloranta and Marja-aho (1982) lowest limit of macrophytes lies at the level 4.5 % of incident red light.

Water colour and red light extinction relationships were calculated from the original measurements of light penetration presented by Eloranta (1978):

$$E_r = 0.25 A^{0.42}, \quad (\text{with } r = -0.82, n = 30) \quad (1)$$

where: E_r = extinction coefficient of red light, A = water colour (mg Pt/l).

Hellsten (1997) applied 4,5 % of incident red light as an indicator of the lowest limit of productive littoral. The depth of the zone (D_r) reached by 4.5 % of incident red light (627 nm) can be calculated from the Lambert-Beer law:

$$D_r = -\ln(0.045) / E_r. \quad (2)$$

The light zones can be assessed according to the Lambert-Beer law:

$$L_D = L_0 \exp(-E_r D) \quad (3)$$

where: L_D = intensity of red light at a depth of D , L_0 = intensity of red light just below the surface

Calculated and observed maximum growing depth

Large isoetids such as *Isoetes echinospora*, *Isoetes lacustris* and *Lobelia dortmanna* forms often deepest growing population of aquatic macrophytes in soft water lakes. These plants are perennial and therefore they reflect relatively well also ecological condition of lakes. Kanninen et al. (2009) investigated several polyhumic (colour 40 – 100 mg Pt/l) small lakes in Central-Finland representing large variety of humic content and nutrient enrichment (Table 4.3). In addition to water quality parameters, deepest growing depth of large isoetids was measured by main belt transect method carefully by using rake or subaquatic drop-down video equipment.

Table 4.3 Investigated lakes in Central-Finland (Kanninen et al. 2009)

Lake	Status	Max growth (m)	TotP (ug/l)	Colour (mgPt/l)
Valkeinen	Ref	2,6	8,0	40
Pieni-Myhi	Ref	2,1	14,0	80
Ahveninen	Ref	2,4	19,0	80
Mataroinen	Ref	2,7	7,0	55
Haukijärvi	Ref	2,45	10,0	45
Härkäjärvi	Ref	1,8	15,5	85
Viipperonjärvi	Ref	2,1	12,5	60
Löytönen	Ref	2,2	7,5	50
Harvanen	Ref	2,2	10,0	75
Suurijärvi	Imp	1,1	21,0	40
Pieni-Varpanen	Imp	1,2	29,5	65
Oinasjärvi	Imp	1,3	12,0	95
Syväjärvi	Imp	1,2	25,5	90
Liesjärvi	Imp	1,4	22,0	90
Niskajärvi	Imp	1,5	19,0	100
Vihtanen	Imp	1,5	14,0	80
Korppinen	Imp	2,4	11,5	40
Pieni Saittajärvi	Imp	1,3	18,0	50

Calculated maximum growing depth based on assumption that 4.5 % of incident red light (D_r) defines border, is plotted against observed growing depth in Fig. 4.12. Developed equation describes relatively well potential growing area of aquatic macrophytes showing reduced light climate caused by humic substances.

Further the response against eutrophication can be assessed by comparing total phosphorous content of water and maximum growing depth (Fig. 4.13). Average maximum growing depth in reference lakes was 2.28 meters whereas it was in impacted lakes only 1,43 meters. However, it should be noted that that also humic content of water was slightly higher in impacted lakes (average 72 mgPt/l) compared to reference one (average 63 mgPt/l). Obviously most of these small lakes are impacted by forest ditching causing both increase in humic substances and total phosphorous.

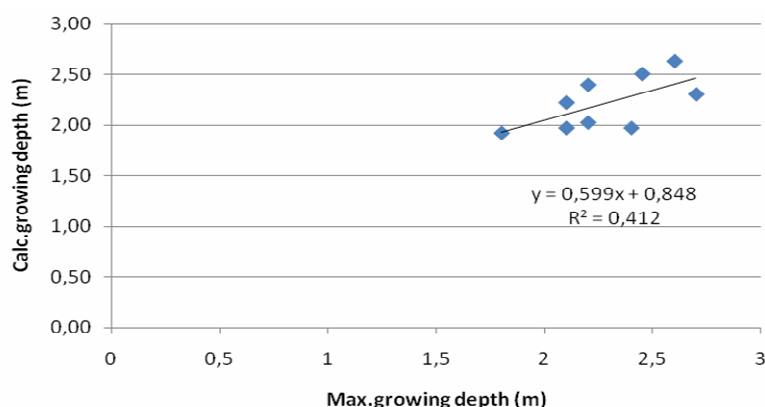


Fig. 4.12. Observed and calculated maximum growing depth in some humic reference lakes (n = 9)

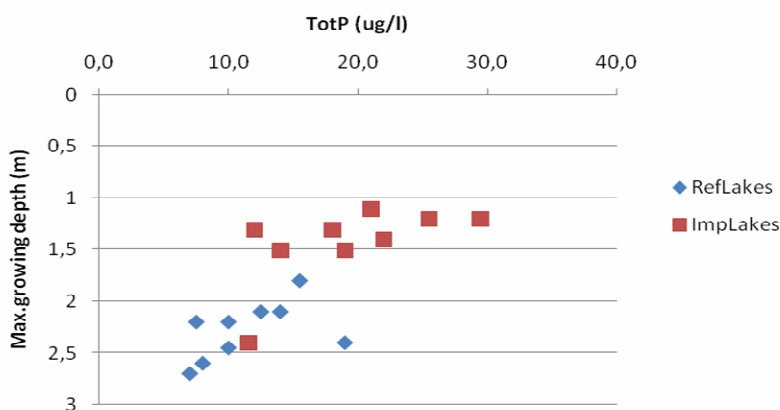


Fig. 4.13. Maximum growing depth of large isoetids and total P content in reference (n = 9) and impacted (n=9) lakes

Ecological quality ratios can be developed also for humic lakes by setting average value of maximum growing depth as reference values and dividing other status classes evenly (Fig. 4.14). Obviously these calculations and border values fits only in polyhumic lakes.

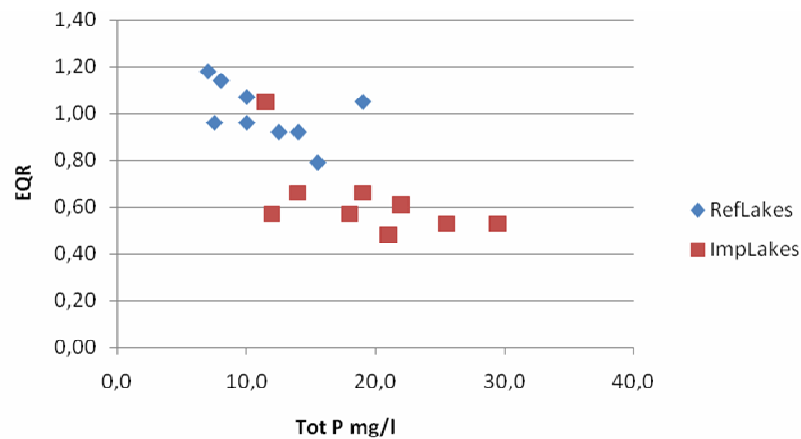


Fig. 4.14. Ecological quality ratios plotted against total phosphorous content of water

4.4.5 C_{max} in and latitude (a preliminary study)

At latitudes above approximately 60°, PAR steadily decreases and earlier analysis indicate smaller maximum depth of colonization (C_{max}) in these lakes. Based on lakes at different latitudes (37-74°), Middelboe & Markager (1997) found a relationship between latitude and max depth of colonization for charophytes and estimated an average decrease in max depth of 0.12 m per degree increase in latitude. This is similar to the results found by Duarte & Kalff (1987), who examined lakes at 15-60 degrees. Schwarz et al 2000 found no such relationship in New Zealand lakes, however, the latitude range for these lakes were only 9 degrees (37-46).

Material and methods

To test of the latitude effect with a small dataset containing lakes from Norway and the Faroe Island. We divide the lakes into three latitude groups; latitudes 55-60 (southern part of Norway), latitudes 62-64 (middle parts of Norway and the Faroe islands), and latitudes 66-69 (Northern Norway). All lakes were boreal-lowland lakes, except one high-altitude lake in southern part (700 m.a.sl), and three lakes north of the timberline in the Northern Norway.

A total of 98 lakes are included, of which 20 are surveyed by scuba divers and underwater photos (Table 4.4). The rest of the lakes (70) are surveyed by the Norwegian standard method; random sampling with boat, aquascope and rake. We expect that C_{max} to some extent can be underestimated in some of the last lakes, especially in clear water, oligotrophic lakes with C_{max} exceeding 5-6 metres. However, all latitude groups include scuba diver surveys.

Table 4.4 Number of lakes included in the latitude analyses.

Latitude	Total number of lakes	Lakes with underwater photos
55-60	47	11
62-64	13	6
66-69	38	3

Results

The correlation between secchi depth and C_{max} seem to vary with latitude, especially for *Nitella* species (mainly *Nitella opaca*), and for isoetids (mainly *Isoetes lacustris*). The difference is less for elodeids, while no difference could be seen for *Chara*-species (Fig. 4.15). The regression results, however, show $r^2 > 0.5$ for the *Nitella*-regressions and for the isoetids at low latitudes (table 4.5).

Isoetes lacustris, which is the deepest growing isoetid, has a maximum colonisation depth at 7-8 m (Rørslett & Brettum 1989). These large isoetids are rare in Northern Norway, and are not included in the lakes north of the timberline.

The C_{max} in the mountain lake in the southern part is close to the regression line for the lakes in North Norway. It may indicate that the difference is partly altitude based. However, the C_{max} in the northern lowland-boreal lakes and the lakes north of the timberline show no significant differences.

The regressions are based on a small dataset. However, these preliminary results indicate an effect from the latitude. The largest difference seems to be between the latitude groups 60-64 and 66-69. Whether there is a difference between lakes in the southern part of Norway (latitude group 55-60) and Central Europe is not known.

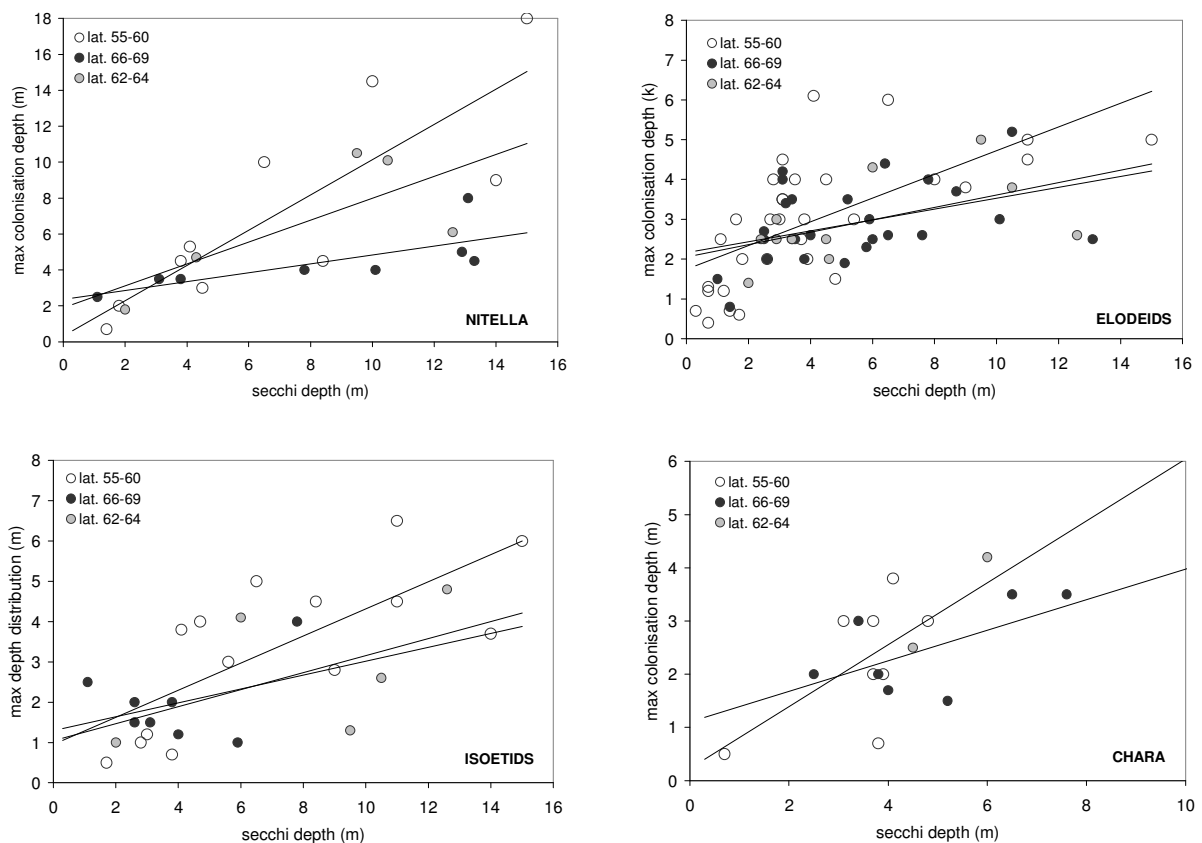


Fig. 4.15. Regressions between Secchi depth and C_{max} for the charophytes *Nitella* (mainly *Nitella opaca*) (upper, left), the isoetids (mainly *Isoetes lacustris*) (down, left), the elodeids (upper, right) and the charophytes *Chara* spp. (down, right). The regression lines are in the same order in all graphs; latitudes 55-60 (upper), 62-64 (middle) 66-69 (lower)

Table 4.5 Results from the linear regressions Secchi depth – C_{max} for 4 growth form groups and 3 latitude groups.

Species/latitude	Equation	R ²	p	n
<i>Nitella</i> latitudes 55-60	$y = 0.9822x + 0.3239$	0.6996	<0.001	10
<i>Nitella</i> latitudes 62-64	$y = 0.609x + 1.9023$	0.5397	0.048	5
<i>Nitella</i> latitudes 66-69	$y = 0.247x + 2.3617$	0.5549	<0.001	8
isoetids latitudes 55-60	$y = 0.3366 + 0.9529$	0.5537	< 0.001	14
isoetids latitudes 62-64	$y = 0.2109x + 1.0477$	0.276	0.161	5
isoetids latitudes 66-69	$y = 0.1724x + 1.2965$	0.1449	0.048	8
elodeids latitudes 55-60	$y = 0.298x + 1.7423$	0.4335	<0.001	31
elodeids latitudes 62-64	$y = 0.156x + 2.049$	0.2952	<0.001	11
elodeids latitudes 66-69	$y = 0.137x + 2.1643$	0.1748	< 0.001	27
<i>Chara</i> latitudes 55-60	$y = 0.5822X + 0.227$	0.3633	0.004	8
<i>Chara</i> latitudes 62-64	not enough data	-	-	2
<i>Chara</i> latitudes 66-69	$y = 0.2871 + 1.1036$	0.3715	0.012	7

4.4.6 C_{max} and year-to-year variations

Often data on macrophytes are based on one or a few years of measurements, but the question is how much C_{max} varies from year to year. In an analysis of 18 Danish lakes it has been shown that the variability can be quite considerable (Søndergaard et al., 2010), (Fig. 4.16). Determination of C_{max} in a lake should therefore preferably be based on more than one year’s measurements or otherwise documented that lake conditions are stable.

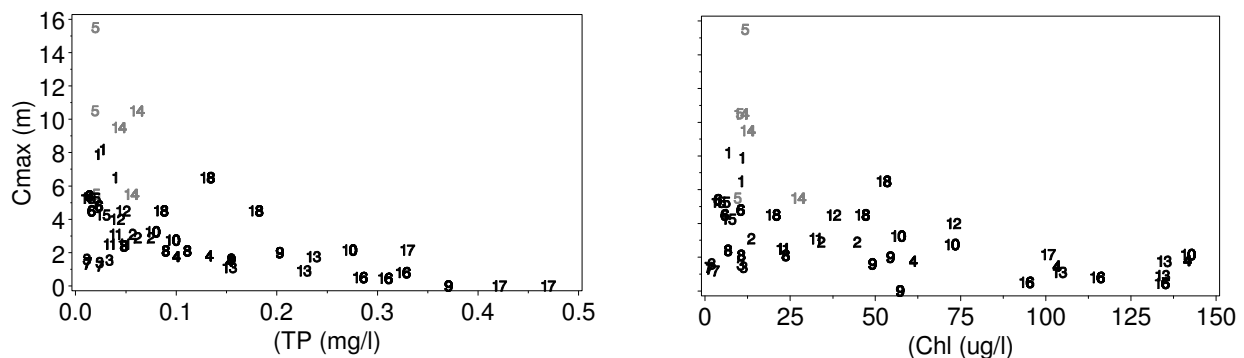


Fig. 4.16 Changes in C_{max} during a 3 years monitoring period from 18 Danish lakes. From Søndergaard et al., 2010

In another Danish example C_{max} has been monitored for 15 years in three lakes where Secchi depth during the same period was relatively stable (Fig. 4.17). In all three lakes C_{max} varied with at least a factor 2 during the 15 years of sampling. Often no clear positive relationship could be seen between Secchi depth and C_{max} the individual years.

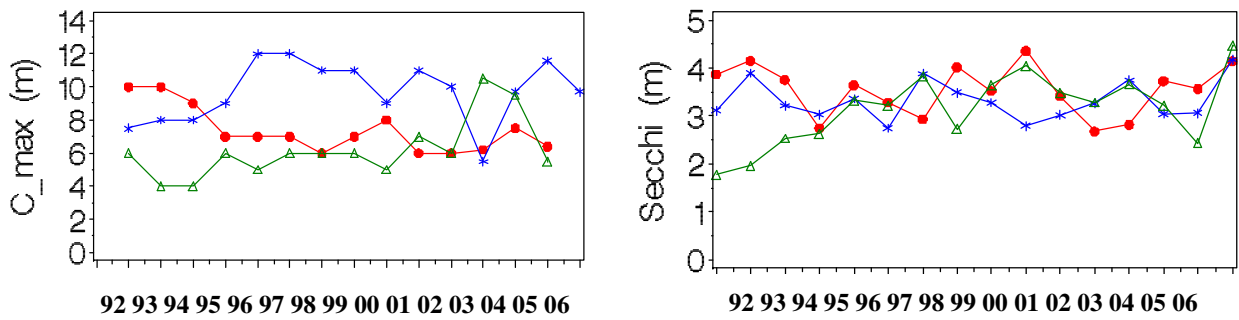


Fig. 4.17. Changes in C_max in three Danish lakes (red dots: Lake Nors, blue stars: Lake Ravn, green triangles: Lake Fure) during a 15 years period of monitoring (1992-2006). Right: Mean summer Secchi depth in the same three lakes and during the same period. In a linear regression there is no significant ($p > 0.05$) relationship between Secchi depth and C_max for the three lakes

4.5 Coverage

4.5.1 Coverage along a eutrophication gradient

Mean macrophyte coverage is shown for shallow lakes (mean depth < 3 m) in Fig. 4.18. It shows a clear decrease in mean macrophyte coverage at increasing nutrient concentrations and chlorophyll a.

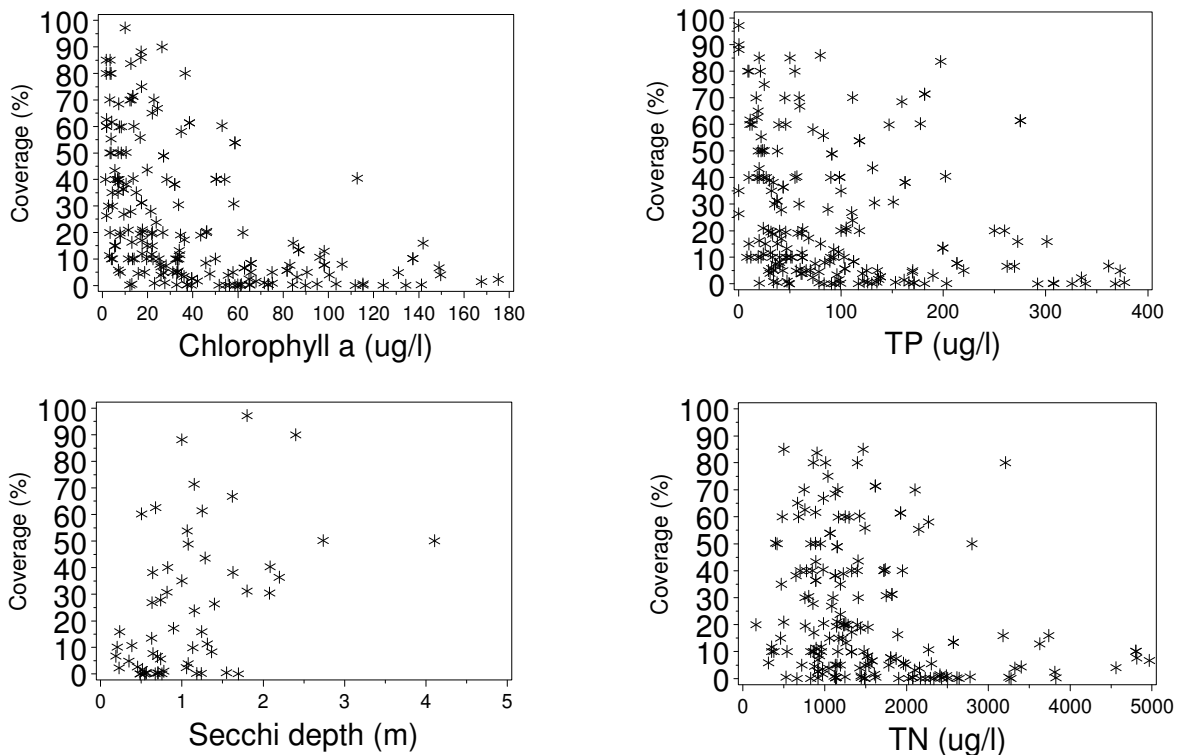


Fig. 4.18. Mean coverage of submerged macrophytes along a nutrient gradient and a chlorophyll a and Secchi depth gradient. All lakes with mean depth < 3 m

The figures also indicate some thresholds above which the coverage of macrophyte usually is low, for example at chlorophyll a concentrations above 30 $\mu\text{g/l}$, TP above 50 $\mu\text{gP/l}$ and TN above 1.5 mgN/l . The relationship to Secchi depth might be difficult to use because Secchi depth might reach the lake bottom in these shallow lakes at low nutrient concentrations. There are too few alkalinity data to split the dataset into low and high alkalinity lakes.

4.5.2 Coverage and year-to-year variations

As for C_{max} there is a considerable year-to-year variation in the coverage of submerged macrophytes in individual lakes, which do not necessarily relate to changes in Secchi depth, but can be due to several other factors such as climatic variability between years which influences growth conditions and changes in other biological components (fish, waterfowl, etc.). An example from a Danish study illustrates the variability in coverage in Fig. 4.19.

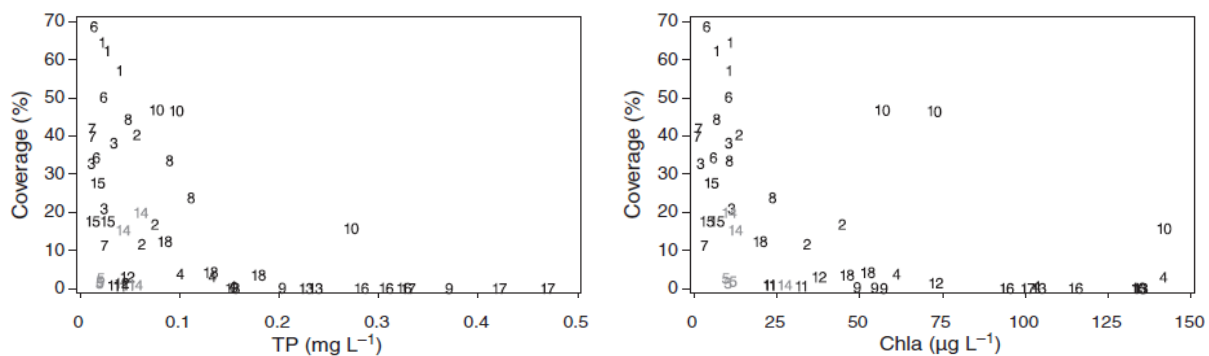


Fig. 4.19. Changes in submerged macrophyte coverage during a 3 years monitoring period from 18 Danish lakes. From Søndergaard et al., 2010

4.6 Conclusions

1. The analyses on abundance demonstrate that macrophyte abundance responds very significantly to eutrophication stressors.
2. There is a large variability in C_{max} for reference lakes both between countries and within individual countries. In the future work it would be valuable to conduct more detailed investigations into how the definitions were made for reference lakes (and H and G classes) – especially if these are used as basis for defining ecological boundaries.
3. It is recommended that C_{max} is used as a macrophyte abundance metric in lakes with maximum depths above 6 m (or mean depths above 3 m).
4. It is recommended that coverage of submerged macrophytes is used in shallow lakes (mean depth <3 m).
5. For both C_{max} and coverage there is considerable year-to-year variation which needs to be acknowledged (using for example data from at least three sampling years) to reduce the risk of misclassification of lakes.

6. The analyses were mainly conducted on siliceous deep or shallow lakes (due to data availability), but other lake types, as for example coloured lakes have other macrophyte characteristics - as also demonstrated here, and should be analysed in more details.
7. At latitudes above approximately 60 ° C_{max} may be reduced due to decreased PAR.
8. Clear relationships can be established between macrophyte abundance (C_{max} and coverage) and chlorophyll *a* and Secchi depth, but it is recommended that C_{max} and coverage are defined independently from other metric boundaries (such as chlorophyll *a*) to avoid circular conclusions and simple “translation errors” between metrics.

4.7 References

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5. Macrophyte metrics for hydromorphological pressure

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5.1 Background

5.1.1 Water level fluctuation and species diversity

Hydromorphological pressures in lakes are related to the human need to control the water levels of the lakes and flows of the rivers in such way that benefits various users of water courses. The reasons for controlling water levels and flows include production of hydropower, flood prevention, recreation, navigation, and supply of water for agricultural or human consumption. Regulation practices vary and depend on the objectives of regulation.

Macrophytes are one of the key indicators of hydromorphological changes in lakes. Because macrophytes grow in the littoral zone they are sensitive for changes in water level fluctuation regime. A general zonation of macrophytes can be based on life-form distribution; helophytes grow in the uppermost zone and isoetids, elodeids and charids occupy deeper areas of lakes. Even small changes in the dynamics of water level fluctuation can affect distribution and the elevation of zones. Morphological changes of the littoral zone, caused for example by dredging or embankments, significantly disturb the development of vegetation.

Regarding the heavily regulated lakes and rivers biodiversity indices do not give very promising results, because most of the species are also present in modified parts as long as regulations are not too extreme (Keto et al. 2006). On the other hand Rørslett (1989) showed in his analysis of 17 Norwegian hydro lakes that the species richness (S) followed equation:

$$S = 16.4 - 1.34 \Delta W - 0.013 H + 0.085 A,$$

where ΔW = mean annual range of water level (m), H = lake altitude (m a.s.l.) and A = lake area (km²).

Further in his analysis of 641 lakes from Norway, Sweden, Denmark and Finland, he found that lake area was best predictor of species diversity (Rørslett 1991), which is linked to available habitat diversity. Stepwise prediction model included also hydromorphology related variables such as water level range and lake lowering with water conductivity and lake elevation values.

Rørslett (1985, 1989) found lower diversity of macrophytes in Norwegian lakes with extended water level fluctuation. Hellsten (2001, 2002) showed similar trend in Finnish regulated lakes. Hill et al. (1998) demonstrated lowered diversity in lakes with fluctuating water level in Canada. Nilsson et al. (1997) found that biodiversity was much lower in Swedish river reservoirs compared to free flowing sites. This relationship, however, is not linear. Extensive literature survey of Scandinavian lakes showed that general biodiversity correlated mainly with draw-down of water level, but regulation amplitude between 1 and 3 meters supported highest biological diversity (Rørslett 1991). In the Netherlands too, the natural water level fluctuation during the year before regulations was in general approximately 1 meter and supported high biodiversity. Today with the water level fluctuations strictly managed, absent or 'reversed'

(higher levels in summer than in winter), the biodiversity of especially the helophyte community has decrease as a result of that change (Van Geest et al. 2005).

A slight increase in disturbance could even create suitable habitats for aquatic macrophytes as noted by Murphy et al. (1990). Similar phenomenon was found in hydrolakes of New Zealand, where increasing monthly water level fluctuation range even increased biodiversity (Riis and Hawes 2002).

Depth variations are usually related to an artificial increase or decrease of water level. Water levels are increased to extend storage capacity of reservoirs or regulated lakes. A sudden increase of water level will initiate erosion processes, which lower biodiversity (Nilsson, 1981; Hellsten 1997). It should be noted that taxonomic composition is a poor indicator of water level increase, because most of the species are still present after water level increase, although abundance may differ significantly (Nilsson & Keddy 1988; Hellsten & Riihimäki 1996). Effects of raised water level also depend on ageing; after inundation shock of Swedish reservoirs species diversity was highest 30-40 years subsequent to the initiation of the regulation (Nilsson et al. 1997). In most cases diversity is slightly increased after inundation due to stabilization of the shoreline.

In general, lowering of water level will lead to increased diversity, as found in several studies (Lohammar 1949; Toivonen & Nybom 1989, Rørslett 1991). The main reason for increased diversity is that a newly exposed littoral zone or general shallowness allows the sublittoral zone to cover the entire water body. Several shallow water lake studies have demonstrated a sensitive balance between different species groups (Best 1987; Van den Berg, 1998).

Several studies indicate that abundance is a much more sensitive indicator for hydrological change than species composition (Nilsson & Keddy 1988; Coops et al. 1996, Hellsten et al. 1996, Hellsten 2001). Generally, water level fluctuation affects zonation patterns, which are a function of the relative abundances of different species with different degrees of adaptation to stress caused by depth and drying. Therefore, changes in the amount of water level fluctuation are reflected by changes in distribution of species.

5.1.2 Effects of seasonal water level dynamics and variation

In addition to the range of water level fluctuation, the dynamics of the fluctuation affects significantly the abundance of macrophytes. For example, the timing and range of the spring flood affects clearly the zonation of sedge species in northern areas (Walker & Wehrhahn 1970, Sjöberg & Danell 1983, Hellsten, 2001). The generally observed increase of common reed (*Phragmites australis*) abundance in Scandinavia may be related to lowered early spring water level (Rintanen 1996; Partanen & Hellsten 2005, Partanen et al. 2005). Reeds also benefit from stabilized water levels and growth periods (Coops et al. 1994, 1995, 1996).

Lowering of the water level while a lake is ice-covered will have significant effects, especially on large sized isoetids such as *Isoetes lacustris* and *Lobelia dortmanna*. Reports of their decline cover northern Scandinavia (Quennerstedt 1958; Rørslett 1984, Rintanen 1996,

Hellsten 2002) and Scotland (Smith 1987, Murphy 1990). Additional to the effect of freezing, changes in sediment quality will also significantly affect their distribution (Murphy 2002).

Apart from this new development, there are few classification schemes related to relationships between seasonally distributed hydro-morphological factors and macrophytes. The direct response of *Isoetes lacustris* to ice penetration enables its distribution to be used for classification purposes (Rørslett 1989, Rørslett & Johansen 1996; Hellsten 2002). The deepest growing areas of *I. lacustris* are also sharply limited by lack of light and therefore their growing niche is easy to predict (Rørslett 1988). The distribution of other large isoetids such as *Isoetes echinospora*, *Lobelia dortmanna* and *Littorella uniflora* can also be used for classification purposes, because they are all relatively weak against ice erosion and changes in sediment structure (Rørslett 1989, Murphy 2002).

The effects of depth changes have been generally used in simple calculation procedures to describe the available growth area for macrophytes. Known relationships between deepest growth limits of bottom-rooted helophytes have produced a large number of different applications for Finnish lakes (Hellsten et al. 2002). Hudon (1997) developed similar relationships between average water level scenarios and areas dominated by different vegetation types in floodplain lakes of St Lawrence River. In general there is a growing demand for water level related indices, see for instance (Wantzen et al (2008).

5.1.3 Stabilized water level and complete drawdown – specific problems of delta lakes

Many characteristics of water-level fluctuations can potentially affect the macrophyte abundance, composition and diversity in lakes. In undisturbed wetlands in temperate regions, high water-levels during spring prevent the establishment of woody plants, and low water levels allow periodic recruitment from shoreline seed banks for many macrophyte species (Brock et al., 1987; Coops & Van der Velde, 1995; Hill et al., 1998; Keddy & Constabel, 1986; Van Geest et al., 2005). The amplitude of water level fluctuations will have a strong and direct impact when they occur in the period of plant growth, viz. between March/April and September. At highly stabilized water levels during the growing season, the vegetation is dominated by a few large species (e.g. Phragmites or Elodea) that exclude many smaller species. With increasing amplitude of water-level fluctuations, the biomass of the dominant species becomes reduced, and species richness increases. If fluctuations become larger than 1-2 meter, however, species richness may decrease because tolerance limits of the individual species are exceeded (Hill et al., 1998; Hudon, 1997; Keddy & Constabel, 1986). Beside variations within one growing season, species occurrence and diversity is also affected by long-term (among years) variability of water levels. Hill et al. (1998) showed for 50 different types of lakes in Canada that macrophyte species richness was maximal at within-year water level variations of 1-2 meter, and at among-year variations with the range of 20-50% of within-year variation.

The natural water-level regime of undisturbed lakes may vary widely. For lakes in Canada, the amplitude of within-year variation of water-levels varied between 0.5 – 3 meter, and was positively related to the catchment area of the lakes (Hill et al., 1998). This large range in amplitude of water level fluctuations in undisturbed lakes has several implications for the

development of a metric for water-level fluctuations. As the occurrence of many macrophyte species is strongly related to the hydrological regime of the lakes and lakes vary widely in their natural water level regime (Oosterberg et al., 2000), no ‘standard’ list of species can be given that can be applied as a reference for all lakes. Furthermore, hydrological regimes of regulated lakes may deviate from undisturbed systems by either hypovariable or hypervariable for both within-year and among-year fluctuations in water level. Artificially decreasing the amplitude of water-level fluctuations is a common goal for lakes that are used for hydroelectricity, while lakes that are used as storage reservoirs often have strongly increased fluctuations compared to their natural water-level regime.

5.2 Developing water level regulation index for "hydrolakes"

5.2.1 Definition of water level regulation practices

Rørslett (1988) defined hydrolake as a water body where the water levels are operated for generating hydro-electric power (HEP). He also suggested a classification of the hydrolakes compared with natural lakes (see Table 5.1).

Table 5.1. Classification of hydrolakes and natural waterbodies. Slightly modified from Rørslett (1988).

category	through-flow time	level range*	winter stages
Hydrolakes (H):			
H1: oscillating	very short	small-medium	high
H2: Intermediate (reservoirs)	short	small-medium	high
H3: storage (reservoirs)	long	often very large	drawdown
Natural lakes (N):			
N1: river-run	short	small-large	low
N2: others	often long	small-medium	low

*: suggested values are: small <2m, medium: 2-4m, large: 4-8m, very large: 8-100+m

For our analysis, we have divided the lakes into three groups; H3, H2 and N2. The definition of storage lakes (H3) is the same as in Rørslett (1988), i.e. only lakes (storage reservoirs) regulated for hydro-electric power. The intermediate regulated lakes (H2) include all other types of regulation (i.e. drinking water reservoirs, reservoirs in rivers, and lakes with stabilized water level). The natural lakes (N2) also include the semi-natural lakes sN2.

The differences in hydrological regime for a storage lake (H3) and a natural lake (N2) are illustrated in Fig. 5.1.

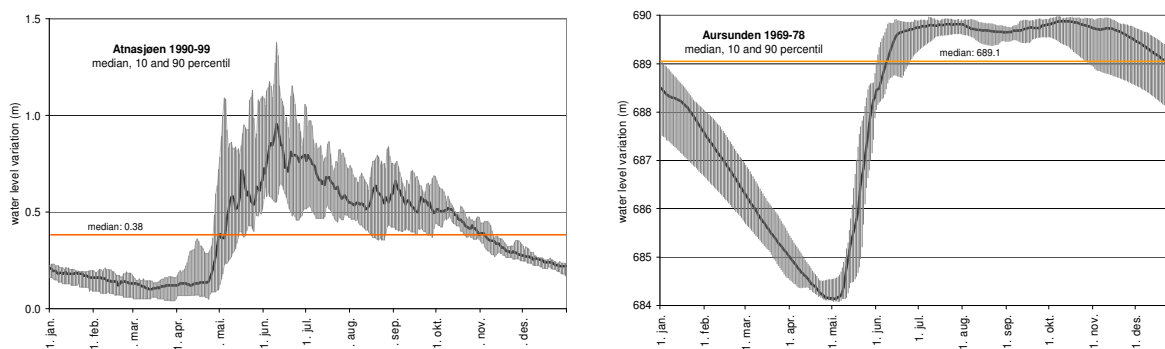


Fig. 5.1. Typical water level variations in a natural lake (left) and a storage lake (right)

5.2.2 Material and methods

A total of 79 lakes from Finland, Norway and Sweden were used in developing the new waterlevel index (WIC). Of these, 37 were storage lakes (H3), 20 other regulated lakes (H2) and 22 natural or semi-natural lakes (see Table 5.2).

Table 5.2. Number of lakes used for developing the new water level index.

Country	Storage lakes H3	Other regulated H2	Natural/seminatural lakes (N2+sN2)	Total
Finland	17	3	9	29
Norway	13	14	10	37
Sweden	7	3	3	13

The Finnish dataset includes low alkalinity, both clear and humic, lakes. Water level fluctuation varied between 0.1 and 6.8 m. The Norwegian dataset consists mainly of clear water, low alkalinity lakes, with water level fluctuations between 0.1 and 5.7 m. Swedish dataset sampled by Wallsten (2010) includes low alkalinity lakes in Värmland with wide range of humic substances. All lakes in the dataset are oligotrophic – slightly mesotrophic lakes.

Only aquatic macrophytes (isoetids, elodeids, nymphaeids, lemnids and charophytes) are included in further analysis. Helophytes are not included in the field survey in all countries, and is therefore excluded from the analysis.

The aquatic macrophytes in Finland were surveyed by the main belt transect method (Keto et al. 2006) in the period 1996-2004, while the surveys in Norway includes both random sampling method (Mjelde 2008) and underwater photos (Rørslett et al 1978), in the period 1976-2003. In addition, old literature data from 1940-41 (Tesaker 1942), surveyed with random method, were included in the Norwegian dataset. In Sweden, a virtual transect method (zone analysis) were used (Wallsten 2010). All countries include species composition, frequency and abundance.

The waterlevel data was collected from the Hertta database (SYKE) in Finland, NVE database in Norway and Fortum database in Sweden, excluding natural lakes with modelled values by SMHI. In Finland water level data from 1980-1999 were used, whereas Norwegian data were more scattered and usually 5-10 years prior to the macrophyte survey. Water level data from Sweden contains 10 years prior to the macrophyte survey.

5.2.3 Winter drawdown as an indicator for water level regulation

We use winter drawdown as an indicator of water level regulation amplitude (for arguments, see Hellsten 2001, Keto et al. 2006, 2008). Winter drawdown was calculated as the average difference between highest water level in October-December and lowest level during the following April-May. Other indicators, such as annual MW–MNW, were also discussed and compared with the winter drawdown, however, without improving the result.

5.2.4 Species diversity and species composition

In total, 69 species of aquatic macrophytes were recorded in the lakes, 49 species in the storage reservoirs (H3), 59 in other regulated lakes (H2) and 56 in natural lakes (N2 and sN2).

Comparison of total number of species to winter drawdown showed no significant correlation, although it was a trend for decreasing number of species with increasing winter draw-down (Fig. 5.2). It was some evidence that increased water level fluctuation (1 – 2 meters) even increased the diversity. However, the lake area, which is one major factor exterminating the species number (Rørslett 1991), differ highly among these lakes.

The macrophyte community was dominated by isoetids (*Ranunculus reptans*, *Isoetes echinospora*, *Eleocharis acicularis*, *Isoetes lacustris*, *Subularia aquatic*, *Lobelia dortmanna*), the nymphaeid *Nuphar lutea*, and the two elodeids *Juncus bulbosus* and *Myriophyllum alterniflorum*. Species composition indicates low alkalinity, oligotrophic lakes. Our further analysis and index assessment should therefore only be used for such lakes.

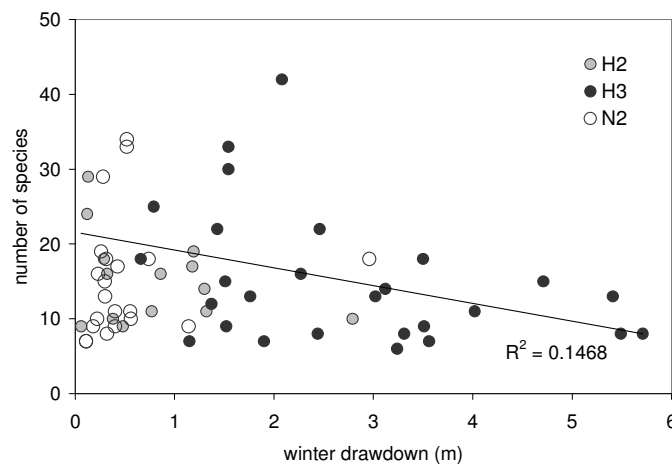


Fig. 5.2. Species diversity in relation to winter drawdown. All lakes in all three countries.

5.2.5 Identifying sensitive and tolerant species

One of the most common approaches to identifying or describing the current state of the macrophyte community is to apply an index which uses a calculation based on the relative number of sensitive vs. tolerant species. It is therefore important to clearly define the method by which sensitive and tolerant taxa were identified, before any index is applied, an issue which is often overlooked in literature on the applicability of such indices.

Hellsten and Mjelde (2009) made a preliminary list of sensitive, tolerant and indifferent species, based on expert judgement. Our approach is to hereby improve this list of sensitive and tolerant species.

The analysis of sensitive and tolerant species is based on an extract of the original dataset (see chapter 5.2.2). To avoid eutrophication effects, we only include oligotrophic or slightly mesotrophic lakes. In addition, only low alkalinity lakes are included in the analysis. Most HEP regulated lakes have low alkalinity, and the bicarbonate species, most of the elodeids, are naturally rare in these lakes. Also, some of the lakes in the original dataset are missing water level data, and are therefore not included here. A total of 66 lakes are used for the percentile analysis; 29 Finnish lakes, 25 Norwegian lakes and 12 Swedish lakes.

We suggest the following description of sensitive and tolerant species:

- Sensitive species: species which prefer or only appear in reference lakes. Decreased frequency and abundance (often disappearance) when increased water level fluctuations
- Tolerant species: species with increased frequency and abundance when increased water level fluctuations. Often less frequent in reference lakes.

In addition, some of the sensitive species seem to be less effected by the winter drawdown. We call these species less sensitive species, due to the fact that they suffer at some extent of water level fluctuation.

Identification of sensitive and tolerant taxa is based on a relatively simple approach, analysing the species occurrence along the winter drawdown gradient, using percentiles. To distinguish between sensitive and tolerant species we use the 75th percentile. This is based on expert judgement, and place e.g. *Isoetes lacustris* within the sensitive group and *Juncus bulbosus* among the tolerant species.

The level of winter drawdown used to separate the two groups is mainly based on expert judgement. We have used changes in frequency and abundance of well known reference or tolerant species to help us decide which level to use. Based on this method we can identify the most sensitive species as: all with 75th percentiles ≤ 1.6 m (winter drawdown), while the most tolerant species seem to be all species with 75th perc > 2.6 m (winter drawdown) (Fig. 5.3).

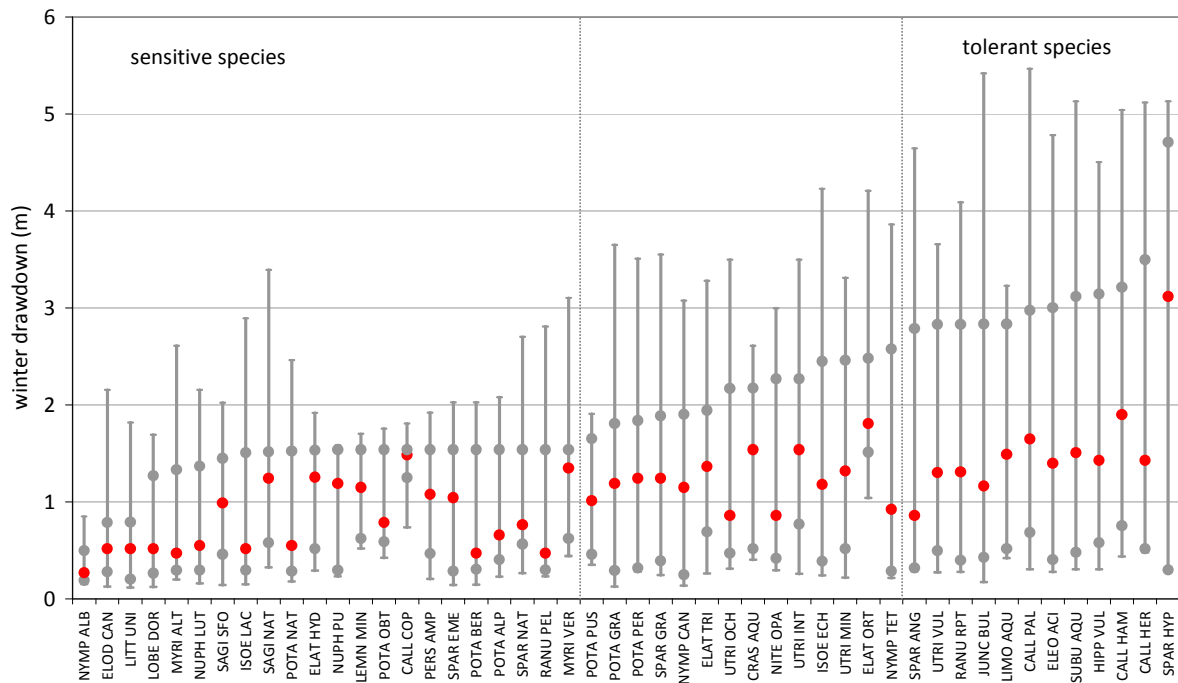


Fig. 5.3. Distribution of sensitive and tolerant species due to winter drawdown, based on Finnish, Swedish and Norwegian lakes. The graph includes 10, 25, 50, 75, and 90th percentiles. Species with less than 4 localities are excluded in the graph. The species are sorted by the 75th percentile

On both sides of such a border there will always be species with similar demands. Such “problems” will appear regardless of which border we use, and will need some expert judgement when assessing the ecological status. One way to avoid this is to use only the most obvious sensitive and tolerant species, i.e. species on the two ends of the scale. For regulation effects, we choose this approach, defining the most tolerant and most sensitive species. The species in “middle group” is perhaps less sensitive than the others, and is therefore called so.

Based on the percentile analysis 46% of the aquatic macrophytes can be characterised as sensitive while 25% seem to be tolerant (Table 5.3). 29% of the species can be grouped as less sensitive.

All tolerant species, except *Utricularia vulgaris*, are either polymorphic (*Juncus bulbosus*, *Hippuris vulgaris*) or amphiphytic, which enable them to withstand draining and erosion in the littoral zone. Especially *Juncus bulbosus* can occur under wide range of environmental conditions (Hinneri 1976, Rørslett 1989).

The classifications agree to a large extent with earlier knowledge and expert judgement (e.g. Rørslett 1989, Hellsten 2001, Hellsten & Mjelde 2009), and also to a certain degree with the growth strategy classification described by Grime (1977).

Table 5.3. Aquatic macrophytes in Finnish, Swedish and Norwegian lakes – sensitive and tolerant to water level regulation (mainly HEP regulation). Species with < 4 loc. not included

Group	Tolerant species	Sensitive species	Less sensitive
ISOETIDS	<i>Eleocharis acicularia</i> <i>Limosella aquatica</i> <i>Ranunculus reptans</i> <i>Subularia aquatica</i>	<i>Elatine hydropiper</i> <i>Isoetes lacustris</i> <i>Littorella uniflora</i> <i>Lobelia dortmanna</i>	<i>Crassula aquatica</i> <i>Elatine ortosperma</i> <i>Elatine triandra</i> <i>Isoetes echinospora</i>
ELODEIDS	<i>Callitriche hamulata</i> <i>Callitriche hermaphroditica</i> <i>Callitriche palustris</i> <i>Hippuris vulgaris</i> <i>Juncus bulbosus</i> <i>Utricularia vulgaris</i>	<i>Callitriche copocharpa</i> <i>Elodea canadensis</i> <i>Myriophyllum alterniflorum</i> <i>Myriophyllum verticillatum</i> <i>Potamogeton alpinus</i> <i>Potamogeton berchtoldii</i> <i>Potamogeton obtusifolius</i> <i>Ranunculus peltatus</i>	<i>Potamogeton gramineus</i> <i>Potamogeton perfoliatus</i> <i>Potamogeton pusillus</i> <i>Utricularia intermedia</i> <i>Utricularia minor</i> <i>Utricularia oroleuca</i>
NYMPHAEIDS	<i>Sparganium angustifolium</i> <i>Sparganium hyperboreum</i>	<i>Nuphar lutea</i> <i>Nuphar pumila</i> <i>Nymphaea alba</i> <i>Persicaria amphibia</i> <i>Potamogeton natans</i> <i>Sagittaria natans</i> <i>Sagittaria sagittifolia</i> <i>Sparganium emersum</i> <i>Sparganium natans</i>	<i>Nymphaea candida</i> <i>Nymphaea tetragona</i> <i>Sparganium gramineus</i>
LEMNIDS		<i>Lemna minor</i>	
CHAROPHYTES			<i>Nitella opaca</i>

5.2.6 Developing water level fluctuation index for Nordic lakes

Hellsten and Mjelde (2009) suggested a water level index (W_{Ic}) using macrophytes to describe the ecological status or ecological potential for regulated lakes. Based on this preliminary work we have developed an improved water level index – W_{Ic(i)}, with the same equation is the as earlier:

$$WI_{C(i)} = \frac{N_S - N_T}{N} \times 100$$

where W_{Ic} is the water level regulation index, N_S is the number of sensitive species, N_T is the number of tolerant species, and N is the total number of species in the lake, including the less sensitive.

Water level regulation index W_{Ic(i)} correlated very well with winter drawdown in the storage reservoirs (H3) for all countries (Fig. 5.4), respectively r²=0.77, 0.67 and 0.73 for Finnish,

Norwegian and Swedish lakes. Some weaker correlation for the Norwegian lakes may be due to some lakes with littoral zone dominated by stones.

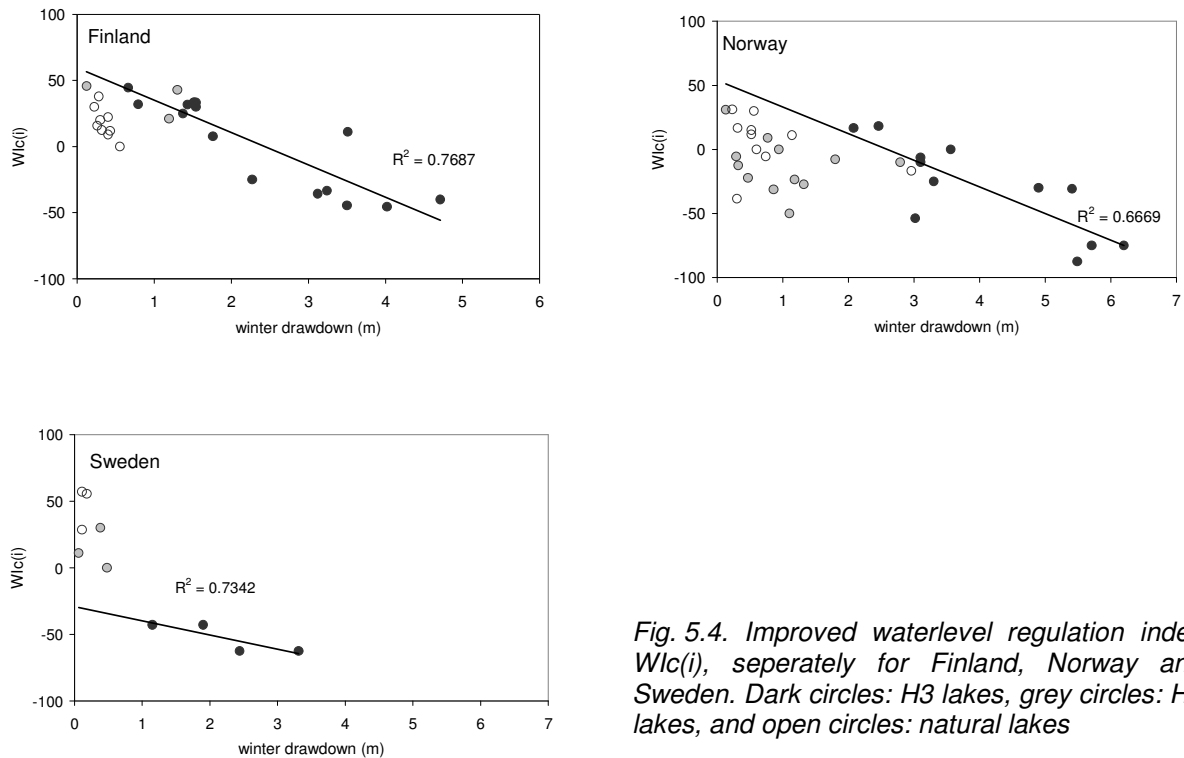


Fig. 5.4. Improved waterlevel regulation index $Wlc(i)$, separately for Finland, Norway and Sweden. Dark circles: H3 lakes, grey circles: H2 lakes, and open circles: natural lakes

Some natural or slightly regulated lakes (H2) seem to have very low index value, especially seen among the Norwegian lakes. This is mainly due to a littoral zone dominated by stones. However, most natural and slightly regulated lakes have index values higher than -20.

The lakes in the H2 group and the natural lakes (N2 and sN2) normally have much less water level fluctuations than the storage lakes. In addition, hydrological regimes are very heterogenous. Therefore, the correlation between $Wlc(i)$ and winter drawdown in these lakes are weak. These lakes will not be included in the boundary setting assessments.

The slope for the Swedish lakes is very different from the Finnish and Norwegian lakes (Fig. 5.5). The reason for this may be the very low number of species observed in some of the Swedish lakes. Swedish method with virtual transects might leave large number of species outside of analysis.

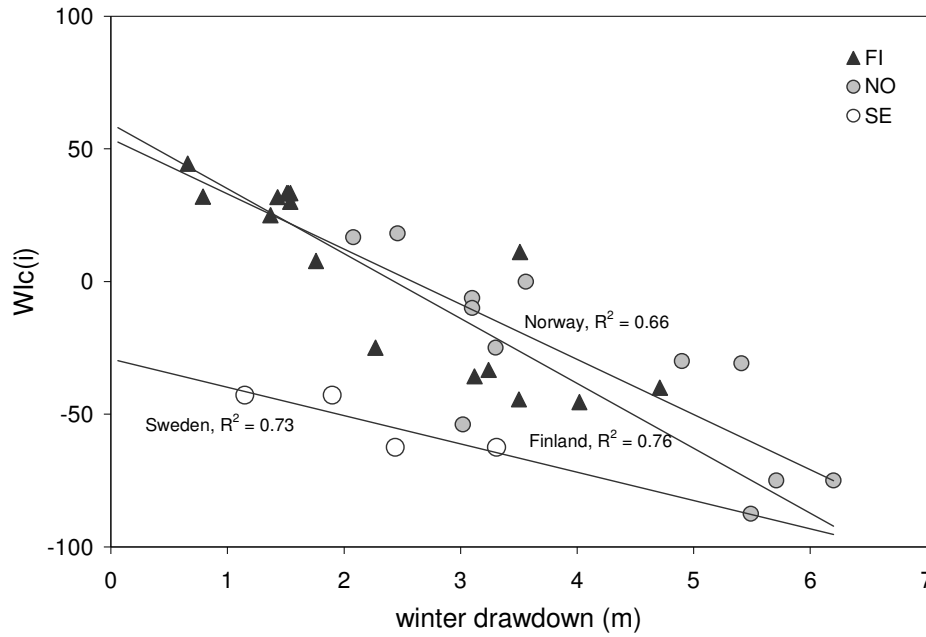


Fig. 5.5. Regression between winter drawdown and the water level index $Wlc(i)$ for the storage lakes (H3). Regression calculated separately for the three Nordic countries

Until this dissimilarity is further investigated, the index and suggested boundaries will only be applicable for Finland and Norway. Figure 5.6 shows the regression between the improved index and the pressure for Finnish and Norwegian storage reservoirs.

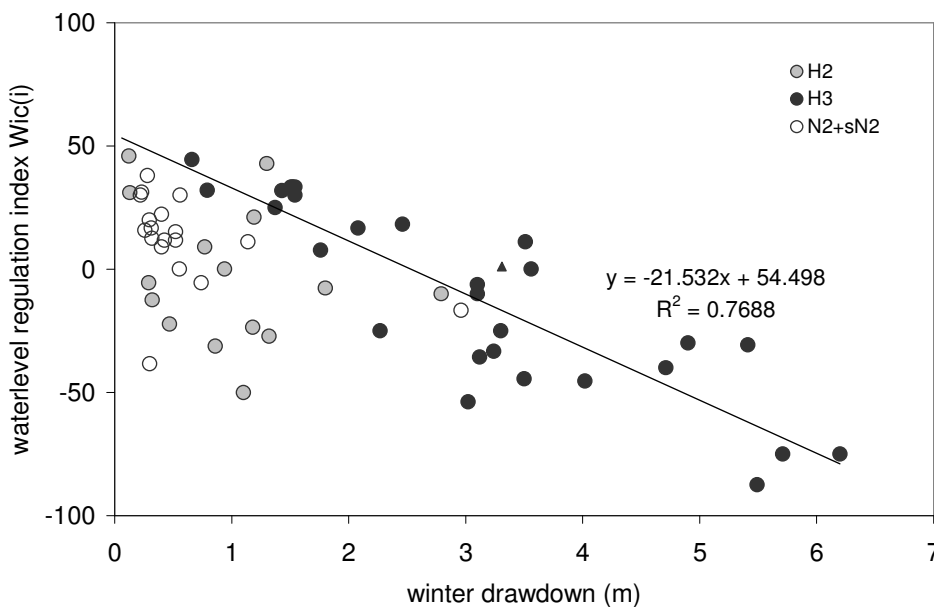


Fig. 5.6. Improved waterlevel regulation index $Wlc(i)$. Lakes with total species number <4 are excluded. In addition, Lake Kemijärvi is excluded, because the large delta-area, with fine substrate that remains unfrozen, despite the winter drawdown

5.2.7 Definition of boundaries

As a reference value we suggest $Wlc(i) = 29$. This represent 75th percentile of the index values for natural and semi-natural lakes (Finnish and Norwegian lakes, only). Further, we suggest a high/good boundary $Wlc(i) = 10$, which is the 25th percentile of the index values for natural and semi-natural lakes.

Stands of *Isoetes lacustris* seem to disappear when winter drawdown exceed 3.4-3.5 m (Fig. 5.7). Therefore, for good/moderate boundary we suggest to use $Wlc(i) = -20$, which correspond to a winter drawdown at 3.4-3.5m (see the equation in Fig. 5.6).

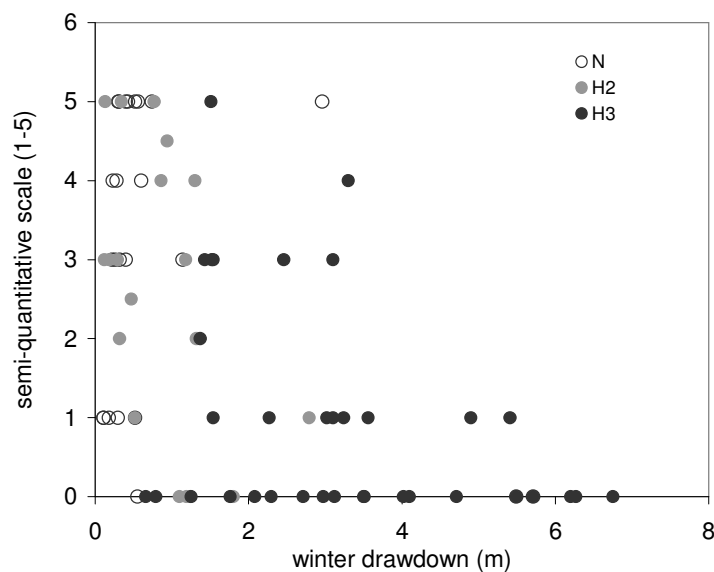


Fig. 5.7. Abundance of *Isoetes lacustris* compared to winter drawdown

Table 5.4 summarizes the suggested boundaries for Finnish and Norwegian storage reservoirs, based on the species composition indeks $Wlc(i)$.

Table 5.4. Suggested boundaries for Finnish and Norwegian storage lakes (H3)

category	$Wlc(i)$ value	Corresponding winter drawdown level (m)
Reference	29	1.2
High/good	10	2.1
Good/moderate	-20	3.5
Moderate/poor	na	na
Poor/Bad	na	na

In addition, when setting boundaries, it is important to take into account the clarity of the lake. Rørslett (1989) discussed the relationship between erosion depth (similar to winter drawdown), Secchi depth and presence/absence of *Isoetes lacustris* in storage reservoirs. Similar, the same relationship can be seen in the lakes analysed here (Fig. 5.8). The figure shows that *Isoetes lacustris* can exist in heavy regulated lakes as long as the secchi depth is high. On the contrary, if the secchi depth is lower, *Isoetes* can disappear also in less regulated lakes. Based on the figure, the good/moderate boundary requires a Secchi depth on at least 5-6 m. If the Secchi depth is lower, a winter drawdown less than 3.4-3.5 m can course loss of *Isoetes lacustris*.

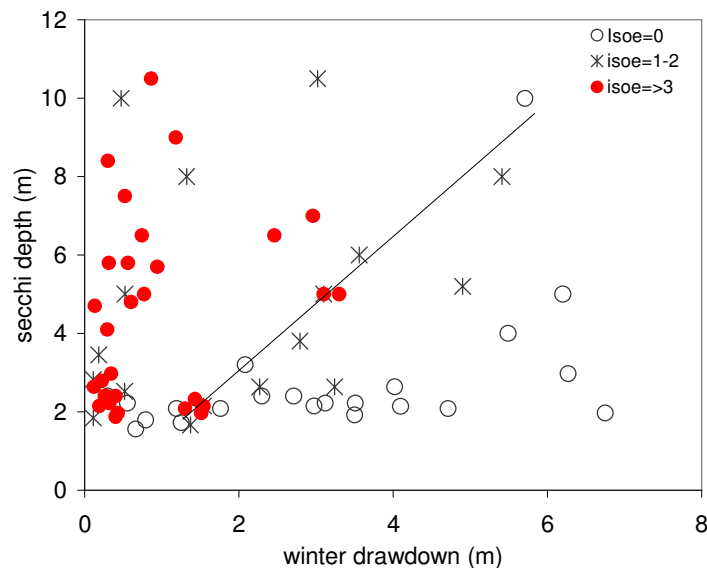


Fig. 5.8. The relationship between winter drawdown, Secchi depth and presence/absence of *Isoetes lacustris*. The presence is based on a semi-quantitative scale, and red dots common-dominant (3-5), stars means rare-sparse and open circles means absent

5.3 Developing water-level fluctuation index for Dutch floodplain lakes

5.3.1 Material and methods

Only limited data were available with regard to the amplitude of water-level fluctuations and macrophyte occurrence. For the development of the water-level fluctuations index, data are used of macrophyte occurrence in floodplain lakes along the Lower Rhine (Van Geest *et al.*, 2005, unpublished results). The method and results of this study are given below. These data have been supplemented with additional data for the occurrence of macrophytes in relation to water-level fluctuations in the river Rhine and Meuse (Maenen, 1989; Aggenbach *et al.* 2007).

In the study of Van Geest *et al.* (2005), the research was confined to lakes that were shallow (mean depth < 2 meter), relatively small (0.01 – 32 ha) and disconnected from the main channel during summer. In 1999, submerged and floating-leaved macrophytes were sampled in 100 floodplain lakes along the Lower Rhine.

Sampling of plots

In July – early August 1999, aquatic vegetation was sampled in 917 plots distributed over 100 floodplain lakes along the Lower Rhine. The number of plots per lake varied (4-31), depending on lake size and apparent complexity of the vegetation structure. Plots were selected to represent the range of vegetation types present in the lake. Plot size varied from 1 m² (submerged vegetation) to 4 m² (nymphaeid vegetation). All plots were sampled from a boat. In each plot, species composition and cover were determined by combining visual estimates and collection. Species cover in each plot was expressed in one of seven classes (1, < 1%; 2, 1-5%; 3, 6-15%; 4, 16-25%; 5, 26-50%; 6, 51-75%; 7, 76-100%), and total percentage cover of submerged plants, floating plants, helophytes and of filamentous algae were estimated in the same way.

Sampling of species richness

Preliminary research indicated that calculation of species richness of aquatic macrophytes on the basis of data from the plots alone would result in an underestimation of species richness in the lakes. Therefore, for the 100 lakes sampled in 1999, species composition and abundance of submerged and floating-leaved macrophyte vegetation of the whole lake area were surveyed by boat until no new species were found. The time spent per lake varied from approximately 30 minutes for very small lakes (< 0.10 ha) to several hours for lakes with a large surface area or a complex vegetation structure. Species abundances were recorded using the Tansley-scale (rare, occasional, frequent, abundant, dominant), and were converted to an ordinal scale ranging from 1 to 5 for statistical analysis.

Abiotic variables

Water depth in July was established at each plot sampled for vegetation (per lake, 4 – 31 plots were sampled for vegetation, see above). The seasonal water level trend between July and October 1999 was measured from a marked rod placed in each lake. Furthermore, the proportion of the surface area of the lake bottom that became exposed between July and October ('drawdown area') was determined by visual estimation.

5.3.2 Results

Water-level fluctuations in relation to species richness

In the lakes sampled, the decrease in water level between July and October ranged between 0.10 and 2.30 meter (25, 50 and 75 percentile value of resp. 0.49, 0.98 and 1,35 meter). Hence, in this dataset the number of lakes with strongly stabilized (< 0.2 meter) or highly fluctuating water-levels (> 1 meter) is relatively low.

The species richness of submerged macrophytes and helophytes, as well as total number of macrophyte species, was significantly related to the amplitude of water-level fluctuations (ANOVA, $p = 0.008$, 0.0139 , and 0.013 , respectively, $F_6 = 2.89$, 3.11 , and 2.85 , respectively). For submerged macrophytes and total number of macrophytes, species richness was lower at fluctuations within the range ≤ 0.2 m compared to $0.4 - 0.6$ m (in both cases $p < 0.05$; Tukey HSD; Fig. 5.9 a,d). For helophytes, species richness was significantly higher at fluctuations of $0.4 - 0.6$ m compared to $1.0 - 1.2$ m ($p < 0.05$, Tukey HSD, Fig. 5.9 b). For floating-leaved macrophytes, no significant pattern between species richness and amplitude of water-level fluctuations was detected (ANOVA; $p = 0.051$), although there was a trend for increased species richness at $0.4 - 0.6$ m (Fig. 5.9 c).

Definitions for index of water-level stabilization

Undisturbed lakes with a natural water-level regime in temperate zones have high water-levels during spring and low water-levels during summer. With decreasing water-levels during summer, parts of lake sediments may become exposed to the air (drawdown). Such drawdown events may influence macrophyte composition and abundance in several ways. For germination, many macrophyte species depend on temporary drawdown. As a result of water-level stabilization, drawdown will not occur and these species may disappear from the lakes. Furthermore, drawdown may reduce biomass of competitive species, giving space for smaller species that would otherwise be absent because of severe competition. Overall, it can be concluded that water-level fluctuations will result in (partial) drawdown of the lakes, which will have a beneficial effect on species richness, as long as the water-level fluctuations are not larger than approximately 1 meter. Water-level fluctuations larger than 0.2 meter already have a positive influence on macrophyte richness because of the occurrence of drawdown in these lakes (Fig. 5.9, 5.10). Therefore, the value of 0.2 meter has been used as a lower limit threshold, below which water level fluctuations are too small to be beneficial for macrophyte development.

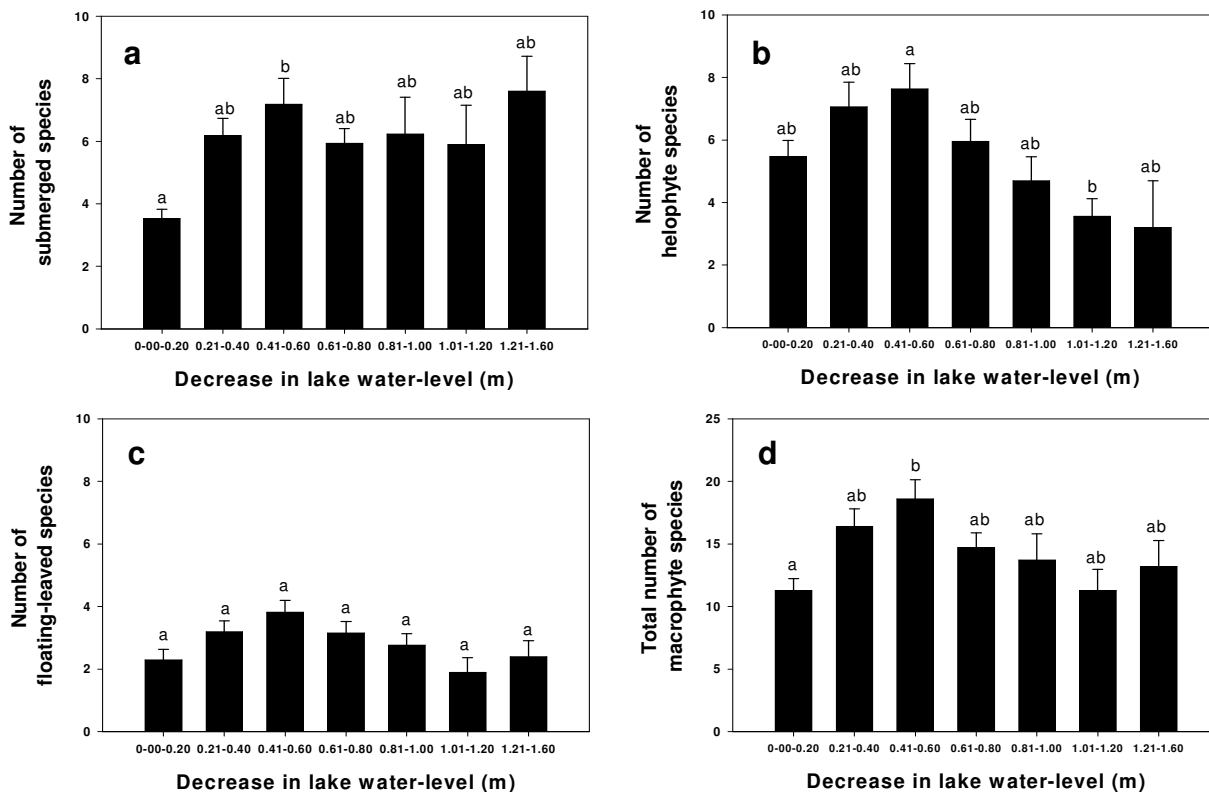


Fig. 5.9. Frequency distribution of species richness of submerged, floating-leaved, helophytes and total number of macrophytes in lakes (\pm standard error) in relation to decline in lake water-level between July – October 1999. Data for lakes with an increase in water-level during July – October 1999 ($n=4$) were excluded. Significant differences are indicated with different letters (post-hoc comparison with Tukey HSD test, $p < 0.05$)

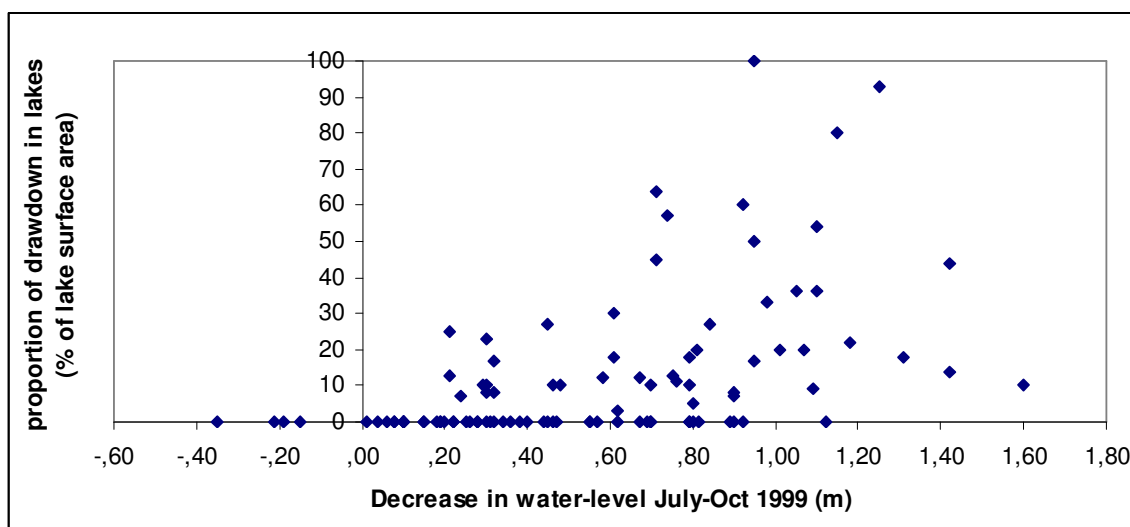


Fig. 5.10. Relationship between amplitude of water-level fluctuations and proportion of drawdown area in the lakes. Lakes with fluctuations less than 0.2 meter have no drawdown area

The definition for sensitive, tolerant and less-sensitive species for stabilized water-levels are:

- **Sensitive species:** a macrophyte species is regarded as sensitive to stabilized water-levels if the presence of this species is decreased at fluctuations between 0 – 0.2 meter compared to fluctuations between 0.2 – 1 meter in the lakes.
- **Tolerant species:** a macrophyte species is regarded as tolerant to stabilized water-levels if the presence of this species is increased at fluctuations between 0 – 0.2 meter compared to fluctuations 0.2 – 1 meter in the lakes.
- **Less-sensitive species:** a macrophyte species is regarded as ‘less-sensitive’ to stabilized water-levels when there is no clear difference in response to the amplitude of water-level fluctuations between 0 – 0.2 meter compared to fluctuations between 0.2 – 1 meter in the lakes.

This classification has been based on Figure 5.11, as well as data of Maenen (1989), Aggenbach *et al.* (2007) and expert judgement.

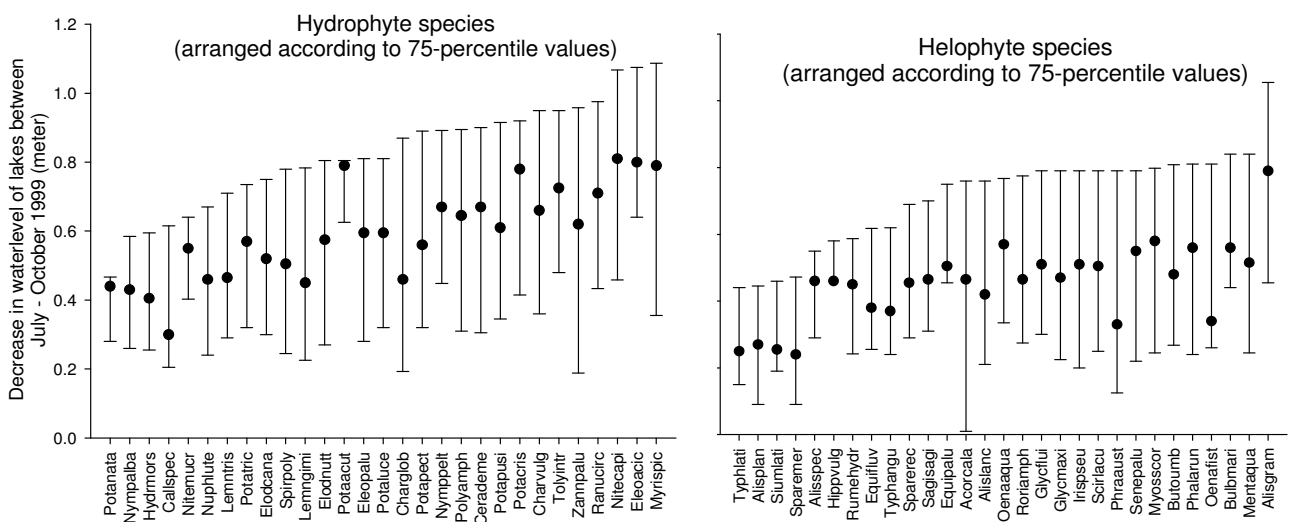


Fig. 5.11. The response of species to decrease in waterlevel between July and October for hydrophytes (upper) and helophytes (lower). 75 percentile values were used as a sorter. Species with less than 3 localities are excluded in the figure.

Definitions for index of increased water-level fluctuations

If water-level fluctuations become too large, species may disappear because tolerance limits are exceeded. For helophytes, the water-levels during spring may be too high for successful establishment, because plants are not able to reach the water surface. For submerged plants, large water-level fluctuations water-levels may result in too high water-levels during spring, which result in a reduced light intensity at the sediment that is too low for successful establishment (Canfield *et al.*, 1985; Middelboe & Markager, 1997).

In the data of Van Geest et al. (2005), only few lakes are present with large water-level fluctuations. Therefore, the data of Maenen (1989) and Aggenbach *et al.* (2007) have been extensively used for the classification into sensitive, tolerant and less-sensitive species. Based on the information presented in the classification schemes of Maenen (1989) and Aggenbach *et al.* (2007), a threshold value of 1 meter has been used (for further definition: see below).

The definition for sensitive, tolerant and less-sensitive species for increased water-level fluctuations are:

- Sensitive species: a macrophyte species is regarded as sensitive to large water-level fluctuations if the presence of this species is reduced in lakes with water-level fluctuations > 1 meter compared to lakes with fluctuations between 0.2 – 1 meter.
- Tolerant species: a macrophyte species is regarded as tolerant to large water-level fluctuations if the presence of this species is increased in lakes with water-level fluctuations > 1 meter compared to lakes with fluctuations between 0.2 – 1 meter.
- Less-sensitive species: a macrophyte species is regarded as ‘less-sensitive’ when there is no differences in presence between lakes with water-level fluctuations > 1 meter compared to lakes with fluctuations between 0.2 – 1 meter.

In Table 5.5, the response of species to water-level stabilization is given (viz. fluctuations < 0.2 meter and – hence - absence of drawdown, see Figure 5.10). In Table 5.6, the response of macrophyte species to increased amplitude of water-level fluctuations (> 1 meter) is shown. Both indices have been calculated based on presence/absence data, as well on abundance of species.

For presence of species, the following formula was used:

$$WI_C = (N_s - N_t * 100) / N_{all}$$

where WI_C is the water-level regulation index, N_s is the number of sensitive species (with decreasing occurrence), N_t is the number of tolerant species (with increasing occurrence), and N is the total number of species, including less sensitive species.

For species abundance, the following formula was used:

$$WI_C = ((\sum A_s - \sum A_t) * 100) / \sum A_{all}$$

where WI_C is the water-level regulation index, $\sum A_s$ is the sum of abundances of sensitive species, $\sum A_t$ is the sum of abundances of tolerant species, and $\sum A$ is the sum of abundances of all species, including less sensitive species.

Table 5.5. Macrophytes organized according to their sensitivity for water-level stabilization (viz. water-level fluctuations < 0.2 meter). Tolerant species and sensitive species show resp. an increase and decrease with water-level stabilization. This classification is based on Figure 5.11, Maenen (1989) and Aggenbach et al. (2007).

Less sensitive species	Sensitive species	Tolerant species
<i>Lemna minor</i>	<i>Acorus calamus</i>	<i>Alisma plantago-aquatica</i>
<i>Lemna trisulca</i>	<i>Alisma gramineum</i>	<i>Elodea canadensis</i>
<i>Myriophyllum spicatum</i>	<i>Alisma lanceolatum</i>	<i>Elodea nuttallii</i>
<i>Phalaris arundinacea</i>	<i>Bulboschoenus maritimus</i>	<i>Epilobium hirsutum</i>
<i>Potamogeton natans</i>	<i>Butomus umbellatus</i>	<i>Glyceria fluitans</i>
<i>Spirodela polyrhiza</i>	<i>Callitriche obtusangula</i>	<i>Hydrocharis morsus-ranae</i>
	<i>Callitriche platycarpa</i>	<i>Nuphar lutea</i>
	<i>Callitriche stagnalis</i>	<i>Nymphaea alba</i>
	<i>Carex acuta</i>	<i>Potamogeton nodosus</i>
	<i>Carex disticha</i>	<i>Potamogeton perfoliatus</i>
	<i>Ceratophyllum demersum</i>	<i>Potamogeton trichoides</i>
	<i>Chara vulgaris</i>	<i>Sium latifolium</i>
	<i>Eleocharis acicularis</i>	<i>Sparganium emersum</i>
	<i>Eleocharis palustris</i>	<i>Typha angustifolia</i>
	<i>Equisetum fluviatile</i>	<i>Typha latifolia</i>
	<i>Equisetum palustre</i>	
	<i>Glyceria maxima</i>	
	<i>Hippuris vulgaris</i>	
	<i>Iris pseudacorus</i>	
	<i>Mentha aquatica</i>	
	<i>Myosotis palustris</i>	
	<i>Nasturtium officinale</i>	
	<i>Nitella capillaris</i>	
	<i>Nitella mucronata</i>	
	<i>Nymphoides peltata</i>	
	<i>Oenanthe aquatica</i>	
	<i>Persicaria amphibia</i>	
	<i>Phragmites australis</i>	
	<i>Potamogeton crispus</i>	
	<i>Potamogeton lucens</i>	
	<i>Potamogeton pectinatus</i>	
	<i>Potamogeton pusillus</i>	
	<i>Ranunculus circinatus</i>	
	<i>Rorippa amphibia</i>	
	<i>Rumex hydrolapathum</i>	
	<i>Sagittaria sagittifolia</i>	
	<i>Schoenoplectus lacustris</i>	
	<i>Senecio palustris</i>	
	<i>Sparganium erectum</i>	
	<i>Tolypella intricata</i>	
	<i>Zannichellia palustris</i>	

Table 5.6. Macrophytes organized according to their sensitivity for large water-level fluctuations during the growing season (> 1 meter). Tolerant species and sensitive species show resp. an increase and decrease with large water-level fluctuations. This classification is based on Figure 5.11, Maenen (1989) and Aggenbach et al. (2007).

Less sensitive species	Sensitive species	Sensitive species (cont.)
<i>Lemna minor</i>	<i>Acorus calamus</i>	<i>Rumex hydrolapathum</i>
<i>Phalaris arundinacea</i>	<i>Alisma lanceolatum</i>	<i>Sagittaria sagittifolia</i>
<i>Spirodela polyrhiza</i>	<i>Alisma plantago-aquatica</i>	<i>Schoenoplectus lacustris</i>
	<i>Bulboschoenus maritimus</i>	<i>Senecio palustris</i>
	<i>Butomus umbellatus</i>	<i>Sium latifolia</i>
<u>Tolerant species</u>	<i>Callitriche obtusangula</i>	<i>Sparganium emersum</i>
	<i>Callitriche platycarpa</i>	<i>Sparganium erectum</i>
<i>Alisma gramineum</i>	<i>Callitriche stagnalis</i>	<i>Typha angustifolia</i>
<i>Chara vulgaris</i>	<i>Carex acuta</i>	<i>Typha latifolia</i>
<i>Eleocharis acicularis</i>	<i>Carex disticha</i>	<i>Zannichellia palustris</i>
<i>Nitella capillaris</i>	<i>Ceratophyllum demersum</i>	
<i>Oenanthe aquatica</i>	<i>Chara globularis</i>	
<i>Persicaria amphibia</i>	<i>Eleocharis palustris</i>	
<i>Tolypella intricata</i>	<i>Elodea canadensis</i>	
	<i>Elodea nuttallii</i>	
	<i>Epilobium hirsutum</i>	
	<i>Equisetum fluviatile</i>	
	<i>Equisetum palustre</i>	
	<i>Glyceria fluitans</i>	
	<i>Glyceria maxima</i>	
	<i>Hippuris vulgaris</i>	
	<i>Hydrocharis morsus-ranae</i>	
	<i>Iris pseudacorus</i>	
	<i>Lemna trisulca</i>	
	<i>Mentha aquatica</i>	
	<i>Myosotis palustris</i>	
	<i>Myriophyllum spicatum</i>	
	<i>Nasturtium officinale</i>	
	<i>Nitella mucronata</i>	
	<i>Nuphar lutea</i>	
	<i>Nymphaea alba</i>	
	<i>Nymphoides peltata</i>	
	<i>Phragmites australis</i>	
	<i>Potamogeton crispus</i>	
	<i>Potamogeton lucens</i>	
	<i>Potamogeton natans</i>	
	<i>Potamogeton nodosus</i>	
	<i>Potamogeton pectinatus</i>	
	<i>Potamogeton perfoliatus</i>	
	<i>Potamogeton pusillus</i>	
	<i>Potamogeton trichoides</i>	
	<i>Ranunculus circinatus</i>	
	<i>Rorippa amphibia</i>	

In Figure 5.12, the relationship between WI_C -stabilization and percentage of drawdown area of the lakes is given for resp. species presence and abundance. In both cases, WI_C was significantly related to lake drawdown (Spearman $R =$ resp -0.45 ($p < 0.01$) and -0.55 ($p < 0.01$). There is however, a large amount of scatter around the regression lines, especially for lakes with no drawdown. Hence, the performance of the index is rather poor, as this index should discriminate between sites with and without drawdown.

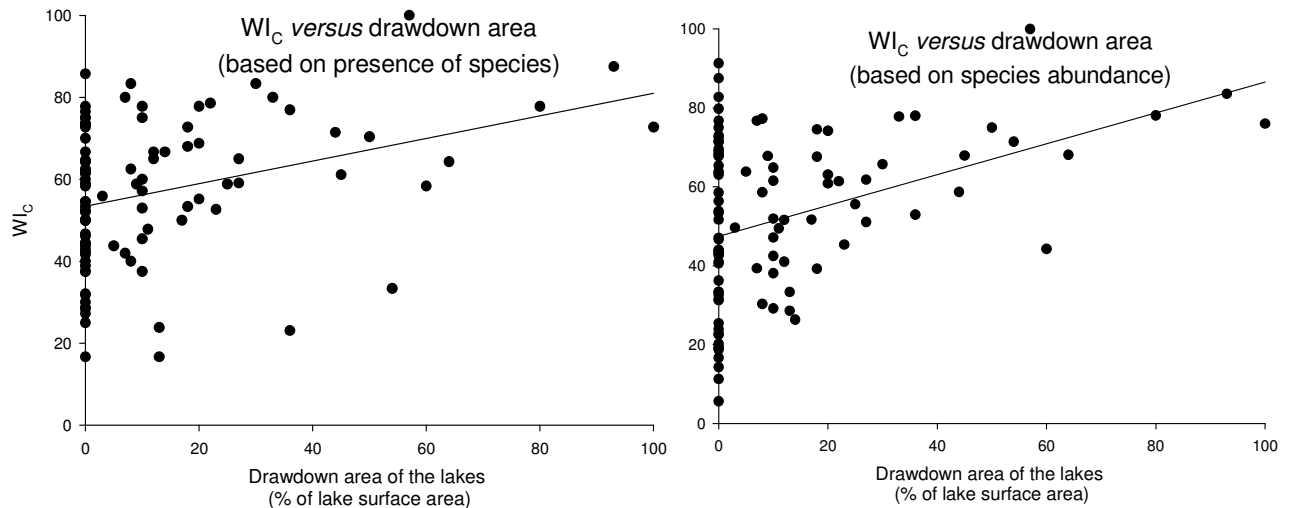


Fig. 5.12. Relationship between WI_C and summer drawdown for floodplain lakes along the lower Rhine in The Netherlands based on **presence** of species as listed in Table 5.5 (left) and **abundance** of species listed in Table 5.5 (right)

In Figure 5.13, the relationship between WI_C and increased amplitude of water-level fluctuations (> 1 meter) is given for resp. species presence and abundance. In both cases, WI_C was significantly related to increased amplitude of water-level fluctuations (Spearman $R =$ resp 0.31 ($p < 0.01$) and 0.31 ($p < 0.01$). Also for these indices, there is a large amount of scatter around the regression line. Furthermore, the interpretation of this index is hampered by the relatively small number of lakes with fluctuations larger than 1 meter.

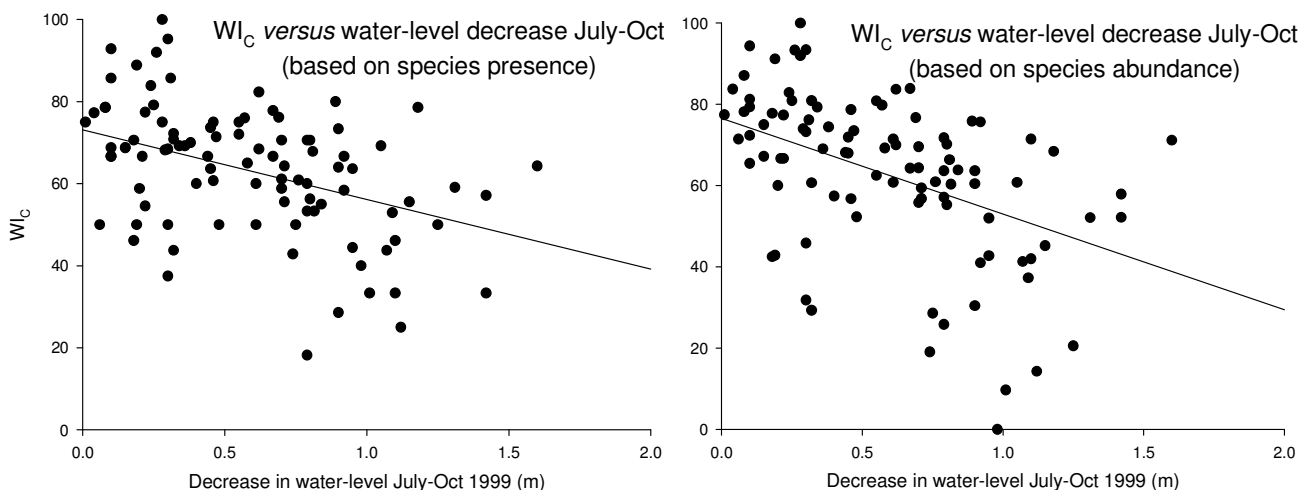


Fig. 5.13. Relationship between WI_C and amplitude of water-level fluctuations for floodplain lakes along the lower Rhine in The Netherlands based on **presence** of species as listed in Table 5.6 (left) and **abundance** of species listed in Table 5.6 (right)

5.3.3 Discussion

Availability of hydrological data and consequences for metric development

There is a huge gap in the availability of data of the water-level regime of lakes in countries from the Central Baltic region. For all lakes that are included in the WISER or other (monitoring) programs, no data were available with regard to water-level fluctuations. Even a classification of the lakes based on different classes of water-level fluctuations could not be made. This shortage of data is not restricted to Europe. Also for wetlands in North-America, there is a huge gap in the availability of data with regard to the water-level regime (Keddy, 2000). Obviously, the lack of data of the water-level regime hampers the development of a water-level fluctuation metric based on macrophyte composition. In this study, only data are used of floodplain lakes along the River Rhine (Van Geest *et al.*, 2005) and macrophytes of the River Rhine and Meuse in the Netherlands (Maenen, 1989; Aggenbach *et al.*, 2007). Because these data are restricted to a small geographic area, the applicability to other areas in the Central Baltic is unknown. Additionally, these data are only derived from eutrophic shallow lakes (average lake depth < 3 meter), and the applicability of the index for deeper, stratified or meso-/oligotrophic lakes with higher water clarity is also unknown. Furthermore, in the datasets only few data were available for sites with either strongly stabilized water levels or high amplitude of water-level fluctuations. Such sites are required for the development of a metric, because the extreme ends of water-level fluctuations will strongly determine the overall response curve of macrophytes to fluctuating water-levels. At last, the validation of the metric should be carried out with data that has not been used for the construction of the metric. Hence, the available data are not sufficient for the development of a ‘general’ water-level fluctuation metric, which is widely applicable in the Central-Baltic.

Performance of water-level fluctuation metrics

In this project, two metrics have been developed: one for stabilized water-levels, and one for increased water-level fluctuations. Both of these indices have been calculated for presence and abundance of species.

The WI_c for water-level stabilization was positively related to the proportion of summer drawdown in these lakes. In these lakes, the absence of summer drawdown can be regarded as a disturbance, as many macrophyte species require temporary exposed soils for germination and/or successful establishment. These species will disappear when summer drawdown in these lakes is absent because of a strong stabilization of water-levels. In addition, summer drawdown may reduce the biomass of strong competitors (e.g. *Elodea*) that cannot resist desiccation, giving space to smaller, less competitive species. For this metric, the correlation between WI_c and drawdown area was higher for the ‘abundance-based’ metric than for the ‘presence-based’ metric (Spearman R = resp. -.55 and -0.45). However, there is still a huge scatter around the regression line, especially for lakes with no drawdown. The causes for this might be found in other pressures present at the site (e.g. nutrient loading difference in relation to the frequency of inundation over multiple years, access to the lake by livestock or differences in soil types). A

combination of the information on water level fluctuation with that of nutrient loading might improve the percentage explained variance.

The WI_c for water-level fluctuations larger than 1 meter (during the growing season) was positively related to the amplitude of water-level fluctuations in the lakes. If fluctuations become too large, species may disappear because tolerance limits are exceeded. For helophytes, this may be caused by complete submergence of the plants, because many helophyte species require that at least the tip of the plant is above the water surface as oxygen from the air needs to be transported to the roots. For submerged plants, a large decrease of the water-level during the growing season implies that the light intensity at the sediment may be too low for growth or successful establishment of macrophytes, because of the high water-levels during spring time (Canfield *et al.*, 1985; Middelboe & Markager, 1997). For this metric, there were no differences between the correlation coefficients of the ‘abundance-based’ and ‘presence-based’ metric (in both cases, Spearman $R = 0.31$). Also for this metric, there is still a huge scatter around the regression line. The causes for this are probably similar to those suggested in the previous paragraph.

Room for improvement of the metrics

In this study, a first step has been made for the development of metrics for resp. water-level stabilization or increased fluctuations. For both metrics, a significant correlation is obtained for resp. the proportion of drawdown and large water-level fluctuations. This result is promising given the limited availability of data. Nevertheless, for both metrics there is a large scatter around the regression lines that needs to be reduced. The performance of the metrics can be improved in the following ways:

1. clearly, more data should be obtained with regard to water-level regime of the lakes in relation to macrophyte composition and abundance (for both hydrophytes and helophytes). Especially the extremes of water-level fluctuations are of high importance (water-level stabilization and high amplitude of water-level fluctuations), as well as information about the variation of water-levels between years.
2. as explained above, many mechanisms may be involved in the response of macrophytes to changing water-level regimes. The approach used in this study is rather descriptive, and doesn't take into account the mechanisms involved. Incorporation of life history strategies (e.g. the R-S-C division of Grime) may resolve this issue, because such an approach facilitates causal explanations between changes in the water-level regime and macrophyte occurrence;
3. water-level fluctuations and connectivity with the river may also result in changes of the foodweb structure in lakes or biogeochemical pathways. These changes may also strongly influence the occurrence and abundance of macrophytes. Examples of these is the occurrence of alternative stable states in shallow, eutrophic lakes (Scheffer, 1998), and the effects of sediment exposure for the availability of phosphate, and – hence – trophic state of the lakes.

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6. General conclusions and recommendations

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- Macrofossils of aquatic plant provide an insight not only into the past species assemblages that might have populated ‘reference’ communities in lakes, but also it can be demonstrated that these assemblages have in many cases shifted away from the relatively stable conditions of pre-1850. Beta-diversity of sub-fossil plant remains can be used to assess the direction and magnitude of change away from pre-defined reference conditions and therefore this palaeo-limnological tool represents a valuable asset for both defining reference conditions and for setting restoration targets.
- The best performing metrics for detecting eutrophication pressure were those based on trophic scores of species – the Intercalibration Common Metric (ICM) and the Ellenberg Index (EI). They can be recommended in many countries and lakes types as common metrics for IC purposes.
- When using EI it is recommended to include helophytes when recorded since the assessment of eutrophication seems to be more reliable when more scored taxa are considered. The higher the number of the species with an indicative value the more reliable the assessment.
- The metrics based on taxa richness or proportions of functional groups responded much less consistently to the eutrophication pressure gradient than those based on trophic scores and their use for IC is therefore very limited.
- The analysis showed that the abundance of submerged macrophytes is potentially a good candidate metric responding very significantly to eutrophication stressors as macrophyte abundance decreases with increasing algal turbidity along the eutrophication gradient.
- The analyses were mainly conducted on siliceous deep or shallow lakes (due to data availability). Other lake types, as for example coloured lakes or lakes at high latitudes above 60° have different macrophyte characteristics or reduced C_{max} due to decreased PAR. For a broader use of macrophyte abundance metrics for detecting eutrophication impacts, other factors affecting underwater light climate such as the content of humic substances, and geographic latitude must be eliminated (or taken appropriately into account).
- There is a large variability in C_{max} for reference lakes both between countries and within individual countries. In the future work it would be valuable to conduct more detailed investigations into how the definitions were made for reference lakes (and H and G classes) – especially if these are used as basis for defining ecological boundaries.
- For both C_{max} and coverage there is also a considerable year-to-year variation which needs to be acknowledged (using for example data from at least three sampling years) to reduce the risk of misclassification of lakes.
- It is recommended that C_{max} is used as a macrophyte abundance metric in lakes with maximum depths above 6 m (or mean depths above 3 m) whereas coverage of submerged macrophytes is used in shallow lakes (mean depth <3 m).
- Clear relationships can be established between macrophyte abundance (C_{max} and coverage) and chlorophyll *a* and Secchi depth, but it is recommended that C_{max} and coverage are defined independently from other metric boundaries (such as chlorophyll *a*) to avoid circular conclusions and simple “translation errors” between metrics.

- Taxonomic composition of macrophytes is a poor indicator of water level changes, because most of the species are still present after water level increase, whereas lowering of water level will lead to increased diversity, as newly exposed littoral zone or general shallowness allows the sublittoral zone to cover the entire water body.
- Abundance of macrophytes is a much more sensitive indicator for hydrological change than species composition. The effect of water level fluctuation on zonation patterns depends, however, on the bathymetry of a lake and thus is a lake-specific reaction.
- A new waterlevel index for Nordic lakes (W_{Ic}) was elaborated based on dividing macrophyte species into sensitive and tolerant species regarding water level fluctuations.
- As the W_{Ic} index was based on data from low alkalinity, oligotrophic lakes, the index should therefore be used for assessment of such lakes only.
- W_{Ic} correlated very well with winter drawdown in the storage reservoirs for all countries with respective R² values of 0.77, 0.67, and 0.73 for Finnish, Norwegian and Swedish lakes. The slope of the regression for the Swedish lakes was very different from that for the Finnish and Norwegian lakes. Until this dissimilarity is further investigated, the index and suggested boundaries will only be applicable for Finland and Norway.
- The reference value and the high/good boundary were set based on natural and semi-natural lakes. The good/moderate boundary was set at W_{Ic} value of -20, which corresponds to a winter drawdown of 3.4-3.5 m at which stands of *Isoetes lacustris* usually disappear.
- For lakes in countries from the Central Baltic region, there is a huge gap in the availability of data of the water-level regime. For the development of a metric of water-level fluctuations in this region, only data were available for floodplain lakes and the main channels of the River Rhine and Meuse in The Netherlands.
- Based on these data, two separate indices have been developed: one for the impact of stabilization of water-levels (< 0.2 meter), and one for the impact of large water-level fluctuations (> 1 meter). These metrics have been calculated separately for 'presence' and 'abundance' data of macrophyte species.
- W_{Ic} for water-level stabilization (fluctuations < 0.2 meter) correlated significantly with the proportion of summer drawdown in the floodplain lakes (Spearman R = -0.55 (abundance-based metric) and -0.45 (presence-based metric); both with p < 0.01). However, there is still a huge scatter around the regression line, especially for lakes with no drawdown.
- W_{Ic} for increased water-level fluctuations (> 1 meter) correlated significantly with the amplitude of water-level fluctuations in the lakes (Spearman R = 0.31 for both 'presence' and 'abundance'-based metric; both with p < 0.01) but also for this metric there is a huge scatter around the regression line.
- Because the data originates from a small geographic area with only shallow, eutrophic lakes, the applicability of the metrics to other areas and lake types in the Central-Baltic is unknown. Additional data are required for further development and testing of the metrics.