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SEVENTH FRAMEWORK PROGRAMME

WISER

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

DELIVERABLE

# Deliverable D3.2-2: Report on uncertainty in macrophyte metrics

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| Dissemination Level | | |
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| RE | Restricted to a group specified by the consortium (including the Commission Services) | |
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## Content

# 1 Introduction

## 1.1 Uncertainty issues within the WFD

The Water Framework Directive (WFD) requires that results of the status assessment and monitoring programmes give estimates of the confidence and precision of the determination. Understanding the effect of sampling variation and other sources of uncertainty on the ecological status class assessment and underlying metrics is key in this process. Sources of uncertainty can be, amongst others, due to natural spatial and temporal variation, sampling methodology or predictive modeling (Clarke and Hering, 2006). A detailed overview of general considerations with regards to uncertainty issues in status assessment is given in the WISER Deliverable D6.1-1: "*Report on a workshop to bring together experts experienced with tool development and uncertainty estimation*" (Clarke and Jones, 2008).

For macrophyte status assessment the sampling methodology is an important source of uncertainty. Quantitative estimates of uncertainty related to bioassessment methods are required to reach accurate waterbody ecological classification decisions. Standardised, objective, and repeatable monitoring methods are essential in monitoring programs with aims to detect anthropogenic impact on lake ecosystems. Results of lake macrophyte surveys are extremely sensitive to errors due to both vertical and horizontal variability of macrophyte communities (Jensen 1977, Janauer, 2002). In addition to spatial variability there are errors related to recognition and identification of individual species and also especially to coverage estimations of vegetation.

This study aims to assess the relative importance of different sources of (spatial) variation in the sampling data on uncertainty in the available metrics. Previous work on uncertainty in macrophyte status assessment methods and metrics (especially from the STAR project on running waters) showed that inter-surveyor differences were low and the influences of temporal variation (years and seasons) and shading slightly stronger (Clarke and Hering, 2006). The strongest variation was due to habitat modifications, but several metrics were of sufficient precision in terms of sampling uncertainty to be useful for estimating the ecological status of rivers (Staniszewski et al., 2006). However, the probability of misclassification of a site was found to be largely associated with classification methodology (Szoszkiewicz et al., 2007, 2009).

## 1.2 General scope and specific aims of the report

The general aim of this study was to assess uncertainty in various macrophyte metrics, which might be used in assessing status of this BQE. This has been achieved by using several sources of information, including a dataset collected as part of the WISER project using a common sampling method from 28 lakes in 10 European countries. A primary focus is to quantify the variation resulting from choices in sampling methodology and the effects of lake typology and environmental pressures (especially total phosphorus concentration in the overlying water).

## Research questions

Several specific research questions were formulated:

- How does the choice of using presence-absence data or abundance data affect metric results and their uncertainty?
- How does the choice of the species list that is used (i.e. full list *vs* selected taxa/ with or without helophytes) affect the results of the metric?
- How does surveying 0-1 m depth zone compare to surveying the whole depth range of potentially colonized area?
- How variable is a metric between lake types, between waterbodies, within a waterbody, and between transects?
- What is the effect of using only subsets of the data on the amount of variation? (e.g. depth zone, species saturation limit adjusted)

A practical aim of this work is to give recommendations on appropriate sampling design and analysis methods that are most likely to reduce uncertainty in status assessment. This study does not address the effects of probability of misclassification of water bodies in status classes as for the used metrics no common status boundaries have been defined.

# 2 Methods

To answer the five questions posed in the introduction, data were collected from three sources; (i) a customised field survey of 28 lakes in 10 countries, (ii) an unpublished Finnish study, and (iii) data available from previous studies in the literature.

## 2.1 Data collection

### 2.1.1 Customised field survey

A dedicated sampling campaign was conducted in the summer of 2009. 28 lowland clear water lakes from 10 countries representing broad geographical and trophic gradient were selected for survey (Table 2.1, Appendix).

*Table 2.1. List of lakes surveyed for macrophytes in 2009 for uncertainty analyses. Information is as per WFD definitions. Where information was not available this si denoted with a dash*

| Country | Lake Name | GIG Region | GIG Type | Alkalinity Type | Provisional Status | Eutrophication pressure | Hydromorpho-logical pressure |
|---|---|---|---|---|---|---|---|
| Germany | Roofensee | CB | LCB1 | High | H/G | Low | Low |
| | Grienericksee | CB | LCB1 | High | G/M | Medium | Medium |
| | Glindower See | CB | LCB1 | High | P/B | High | Medium |
| Denmark | Fussingsø | CB | LCB1 | High | - | Medium | - |
| | Nordborgsø | CB | LCB1 | High | - | High | - |
| Estonia | Saadjärv | CB | LCB1 | High | H/G | Low | Low |
| | Viljandi | CB | LCB1 | High | G/M | Low | Medium |
| Poland | Kiełpińskie | CB | LCB1 | High | G | Medium | Low |
| | Rumian | CB | LCB1 | High | M | Medium | Low |
| | Lidzbarskie | CB | LCB1 | High | P/B | High | Low |
| United Kingdom | Rostherne Mere | CB | LCB1 | High | P/B | High | Low |
| | Loweswater | N | LN2a | Medium | M | Medium | Low |
| | Grasmere | N | LN2a | Medium | M | Medium | Low |
| Finland | Sääksjärvi | N | LN1 | Medium | G/M | Low | Medium |
| | Vuojärvi | N | LN1 | Medium | M/P | High | Medium |
| | Iso-Jurvo | N | LN2a | Low | H/G | Low | Medium |
| Norway | Nøklevann | N | LN2a | Low | H | Low | Low |
| | Longumvatnet | N | LN2a | Medium | G/M | Medium | Low |
| | Temse | N | LN2a | Medium | M | Medium | Low |
| Sweden | Västra Solsjön | N | LN2a | Low | H | Low | Low |
| | Fiolen | N | LN2a | Low | M | Medium | Low |
| | Skirösjön | N | LN1 | Medium | P | High | Medium |
| France | Aulnes (étang des) | Med | L-M1 | High | M | High | Low |
| | Salagou (lac du) | Med | - | High | M | Medium | Medium |
| Italy | Segrino | Med | AL5 | High | H/G | Low | Medium |
| | Lago di Monate | Med | AL5 | Medium | G | Medium | Medium |
| | Candia | Med | AL5 | Medium | G/M | Medium | Low |
| | Alserio | Med | AL5 | High | M/P | High | Low |

A common sampling procedure was devised, based on boat transect methods. Within each selected lake, six localities evenly distributed along a shoreline were identified (the first assigned arbitrarily, and the other five at regular intervals around the shore). Within each locality three parallel transects were surveyed, each being 5 m from its neighbour and each starting at the shore and proceeding towards the centre of the lake (Figure 2.1). Each transect was divided into depth zones of 1 m depth intervals down to the macrophyte colonisation depth limit and in each depth zone five macrophyte sampling sites were used. At each sampling site a single sample was gathered from a rake dragged along the bottom for approximately 2 m, and supplemented by observation through a bathyscope, where this was possible. In each sample all species were identified and their abundance was estimated using a continuous percentage scale.

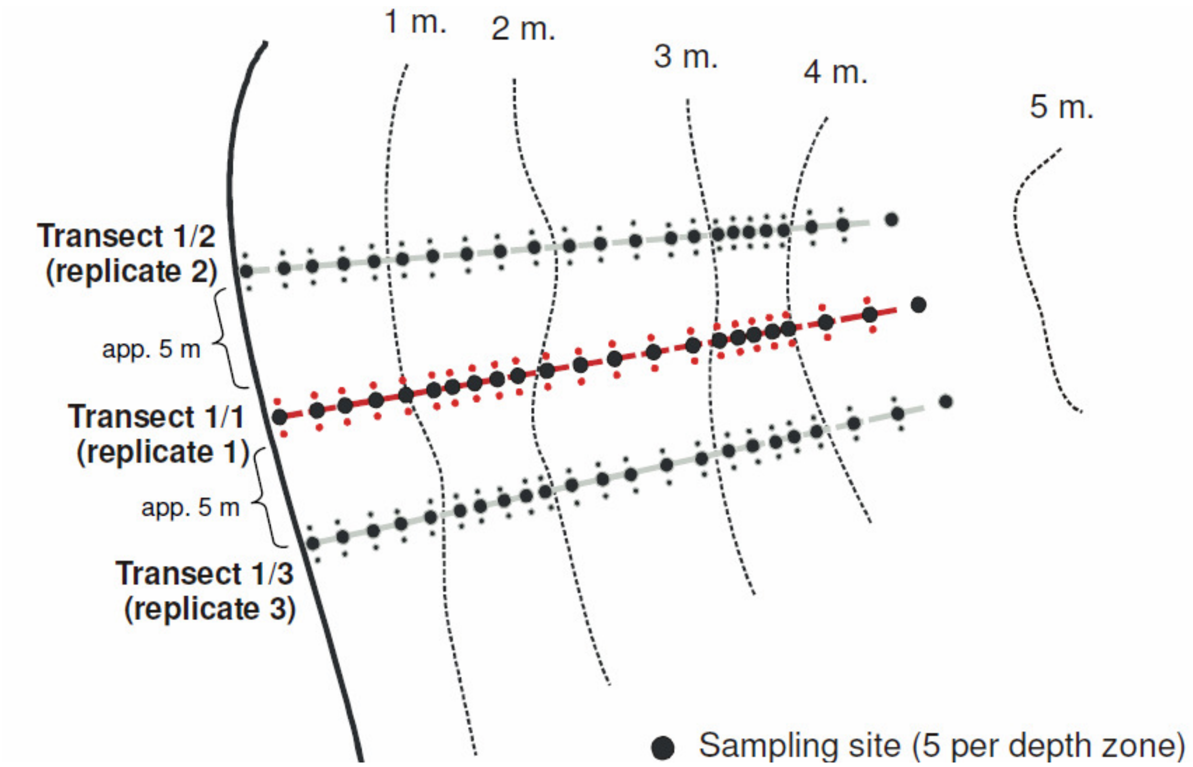


*Fig. 2.1. Diagram of sampling design used in the WISER common field sampling protocol, employed in 2009*

A more detailed description of the sampling procedure and common protocol are available in the WISER Deliverable 3.2-1: "*Overview and comparison of macrophyte survey methods used in European countries and a proposal of harmonized common sampling protocol to be used for WISER uncertainty exercise including a relevant common species list*" (Kolada et al., 2009).

Data collected during the field campaign were compiled using the WISER common database format. This maintained the hierarchical structure of the data in a form analogous to the sampling design. In this format, each observation of a taxon in a sample was given a separate record. Data were extracted from the database at various levels to enable analysis. These levels were depth-zone within transect, transect, locality, and whole lake. Within each of these levels a taxon was deemed to be present if it was recorded at least once. Abundance at each level was relative point frequency, which for each taxon was the number of observations of the taxon, divided by the total number of observations of all taxa at that level.

### 2.1.2 Finnish study on uncertainty

As an additional set of information for use in the uncertainty assessment for macrophyte metrics the results of an unpublished study in Finnish lakes is presented to complete some of the questions that were difficult to answer with the data from the common WISER database. This study assessed the effect of different sources of error variation on the metric values and consecutive classification results in the Finnish macrophyte based ecological classification scheme (Kanninen et al., 2009a,b). The Finnish approach to the classification of lakes based on macrophytes involves the use of three individual metrics; being (i) Proportion of Type Specific Taxa (PTST), (ii) Percent Model Affinity (PMA) and (iii) Trophic Index (RI). Each of these metrics has type specific expected (E) values and ecological class boundaries. The median of the metric-specific classification results is used as the final classification outcome.

The field campaign consisted of macrophyte surveys of 18 small (area <5 km$^2$) humic (water colour 30-90 mg Pt/l) lakes in Eastern Central Finland. The lakes represented reference conditions (n=10) and lakes impacted by nutrient loading (n=8). On each lake fifteen macrophyte transects were surveyed in June-August 2003-2005. The main belt transect method with minor modifications has been applied in the Finnish lake biological monitoring programs since 2006. In the main belt transect method (Kuoppala et al., 2008), the transect is divided to zones according to macrophyte life forms or dominant species. In each zone the species are recorded and the frequency and coverage of each taxa are estimated on a percent scale.

In five of the lakes a whole lake (areal) survey of the macrophyte species and their abundance was also employed. Whole lake surveys are a traditionally used method in Finnish macrophyte studies and the method is used in certain long-term monitoring cases. The data were used to compare the Ecological Quality ratios (EQRs) produced by the different methods.

Data from 29 transects (5-6 transects from each of six lakes) were used to estimate the variation related to different observers by repeating the transect survey at same sites by three different field teams. All teams had good prior expertise in using the method and an *intercalibration session* preceded the field work. On another five lakes the between-observer variation of the whole lake survey method was explored. In this case 3/5 of the shoreline length of each lake was repeated by different field teams.

## 2.2 Data analyses

This section describes the methods used to carry out the analyses on the WISER dataset for uncertainty issues. First is a description of some general data exploration and derivation of macrophyte metrics. This is followed by a description of the methods used to answer the specific research questions.

### 2.2.1 General data-exploratory work

***Multivariate analyses***

A multivariate analysis (clustering and ordination of samples) was performed for quick exploration of the data available within the WISER dataset. Species abundance data were averaged per lake and used for exploratory multivariate analyses with the statistical software programme PRIMER. A similarity matrix was calculated using the Bray-Curtis Similarity index on the non-transformed abundance data and on the presence/absence data. Dendrograms were made by clustering using group average and ordination plots were made using on-metric Multi Dimensional Scaling (NMDS).

### 2.2.2 Calculation of indices

- ***ICM-LM trophic index and Ellenberg index***

Taxon-specific trophic rank scores, also known as Intercalibration Metric scores and referred to in this report as ICM-LM (Intercalibration Common Metric for Lake Macrophytes) scores, were supplied by Nigel Willby, of the University of Stirling, UK. These scores are in use by the Water Framework Directive Intercalibration Exercise as a means of comparing lake macrophyte condition across Member States, where assessment methods are not consistent. For submerged aquatic plant taxa, scores were derived using methods similar to those used by UKTAG (2009), and Birk and Willby (2010). In general, scores were calculated by rescaling the median of the logarithms of the concentration of total phosphorus concentrations in lakes across Europe in which the taxon is found. These scores are available in the WISER Deliverable 3.2-3: „*Report on the most suitable lake macrophyte based assessment methods for impacts of eutrophication abnd water level fluctuations"* (Kolada et al., 2011). Scores are combined across sites to form a metric, either as a simple mean, or by using some measure of abundance to weight the mean (see formula below). The site metric is intended to be representative of the nutrient status of the nutrient status of the water, as experienced by the macrophyte flora.

The ICM-LM scores were only available for real hydrophytes, as this metric was only ever designed to be used with submerged taxa. Ellenberg's Nitrogen values for soil fertility (scores from 1 to 9; Ellenberg et al., 1991) were compiled for all taxa in the dataset in order to test the use of a metric with and without helophytes. We supplemented the original values with British values where original values were missing (Hill et al., 1999). Even with these supplements, there were 13 taxa, notably charophytes, for which no score was available. Scores for the missing taxa were derived by making a regression analysis of the ICM-LM and Ellenberg values for all species with both values and used the regression line to fill in the blanks for charophytes. These Ellenberg values were then used to calculate an average Ellenberg-N metric per lake. There were 16 taxa for which neither Ellenberg nor ICM-LM scores were available, and these were excluded from further analyses.

In this study, ICM-LM and Ellenberg metrics were calculated from scores both as simple averages of the scores of the taxa found (unweighted), or as weighted averages,

$$M = \frac{\Sigma S_i \cdot A_i}{\Sigma A_i}$$

where M is the metric, $S_i$ is the score for taxon i, and $A_i$ is the abundance of taxon i.

In many cases, these metrics have been calculated for subsets of the macrophyte community, such as “submerged only”, or “helophytes”.

- ***Maximum depth of colonisation***

Maximum depth of colonisation (C_max) was determined as the greatest depth in which rooted plants were found, using the common survey method. This value was used at the transect, station and lake levels.

- ***Species richness***

Species richness was expressed as the number of all macrophyte taxa identified within a lake, transect or station.

### 2.2.3 Uncertainty assessments

The WISER lake macrophyte data was used to examine variability associated with the varying levels of the hierarchical sampling scheme: transects within stations within waterbodies within countries. We assessed this for several response metrics, including:

- Lake Macrophyte Intercalibration Metric for lake macrophytes (ICM-LM),
- modified Ellenberg score,
- maximum growing depth (C_max),
- species richness.

Each metric was calculated for each transect. We used lake level alkalinity and total phosphorus concentration as covariates in the analyses for two reasons. Firstly, these variables define strong gradients in the dataset. Secondly, the response of the macrophyte metrics to lake-level TP, accounting for variations in alkalinity, and for uncertainty in surveying lakes, is of considerable interest in itself. As these covariates were measured at the waterbody level they explain variance between waterbodies and countries, but not within waterbodies. We examined correlation between metrics at the various levels in the sampling hierarchy, and correlations between explanatory variables.

Alkalinity information for Étang des Aulnes (France) was not available. Data from this lake were excluded from further analyses.

We undertook the uncertainty analyses using linear mixed effects models, fitted using the nlme package in the R environment for statistical computing (R Development Core Team, 2008). The levels of the sampling hierarchy were specified as nested random effects, with the lowest level, variation between transects, forming the residual. We used TP and alkalinity, measured at the lake level, as explanatory variables in all analyses because of their strong relationship to the metrics studied. Depending on the focus of the analysis, we used additional explanatory variables. For example when looking at metric values for different depth zones, an indicator variable for depth zone was used. TP and alkalinity were log transformed, and richness was square root transformed prior to model fitting.

Correlations between metrics were examined by first creating a data matrix with a single column for metric value and second column containing an indicator variable for the metric name. Analysis then considered the responses of pairs of metrics modelled as a bivariate normal distribution which can consider correlations between metrics at each of the levels of the sampling hierarchy. Models were fitted using Residual Maximum Likelihood Estimation (REML) to produce unbiased estimates of random effect variances, but any comparison of models differing in their fixed effects was undertaken using Akaike's Information Criterion and models fitted by standard Maximum Likelihood.

### 2.2.4 Finnish data analyses

In the unpublished Finnish study, the estimates of variance (measured as standard deviation) from different sources (observer in different methods, shore type) were used to calculate probabilities of misclassification due to different sources, using STARBUGS software (Clarke, 2005) and following methods described by Clarke and Hering (2006).

The effect of shore type on the ecological quality metrics and their variation was explored by quantifying the variation of the metric EQRs metrics between three shore type categories (5 transects per lake per category, 12 lakes).

# 3 Results

## 3.1 General exploratory work on the data

### 3.1.1 Ordination of the analysed lakes

The list of taxa identified in all 28 lakes included 127 taxa; 115 recorded at species level, 11 at genus level and one undefined moss. The list was comprised of 81 submerged taxa (including 4 algae, 6 mosses, 8 isoetids and 13 charids) and 46 others (including helophytes, supralittoral and even 4 terrestrial taxa). Filamentous algae, woody species (A*lnus* sp.*, Salix* sp.*,* etc.) and taxa recorded at genus or higher level were excluded from analyses.

The clustering of species data including helophytes per lake is shown for the abundance data on species composition only, as results from the presence absence were very comparable. Figure 3.1 shows the similarity between samples in a dendrogram graph and Figure 3.2 shows the resulting ordination of sites in a 2D plot (stress 0.16).

Three main groups of samples can be distinguished in the similarity analyses:

1 mainly higher alkalinity central European lakes (FR, EE, PL, DE, GE), with 2 more eutrophic, moderate alkaline lakes from the Northern GIG (1 UK, 1 FI),

2 a small group of higher altitude lakes (all Italian lakes and 2 Norwegian lakes),

3 Nordic moderate and low alkaline lakes (FI, SE, NO, UK).

Similarity between samples was never more than 60%. There was one outlying sample, the Swedish lake Skirösjön. Only one registered submerged species was recorded from this lake so it might therefore fall outside any of the three groups.

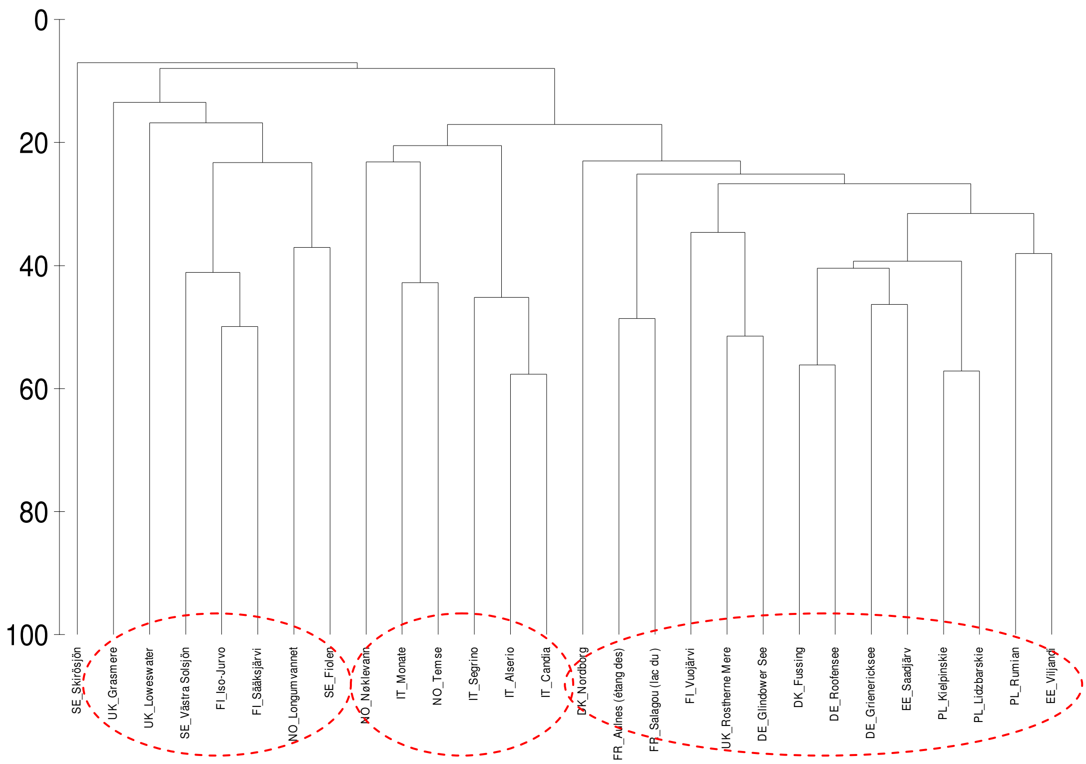


*Fig. 3.1. Hierarchical clustering of the lakes, with grouping of lakes into three main groups*

The three distinguished groups based on the clustering of species data can also be seen in the NMDS ordination diagram. Imposed on that is a vertical axis that appears to represent a trophic gradient with lakes of higher trophic status in the upper part of the plot and lakes of lower trophic status in the lower part of the plot. The horizontal axis appears to represent an alkaline gradient, with the lower alkalinity sites on the left part of the plot and the higher alkaline lakes on the right site of the plot.

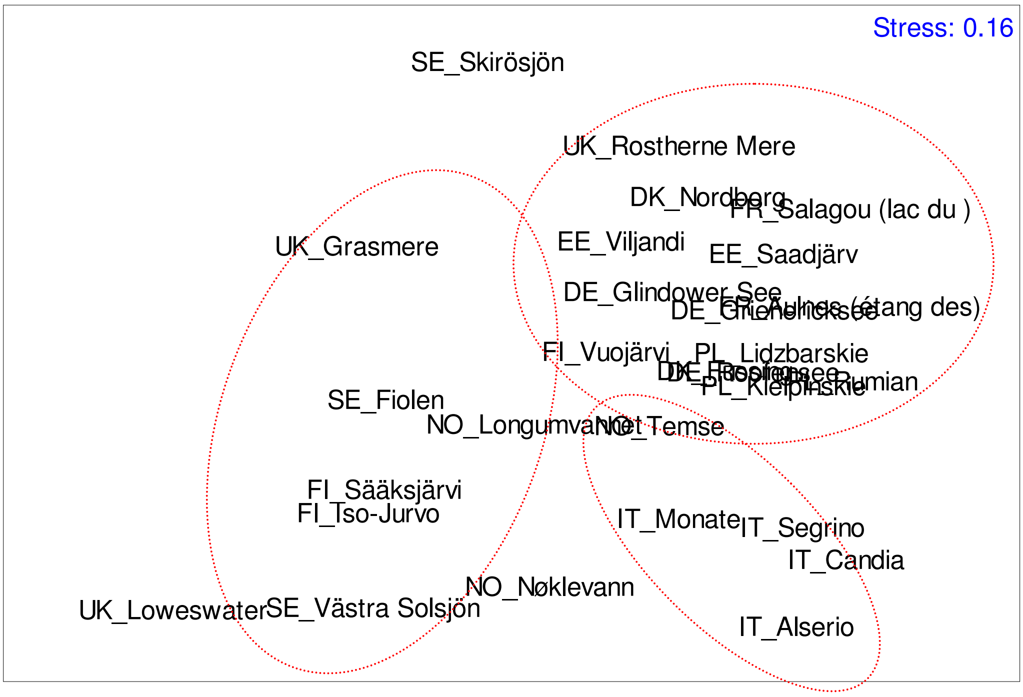


*Fig. 3.2. Ordination of 28 lakes from 10 European countries used for WISER uncertainty exercise based on NMDS, showing the three dominant groups in the total database and the 1 outlying Swedish lake*

## 3.1.2 Effects of alkalinity

Alkalinity has a strong influence on the metric:TP relationship. Unfortunately, we do not have enough low alkalinity lakes in this data set to really separate them for statistical analyses per alkalinity group. Plotting logTP against ICM-LM index for the three different alkalinity groups shows this relationship between alkalinity and TP (Figure 3.3). All high alkalinity lakes have values of ICM-LM between 4.5 and 7.5 with no clear relationship between logTP and ICM-LM. At high TP the relationship flattens right off, especially given that there are fewer transects for which an ICM-LM is calculable at all. The plot also highlights the wide variation in this metric within lakes. It is notable that even for the quite high TP lakes, some transects have much lower ICM-LM scores than others which is due to the number of species in these samples rather than the type of the species. This also indicates this metric should not be used for lakes at the high end of the pressure scale.

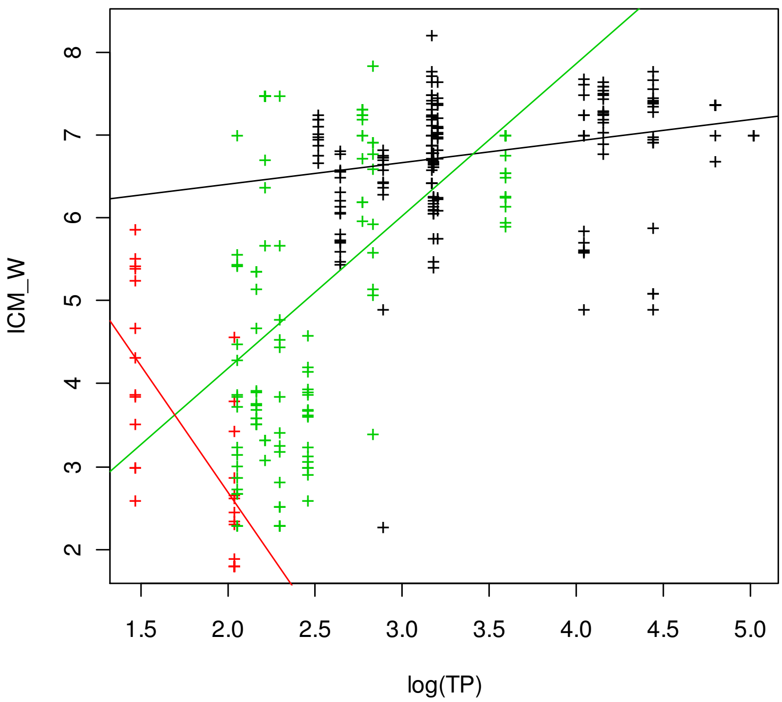


*Fig. 3.3. Relationship between log(TP) and ICM-LM, as calculated at the station level, for the three WFD alkalinity classes low (red), moderate (green) and high (black)*

A similar pattern was observed when comparing average lake Ellenberg values (for submerged taxa only) to lake alkalinity (Figure 3.4). At low alkalinities, there is a sharp increase in the Ellenberg metric associated with increasing alkalinity, but at alkalinities above 0.5 meq/L the Ellenberg score is consistently high, and does not appear to increase further.

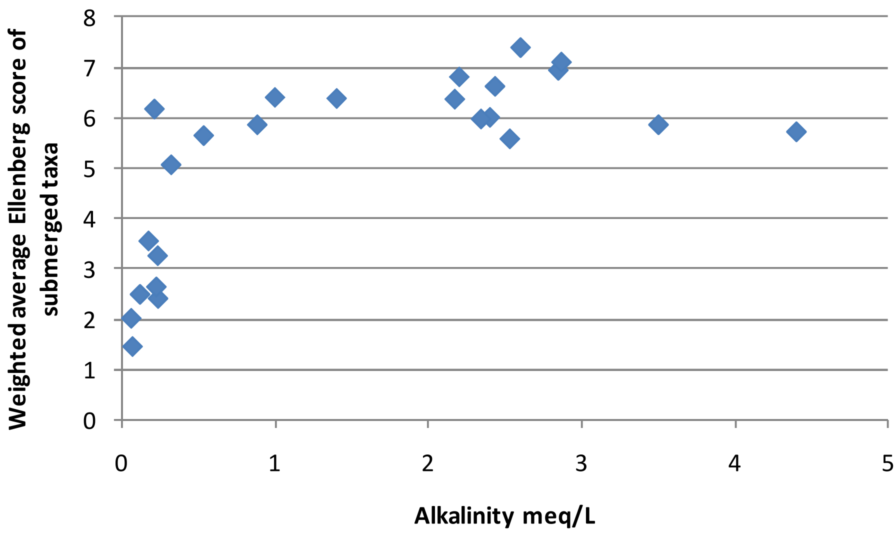


*Fig. 3.4. Weighted average (for each lake) Ellenberg scores compared to alkalinity*

### 3.1.3 ICM-LM scores vs. Ellenberg values

Both Ellenberg and ICM-LM values were available for 51 of all 127 taxa included in the analysed taxa list. In the regression of ICM-LM scores against Ellenberg values (Figure 3.5), the determination coefficient ($R^2$) was 0,64 and the regression equations were:

- Ellenberg score = 0.224+0.789*ICM-LM score
- ICM-LM score = 1.88+0.810*Ellenberg score

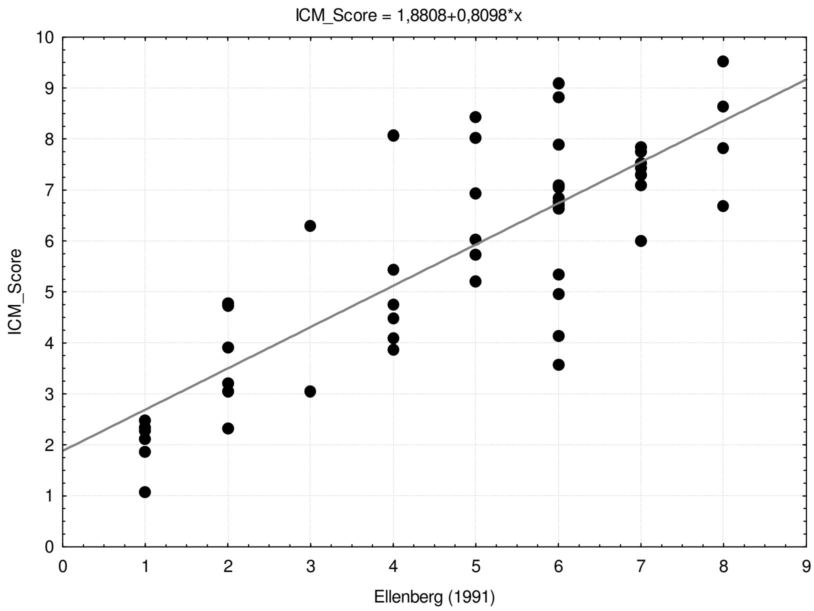


*Fig. 3.5. Regression of ICM-LM score against Ellenberg-N score for the 51 taxa recorded in this study for which both scores were available*

### 3.1.4 Combined response of ICM-LM and Ellenberg to TP

Table 3.1 illustrates the parameters of a bivariate model where both ICM-LM and Ellenberg score are modelled as a function of TP and alkalinity, including their covariance at all levels of the model. Country is not included here as its variance is minimal. The results show that in this model, ICM-LM responds more steeply than Ellenberg (0.48 vs 0.38). P-values for these parameters are only marginally different (0.094 vs 0.090), probably because of the lower residual variance in Ellenberg score at each level of the model. This in turn may be because in each instance, Ellenberg score is based on more taxa than ICM. Correlation between ICM-LM and Ellenberg score is estimated at each of the levels of the model, and is strongest at waterbody scale (0.71), decreasing as one moves to stations within waterbodies (0.64) and transects within stations (0.44). This correlation is in part due to the overlap in species used to calculate the two scores. The relationship between alkalinity and both metrics is virtually identical.

*Table 3.1. Parameters for multivariate model of ICM-LM and Ellenberg score vs alkalinity and TP. Random effects for ICM-LM and Ellenberg presented as standard deviations, fixed effects presented as parameter value with standard error in brackets*

| | ICM | Ellenberg | Correlation | Number of observations |
|---|---|---|---|---|
| ***Random effects*** | | | | |
| Waterbody | 0.84 | 0.65 | 0.71 | 22 |
| Station | 0.73 | 0.64 | 0.64 | 123 |
| Transect | 0.50 | 0.44 | 0.42 | 677 |
| ***Fixed effects*** | | | | |
| Intercept | 4.54 (0.90) | 4.67 (0.70) | | |
| alkalinity | 0.69 (0.21) | 0.68 (0.17) | | |
| TP | 0.48 (0.29) | 0.38 (0.22) | | |

## 3.2 Results specific to research questions:

### 3.2.1 Research question 1: How does the choice of using presence-absence data or abundance data affect the metric results and their uncertainty?

The REBECCA project (Penning et al, 2008a), found very little difference between metrics calculated using presence/absence data, and those calculated using a simplified abundance scale, partially because the data came from many different sources. Relationships between metrics and their associated pressures became weaker when metrics were weighted by abundance, rather than being calculated only on presence/absence data.

Analysis of WISER field campaign data showed that presence/absence and abundance weighted scores are highly correlated (Figure 3.6). This correlation is high especially at the waterbody scale, and progressively less correlated as one moves to the finer scales of station and transect within the lake (Table 3.2).

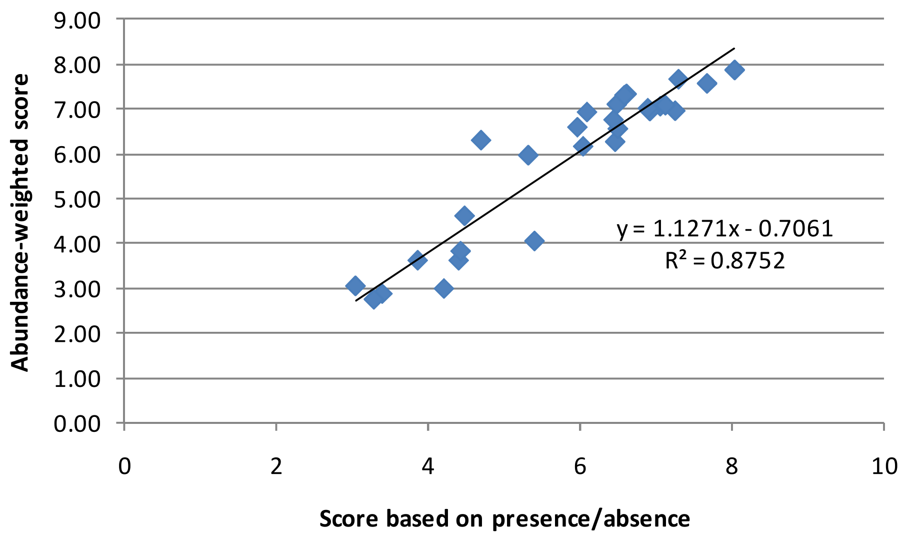


*Fig. 3.6. Comparison of ICM-LM scores, calculated at the lake level for submerged and helophyte taxa, based on presence/absence data and scores weighted by abundance*

Table 3.2 shows the parameters of a bivariate model where both abundance weighted and presence/absence ICM-LM are modelled as a function of TP and alkalinity, including their covariance at all levels of the model. Note that the unit of observation is the transect, hence there is an implicit weighting at the station and waterbody scale based on the number of times a taxon was observed. Compared to presence/absence ICM-LM, the abundance weighted ICM-LM gives a steeper (0.46 vs 0.38) but slightly less precise (standard error of 0.30 vs 0.28) response to TP, while response to alkalinity is very similar. Presence/absence ICM-LM shows greater variance at waterbody scale than weighted ICM; variances are progressively similar at station and transect level.

*Table 3.2. Parameters for multivariate model of transect-level abundance-weighted and presence-absence ICM-LM vs alkalinity and TP. Random effects for abundance weighted and presence absence presented as standard deviations, fixed effects at the waterbody scale are presented as parameter value with standard error in brackets*

| | Abundance Weighted | Presence-absence | Correlation | Number of observations |
|---|---|---|---|---|
| ***Random effects*** | | | | |
| Waterbody | 0.84 | 0.78 | 0.99 | 22 |
| Station | 0.75 | 0.72 | 0.94 | 113 |
| Transect | 0.49 | 0.50 | 0.84 | 634 |
| ***Fixed effects (waterbody scale)*** | | | | |
| Intercept | 4.57 (0.92) | 4.77 (0.86) | | |
| alkalinity | 0.71 (0.22) | 0.69 (0.20) | | |
| TP | 0.46 (0.30) | 0.38 (0.28) | | |

### 3.2.2 Research question 2: How does the choice of the species list that is used affect the results of the metric?

The comparison of weighted average Ellenberg scores when using submerged species only vs. all taxa (including helophytes) shows a strong relationship between them (Figure 3.7). However, it appears that results of the helophyte scores offer little extra information (**Błąd! Nie można odnaleźć źródła odwołania.**Figure 3.8). Metrics based on submerged taxa only show a stronger relationship with the pressure variable TP, and are also more closely related to alkalinity (Table 3.3)

Table 3.3 illustrates the parameters of the bivariate model where weighted Ellenberg scores for only helophytes and only submerged taxa are modelled as a function of alkalinity, including their covariance at all levels of the model. Alkalinity clearly shows strong relationships between both metrics, but a considerably steeper relationship with submerged taxa than helophytes. TP was not fitted as a covariate in this model as with alkalinity already included, relationships with the metrics were weak. However, fitting TP to both metrics did seem to show that the relationship with submerged taxa was stronger than the relationship with helophytes only. While the former was in the expected positive direction, the point estimate for the latter was actually negative. Considering residual correlations between the metrics, these were generally low, although there was some evidence of a negative correlation (-0.31) between the metrics at the waterbody scale.

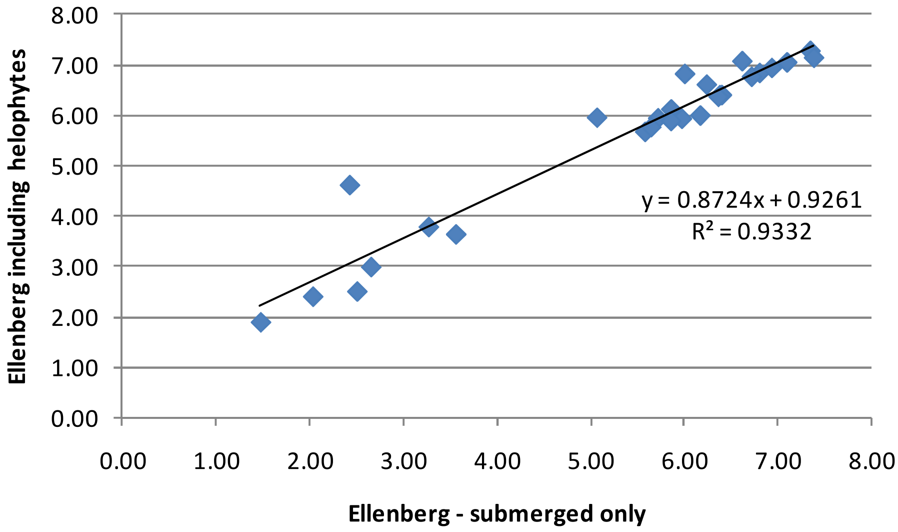


*Fig. 3.7. Comparison of weighted average Ellenberg scores when calculated using only submerged taxa (x-axis), and all taxa (y-axis)*

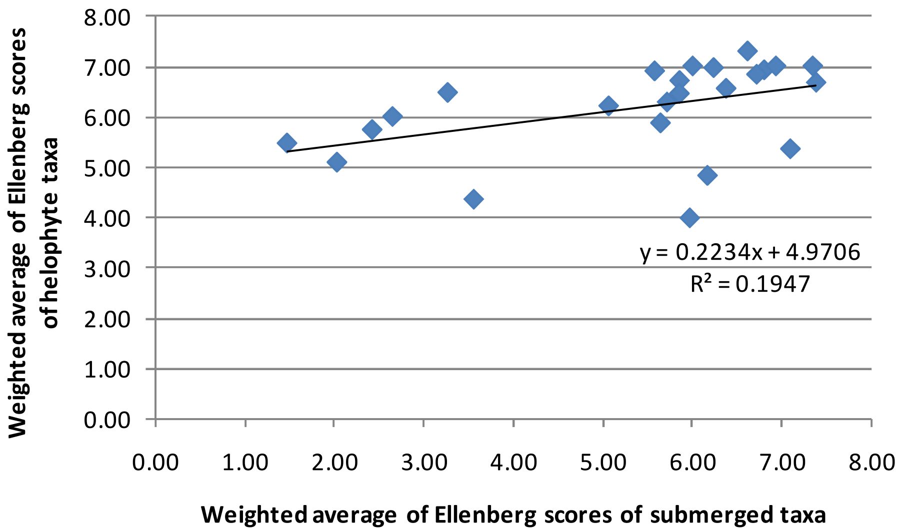


*Fig. 3.8. Comparison of weighted average Ellenberg scores when calculated using only submerged taxa (x-axis), and only helophytes (y-axis)*

*Table 3.3. Parameters for multivariate model of separate Ellenberg scores for helophytes and submerged taxa vs TP and alkalinity. Random effects for the two metrics presented as standard deviations, fixed effects presented as parameter value with standard error in brackets*

| | Submerged | Helophytes | Correlation | Number of observations |
|---|---|---|---|---|
| ***Random effects*** | | | | |
| Waterbody | 0.91 | 0.46 | -0.31 | 27 |
| Station | 0.69 | 0.85 | -0.01 | 149 |
| Transect | 0.51 | 0.55 | 0.03 | 661 |
| ***Fixed effects*** | | | | |
| Intercept | 5.34 (0.19) | 6.19 (0.13) | | |
| alkalinity | 1.15 (0.14) | 0.36 (0.10) | | |
| TP | -- | -- | | |

Forcing there to be a single relationship with alkalinity for both metrics did lead to a significant partial relationship between the submerged metric and TP, but given that there are in practice clearly different relationships between the metrics and alkalinity, this is not realistic, and highlights that care must be taken in the construction of these multivariate models.

### 3.2.3 Research question 3: How does surveying 0-1 m depth zone compare to surveying the whole depth range of potentially colonized area?

Table 3.4 illustrates the parameters of a bivariate model where weighted ICM-LM scores for submerged taxa for the depth zones up to 1m and greater than 1m are modelled as a function of TP and alkalinity, including their covariance at all levels of the model. Note that for this analysis the <1 m depth zone was compared to the >1 m depth zone. The <1 m depth zone was not compared to the full transect, as this would have used data from the <1 m depth zone twice and created noise in the analysis.

ICM-LM values for depth >1m were less variable at waterbody scale (so there is less variation between lakes), but marginally more variable at station and transect scale. There is fairly high correlation between ICM-LM for the different depth zones at waterbody and station scale, but low correlation at transect scale. Most importantly, ICM-LM scores for deeper water were lower than for shallower water (intercept at 4.51 vs 5.05) indicating that species in the shallow zone are more often representing higher trophic status. Relationships with alkalinity were little different, but the relationship with TP was substantially steeper and more precise for the deep zone than for the shallow zone.

*Table 3.4. Parameters for multivariate model of ICM-LM (abundance weighted for submerged species only) for depth zone <1m and >1m vs alkalinity and TP. Random effects for depth zone presented as standard deviations, fixed effects presented as parameter value with standard error in brackets*

| | < 1m | > 1m | Correlation | Number of observations |
|---|---|---|---|---|
| ***Random effects*** | | | | |
| Waterbody | 0.93 | 0.80 | 0.79 | 22 |
| Station | 0.76 | 0.79 | 0.67 | 113 |
| Transect | 0.54 | 0.50 | 0.10 | 529 |
| ***Fixed effects*** | | | | |
| Intercept | 5.05 (1.03) | 4.51 (0.90) | | |
| alkalinity | 0.72 (0.24) | 0.68 (0.21) | | |
| TP | 0.33 (0.33) | 0.49 (0.29) | | |

### 3.2.4 Research question 4: How variable is a metric between lake types, between waterbodies, within a waterbody, and between transects?

Table 3.5 compares the relative proportion of variance in the selected metrics at each level of the sampling hierarchy, and summarised this variance as proportions between and within waterbodies. Results are presented per metric for models with and without TP and alkalinity as explanatory variables. Not surprisingly, both ICM-LM and Ellenberg show similar behaviour, with 70-75% of variance in the metric occurring between waterbodies and countries in the null model. Including TP and alkalinity in the models reduces this variance to 40-50%. In these latter models, ICM-LM, compared to Ellenberg, illustrates a slightly higher proportion of variance between waterbodies + countries, with correspondingly less variance within waterbodies. Maximum growing depth also behaves similarly to ICM-LM, although the covariates appear slightly more successful in explaining between-waterbody variance. The richness metric follows a completely different behaviour; introduction of the covariates reduces the variance between waterbodies but accentuates the variance between countries and total between waterbody variance remains roughly constant.

*Table 3.5. Proportions of variance at different levels of the sampling strategy for four different metrics and two formulations of the model: with and without TP/alkalinity*

| Metric | Model | Country | Waterbody | Station | Transect | Total Between | Total Within |
|---|---|---|---|---|---|---|---|
| ICM-LM (weighted) | Null | 0.11 | 0.61 | 0.19 | 0.08 | 0.72 | 0.28 |
| | TP + Alk | 0.00 | 0.47 | 0.37 | 0.16 | 0.47 | 0.53 |
| Ellenberg | Null | 0.31 | 0.42 | 0.18 | 0.08 | 0.74 | 0.26 |
| | TP + Alk | 0.00 | 0.41 | 0.40 | 0.19 | 0.41 | 0.59 |
| Max growing depth C_max | Null | 0.39 | 0.31 | 0.21 | 0.08 | 0.70 | 0.30 |
| | TP + Alk | 0.01 | 0.38 | 0.44 | 0.17 | 0.39 | 0.61 |
| Richness | Null | 0.18 | 0.19 | 0.45 | 0.18 | 0.37 | 0.63 |
| | TP + Alk | 0.28 | 0.10 | 0.44 | 0.18 | 0.38 | 0.62 |

Alkalinity showed very strong relationships (looking at the p-values) with all metrics except richness (Table 3.6). Relationships between TP and metrics were always in the expected direction but for both ICM-LM and Ellenberg, there was sufficient imprecision in the relationships for p-values to be above the traditional cut-off values of p=0.05. This general pattern was confirmed through re-fitting models using maximum likelihood (ML) estimation and comparison of Akaike Information Criteria (AIC) values. The significant relationships between TP and both C_max and Richness metrics were notable. We re-fitted the C_max and richness TP/alkalinity models to the subset of data with an ICM-LM score. The C_max-TP relationship was robust to this fitting to a smaller subset of the data, but the richness relationship was not. 49 transects had values for richness but not ICM-LM; these were spread across 12 lakes, the lake with the largest number of transects lost being Glindower See with 15. For C_max, it is notable that the strong relationship with TP was entirely dependent on alkalinity also being in the model, without alkalinity, the C_max-TP relationship was very weak (results not shown).

*Table 3.6. Significance (p-values) for approximate tests for TP and alkalinity fixed effects for models for each metric in Table 3.5, and numbers of samples at each level of the model*

| Metrics | TP | Alkalinity | Country | WB | Station | Transect |
|---|---|---|---|---|---|---|
| ICM-LM (weighted) | 0.144 | 0.007 | 8 | 22 | 113 | 317 |
| Ellenberg | 0.115 | 0.002 | 8 | 22 | 123 | 360 |
| Max growing depth | 0.001 | 0.000 | 8 | 18 | 100 | 282 |
| Richness | 0.027 | 0.191 | 8 | 22 | 125 | 366 |

### 3.2.5 Research question 5: What is the effect of using only subsets of the data on the amount of variation (e.g. depth zone, species saturation limit adjusted)?

Due to the uneven distribution of macrophyte vegetation along shoreline, sampling effort is one of the key factors affecting observed species diversity. The total number of species detected depends heavily on the number of transects, as highlighted by Finnish experiences from resampling the transect data of several small humic lakes (Figure 3.9). In this lake type, lakes impacted by eutrophication have a higher species diversity than reference lakes, which therefore require a higher number of transects to be assessed correctly.

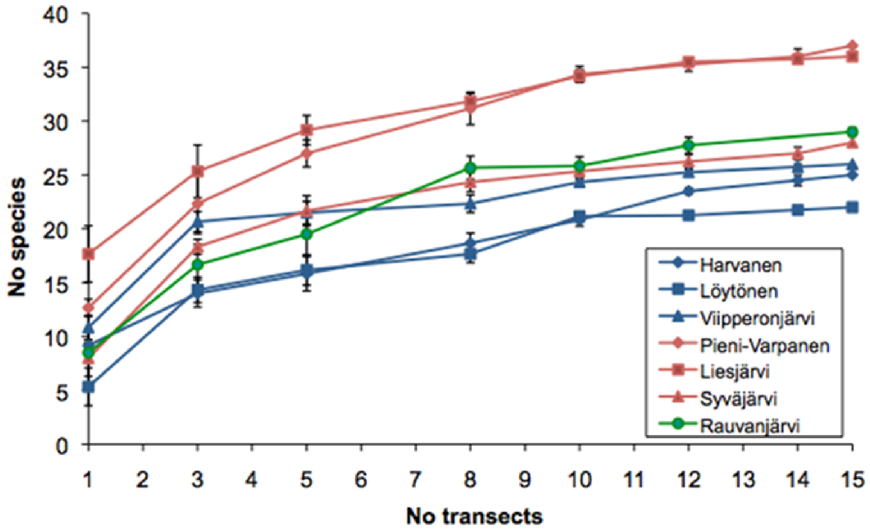


*Fig. 3.9. Relationship between the number of transects surveyed and the subsequent number of species recorded in six Finnish lakes (Kanninen unpublished report). Blue lines represent reference and pink lines impact lakes of the small humic lake type, Lake Rauvanjärvi (green line) is a reference lake for the medium sized humic lake type*

The areal survey method was more efficient than the transect method in producing information on number of species (Figure 3.10). The metric specific Ecological Quality Ratios derived from data from transects and whole lake surveys did not differ in the case of PMA and RI. Whole lake surveys method produced lower PTST metric EQRs than the transect method survey of 15 transects.

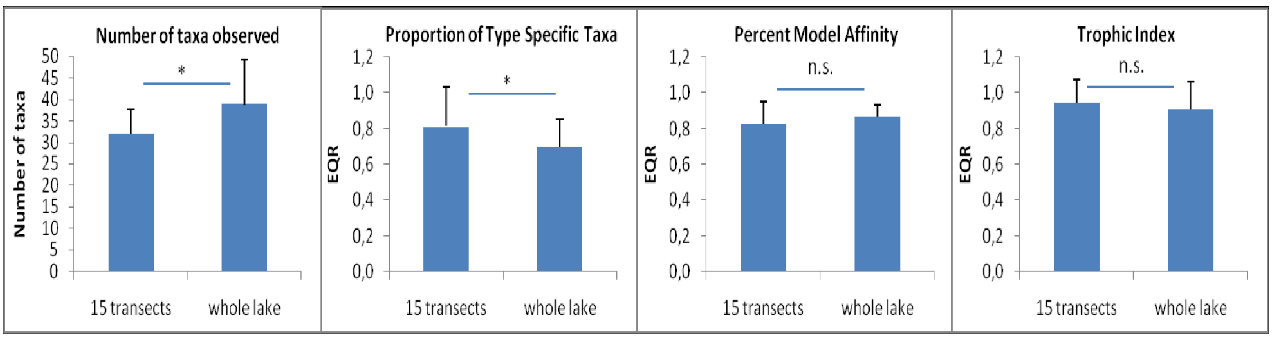


*Fig. 3.10. Values of macrophyte metrics produced by two field methods in several Finnish lakes*

Classification boundaries for the metrics and degree of sampling variation of different sources are shown in the table below (Table 3.7).

*Table 3.7. Classification boundaries for the metrics and degree of sampling variation of different sources*

| | Ecological class boundaries* | | | | Sampling variation in EQRs | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Inter-surveyor variation in transects method | Between habitats variation | Inter-surveyor variation in whole lake survey | Reference condition variation** |
| **Metric** | **high** | **good** | **moderate** | **poor** | **SD** | **SD** | **SD** | **SD** |
| PTST | 0,86 | 0,65 | 0,43 | 0,22 | 0,06 | 0,13 | 0,04 | 0,04 |
| PMA | 0,82 | 0,61 | 0,41 | 0,20 | 0,04 | 0,06 | 0,05 | 0,05 |
| RI | 0,91 | 0,68 | 0,46 | 0,23 | 0,05 | 0,06 | 0,04 | 0,025 |

* EQR-based boundaries for the type "Small humic lake" of the Finnish lake typology system

** estimated as the S.E. of mean of 10 reference lakes

The habitat type categories were differentiated by their average slope, fetch and percentage of organic bottom (Figure 3.11). Shore morphology was the greatest source of uncertainty as it was responsible for the highest degrees of misclassification probability in all of the three metrics according to the STARBUGS simulations. The effect of habitat was most pronounced in the Proportion of type-specific species.

For the metrics PMA and RI all sources of uncertainty and, thus, misclassification probabilities at class midpoints were fairly low. Inter-surveyor variability resulted in fairly low probability of misclassification in all metrics and comparable to the level reported for river MTR-method by Staniszewski et al. (2006). For the metrics using presence-absence data only (PTST and RI), inter-surveyor variability in the transect method was somewhat lower than in the areal survey method. For the abundance based metric PMA, transect data seemed somewhat more repeatable.

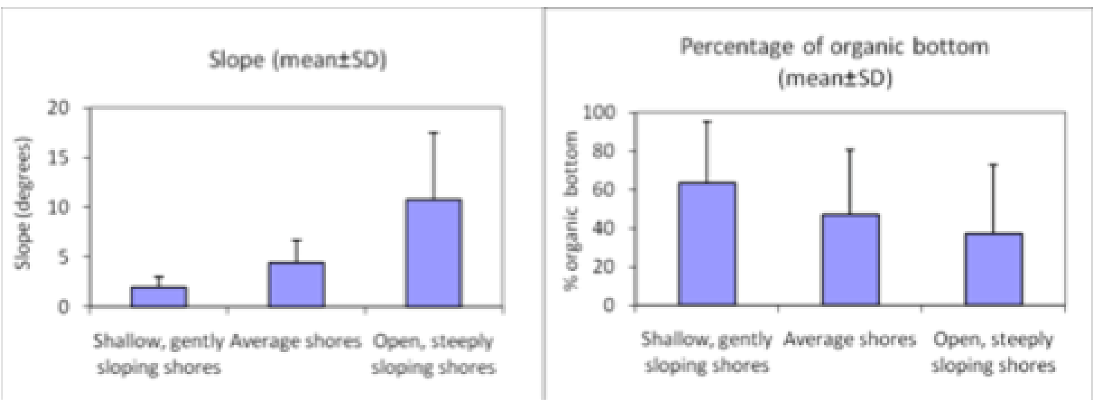


*Fig. 3.11. Average properties of investigated shorelines of Finnish case study lakes*

Habitat type (shore morphology) had an effect also on the detection of human impact (reference vs. impacted lakes) using the metric PTST (Figure 3.12). Metrics PMA and RI were not sensitive to habitat effect in this respect. Also the metric PMA, using the basic 15 transect data, differentiated between reference and impacted lakes, although the response of PMA EQR to the pressure gradient was not as clear as that for PTST (data not shown here). The Trophic Index did not differentiate between reference and impacted lakes nor respond to the pressure gradient in this data.

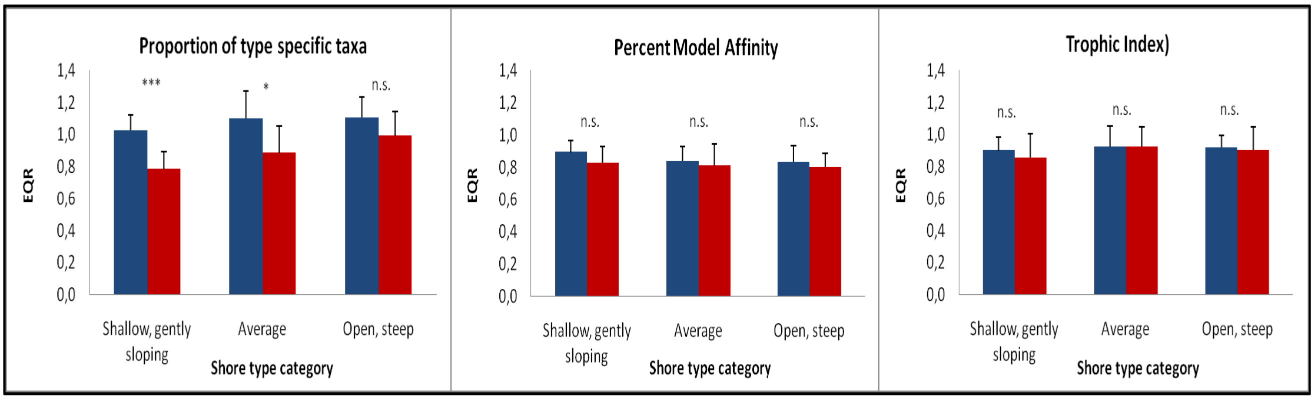


*Fig. 3.12. Effect of shore type on the EQRs produced by different metrics with two different field methods*

# 4. Discussion and conclusions

## 4.1 WISER field campaign dataset

Ordination of the data from the 2009 WISER field campaigned grouped lakes corresponding to differences in alkalinity, altitude, and apparent trophic status. This is in accord with WFD typology and the pressure/response paradigm on which the WFD is based. It was also apparent, within this group of lakes at least, that the response of metrics to nutrient pressure (TP) decreased with increasing alkalinity. Higher alkalinity lakes do not appear to respond as much to increased pressure as low-alkalinity lakes. Unfortunately, it was not considered practicable to conduct separate analyses of the lakes of different alkalinity types, due to the limited number of lower alkalinity lakes in our dataset.

It should also be noted that alkalinity can be increased by eutrophication, an effect that is especially important in low alkalinity lakes. Alkalinity generation in soft water lakes is usually dominated by biological reduction of sulfate and nitrate. Anoxia in the water column is not essential, as reduction can occur in surficial sediments, which are nearly always anoxic (Wetzel, 2001). This means that it is not possible to have a low-alkalinity eutrophic lake, as the process of eutrophication will increase the alkalinity so that it is no longer low.

### 4.1.1 The effect of using abundance data rather than presence-absence data on the metric results and their uncertainty (research question 1)

Although in the REBECCA project (Penning et al, 2008a), there was little evidence of benefit in using metrics calculated using an average scores weighted by abundance, it is important to note that the data collected for the REBECCA study were from multiple sources and collected using disparate sampling and quantification methods. Analysis of the data collected during the harmonized WISER field campaign supports the use of abundance weighted averages as they provide a stronger relationship with the nutrient pressure (TP), but they should only be used in cases where all data has been collected using common abundance scales (and preferably by the same surveyors).

### 4.1.2 The effect of the choice of species list on the metric results (research question 2)

As was concluded in the REBECCA project (Penning et al, 2008b), in this study the use of helophytes in the calculation of metrics appeared to provide little additional information, and metrics based on helophytes do not respond as well to nutrient pressure (TP) as do the submerged species. Helophytes are less affected by water quality as their environment is not sub-aquatic, as their response to eutrophication is obscured by soil trophic characteristics, exposure, shoreline management and especially water level fluctuation dynamics as noted in several studies (e.g. Coops et al., 1994). The use of data from the WISER intensive field campaign provides a stronger basis than has been previously available to answer this question.

It is possible that the use of large datasets collated from multiple sources will provide spurious answers to this question, as it is likely that bias in sampling is related to trophic status. Put simply, it is likely that in regions with lakes where the submerged taxa are highly visible, flourishing and diverse, sampling effort will be concentrated on these plants, in contrast to regions where lakes are more eutrophic, so predominately have few submerged taxa, but a flourishing emergent community, where it is likely that sampling effort will concentrate on the helophyte taxa.

#### 4.1.3 Comparison of shallow (0-1 m) and deeper (1+ m) depth zones (research question 3)

This study has shown that higher ICM-LM scores are obtained from shallow zone samples, than from deeper zone samples, which is very important in the status assessment of lakes. If an assessment method uses only shore-based data (obtained by wading), it is likely to result in an assessment of condition that is worse and less precise than if the method used data from deep water as well (obtained by boat). It was also apparent that metrics calculated on data from deeper samples were more responsive to changes in the pressure (TP). Therefore it seems to be important to include the deeper sites in the survey to get a more precise response to a TP pressure gradient.

#### 4.1.4 Variability of metrics between lake types, between waterbodies, within a waterbody, and between transects (research question 4)

The results illustrate that differences in the number of transects for which metrics may be calculated can have a strong influence on the results. In particular, as TP levels increase, richness decreases, but numbers of taxa for which metrics such as ICM-LM can be calculated decrease even more rapidly. It is a general rule in statistical modelling that the data points at the extremes of the explanatory variables have most influence on the response relationships. Increased imprecision of metrics associated with low richness of indicator taxa, and at the most extreme, non-calculability of such indices can have a significant influence on perceived metric performance. Therefore, to maintain the same degree of uncertainty, more sampling is required at either end of the trophic scale, when there is less vegetation to be sampled.

The results of including both TP and alkalinity in the models are revealing. TP and alkalinity are fairly well correlated in the dataset, hence it is not surprising that in some cases, either variable on its own may show apparent relationships with metrics. In particular, in this dataset, for ICM-LM and Ellenberg, alkalinity is clearly the dominant explanatory variable, and partial relationships with TP are not significant at $p=0.05$. The fact that the significant TP-C_max relationship is conditional on alkalinity also being in the model is notable and highlights the inter-relatedness of these variables.

#### 4.1.5 The effect of using only subsets of the data on the amount of variation (research question 5)

The results of the Finnish study provide evidence of the importance of shore morphology as a source of uncertainty in the Finnish macrophyte based classification system in small humic lakes. The proportion of type specific taxa was the metric that showed most error variation but on the other hand it was also the best metric to differentiate between reference and impacted lakes. When using transects, the effect of shore type on the metric variation should be controlled for by focusing monitoring efforts on shallow or average sloping shores as noted in national quality control project (Kuoppala et al., 2008). The effect of habitat is most likely even more pronounced in larger lakes with more shore morphological variation.

The results suggest that metrics calculated from presence-absence data only may be more effectively derived from survey data of larger areas than transects only. Consideration upon the most cost-effective monitoring method may still be needed. Generally, the effect of inter-surveyor variation on uncertainty of ecological quality metrics was fairly low and can be tackled by surveyor training and implementing other quality assurance protocols.

## 4.2 Recommendations for sampling, data analysis and assessment methods

Analysis of WISER field campaign data presented above supports the following recommendations:

1 Assessment methods should include samples from the entire depth range of aquatic vegetation, as using only shallow samples can result in a worse assessment of ecological status.
2 Assessment methods should use abundance data (not just presence/absence) where possible, but only in cases where all data has been collected using the same methods.
3 Helophytes should not be used in the assessment of the status of lake macrophyte communities, as they do not respond in the same way (as submerged taxa) to nutrient pressure.
4 Assessment methods that rely on trophic status metrics should use data from samples across entire depth range of plants in the lake, else status is likely to be judged worse.
5 Examination of uncertainty in metrics should not be undertaken in the absence of the relationships between metrics and stressors. In the worst case scenario, a metric may illustrate desirable properties of low variance within waterbodies relative to variance between waterbodies, but this may be at the expense of the metric’s desired response to stressors.
6 It is necessary to sample at multiple stations. This is supported by high residual variance in metrics, even after accounting for TP and alkalinity gradients.
7 More sampling is required to maintain the same degree of uncertainty in lakes where macrophytes are scarce or taxa richness is low. At these sites, scores of individual taxa can have a much larger impact than lakes with more macrophyte cover or more taxa.

Examination of the Finnish study on uncertainty supports the following additional recommendations:

1 Shore morphology and exposure have an effect on species composition and therefore careful selection of sites is needed.
2 Intensive areal survey methods might identify more species than transect methods.
3 The number of transects should be high enough to reach species saturation. For some lake types, like the small boreal humic lakes, there is a need for further development of the most suitable metrics for classification, as the three metrics used in the Finnish lakes study are not able to detect human impact. Potential new metrics may include e.g. the maximum depth of isoetids.

## Acknowledgements

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## Appendix - List of lakes used in the analyses with provided and derived properties of each

Columns are Country, Name (of lake), ICM_PA (unweighted ICM-LM score), ICM_W (weighted ICM-LM score), Richness (total number of plant taxa recorded), Helophytes (number of helophyte taxa recorded), EB-S (Ellenberg score calculated on submerged taxa), EB-H (Ellenberg score calculated on helophyte taxa), EB-A (Ellenberg score calculated on all taxa), ICTypeID (WFD Intercalibration type, Altitude (m), Surface Area ($km^2$), Alkalinity (meq/L), Secchi depth (m), and TP (total phosphorus, in µg/L)

| Country | Name | ICM_PA | ICM_W | Richness | Helophytes | EB-S | EB-H | EB-A | ICtypeID | Altitude | Surface Area | Alkalinity | Secchi | TP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DE | Glindower See | 7.05 | 7.05 | 4 | 3 | 6.00 | 7.00 | 6.81 | L-CB1 | 24 | 1.95 | 2.40 | | 151 |
| DE | Grienericksee | 6.09 | 6.92 | 20 | 5 | 6.80 | 6.92 | 6.83 | L-CB1 | 55 | 0.87 | 2.20 | 2.1 | 25 |
| DE | Roofensee | 5.96 | 6.59 | 13 | 4 | 6.23 | 6.96 | 6.60 | L-CB1 | 59 | 0.54 | 2.00 | | 18 |
| DK | Fussing | 7.25 | 6.95 | 12 | 2 | 6.37 | 6.56 | 6.39 | L-CB1 | 15 | 2.17 | 1.40 | 2.2 | 46 |
| DK | Nordborg | 8.03 | 7.87 | 15 | 5 | 5.85 | 6.71 | 6.11 | L-CB1 | 20 | 0.56 | 3.50 | 1.2 | 63 |
| EE | Saadjärv | 6.04 | 6.16 | 15 | 3 | 5.58 | 6.90 | 5.66 | L-CB1 | 85 | 7.10 | 2.53 | 4.6 | 14 |
| EE | Viljandi | 6.46 | 6.26 | 19 | 12 | 5.71 | 6.29 | 5.93 | L-CB1 | 75 | 1.60 | 4.40 | 1.2 | 24 |
| FI | Iso-Jurvo | 3.39 | 2.86 | 17 | 4 | 2.03 | 5.10 | 2.40 | L-N2a | 139 | 2.26 | 0.06 | 3.5 | 8 |
| FI | Sääksjärvi | 3.86 | 3.61 | 15 | 3 | 3.26 | 6.48 | 3.78 | LN-2a | 121 | 0.59 | 0.23 | 3.6 | 12 |
| FI | Vuojärvi | 6.49 | 6.55 | 11 | 6 | 5.64 | 5.88 | 5.76 | L-N2a | 91 | 0.73 | 0.53 | 1.0 | 36 |
| FR | Aulnes (étang des) | 7.29 | 7.66 | 7 | 2 | 7.34 | 7.00 | 7.27 | - | 11 | 0.88 | - | 1.0 | 60 |
| FR | Salagou (lac du ) | 7.67 | 7.56 | 5 | 1 | 6.93 | 7.00 | 6.93 | L-M8 | 139 | 7.20 | 2.85 | 4.3 | 24 |
| IT | Alserio | 6.92 | 6.95 | 7 | 1 | 5.97 | 4.00 | 5.92 | L-AL5 | 243 | 1.23 | 2.34 | 1.0 | 24 |
| IT | Candia | 7.12 | 7.08 | 7 | 0 | 6.39 | | 6.39 | L-AL5 | 226 | 1.69 | 1.00 | 1.8 | 16 |
| IT | Monate | 5.40 | 4.04 | 7 | 2 | 5.85 | 6.45 | 5.88 | L-AL5 | 266 | 2.51 | 0.88 | 4.8 | 9 |
| IT | Segrino | 6.89 | 7.01 | 9 | 0 | 6.36 | | 6.36 | L-AL5 | 374 | 0.28 | 2.17 | 2.3 | 12 |
| NO | Longumvannet | 4.40 | 3.61 | 19 | 13 | 2.42 | 5.74 | 4.61 | L-N1 | 34 | 1.00 | 0.23 | 4.7 | 8 |
| NO | Nøklevann | 4.48 | 4.60 | 22 | 6 | 3.56 | 4.37 | 3.63 | L-N2a | 163 | 0.79 | 0.17 | 5.8 | 4 |
| NO | Temse | 5.31 | 5.96 | 14 | 4 | 5.06 | 6.21 | 5.95 | L-N1 | 15 | 0.62 | 0.32 | 2.2 | 17 |
| PL | Kielpinskie | 6.61 | 7.33 | 17 | 4 | 7.09 | 5.36 | 7.04 | L-CB1 | 120 | 0.61 | 2.87 | 2.9 | 64 |
| PL | Lidzbarskie | 6.44 | 6.75 | 12 | 5 | 6.61 | 7.29 | 7.06 | L-CB1 | 128 | 1.22 | 2.43 | 1.1 | 57 |
| PL | Rumian | 6.48 | 7.10 | 22 | 12 | 7.38 | 6.67 | 7.13 | L-CB1 | 152 | 3.06 | 2.60 | 1.5 | 85 |
| SE | Fiolen | 3.28 | 2.74 | 16 | 7 | 1.47 | 5.48 | 1.89 | L-N2a | 226 | 1.55 | 0.07 | 4.9 | 5 |
| SE | Skirösjön | 3.04 | 3.04 | 6 | 5 | | 6.77 | 6.48 | L-N2a | 146 | 1.05 | 0.51 | 0.9 | 5 |
| SE | Västra Solsjön | 4.20 | 2.98 | 5 | 0 | 2.50 | | 2.50 | L-N2a | 147 | 1.85 | 0.12 | | 45 |
| UK | Grasmere | 4.69 | 6.30 | 15 | 6 | 6.17 | 4.84 | 5.99 | L-N2a | 61 | 0.61 | 0.21 | 3.0 | 9 |
| UK | Loweswater | 4.42 | 3.81 | 11 | 2 | 2.65 | 6.00 | 2.98 | L-N2a | 125 | 0.60 | 0.22 | 3.5 | 10 |
| UK | Rostherne Mere | 6.58 | 7.30 | 7 | 4 | 6.71 | 6.83 | 6.76 | L-CB1 | 27 | 0.48 | 2.44 | 1.5 | 121 |