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A phytoplankton trophic index to assess the status of lakes for the Water Framework Directive

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Abstract

Despite improvements in waste water treatment systems, the impact of anthropogenic nutrient sources remains a key issue for the management of European lakes. The Water Framework Directive provides a mechanism through which progress can be made on this issue. The Directive requires a classification of the ecological status of phytoplankton, which includes an assessment of taxonomic composition. In this paper we present a composition metric, the Plankton Trophic Index, that was developed in the WISER EU FP7 project and demonstrate how it has been used to compare national phytoplankton classification systems in Northern and Central Europe. The metric was derived from summer phytoplankton data summarised by genus from 1795 lakes, covering 20 European countries. We show that it is significantly related to total phosphorus concentrations, but that it is also sensitive to alkalinity, lake size and climatic variables. Through the use of country specific reference values for the index we demonstrate that it is significantly related to other national phytoplankton assessment systems and illustrate for a single European (intecalibration) lake type how it was used to intercalibrate Water Framework Directive boundaries from different countries.

Keywords: phytoplankton, eutrophication, Europe, Water Framework Directive, trophic index, intercalibration, classification, indicator taxa.

Introduction

Phytoplankton is one of the biological quality elements required for assessing ecological status of lakes in Europe according to the Water Framework Directive (WFD). Annex V of the WFD requires phytoplankton to be assessed using biomass, taxonomic composition and bloom metrics. Already many decades before the WFD, lake phytoplankton has been used to assess eutrophication impacts in Europe and North America (Hörnström, 1981; Naumann, 1919; Reynolds, 1980; Taylor et al., 1979; Teiling, 1955; Willén, 2000). The WFD has nevertheless stimulated development and/or improvement of a large array of different national methods (Birk et al., 2012; Brucet et al., in press; Poikane et al., 2009; Solimini et al., 2008), including biomass metrics, such as chlorophyll, cell numbers and biovolume, taxonomic composition metrics, such as proportions of Cyanobacteria or other indicator taxa (Ptacnik et al., 2008; Watson et al., 1997), as well as weighted average of taxa based on their occurrence along the trophic gradient (Mischke et al., 2008; Padisák et al., 2006), or functional composition metrics based on size classes (Lugoli et al., 2012; Morabito & Carvalho, 2012) or functional groups (Metcalfe, 1989; Padisák et al., 2006; Reynolds, 1984). The class boundaries for these national methods are based either on purely statistical principles, dividing the metric data into equidistant classes along the pressure gradient, or using more ecological principles, e.g. change points or thresholds in response curves of indicator taxa along the pressure gradient (Lyche Solheim et al., 2008). Thus, different metrics as well as different boundary setting methods contribute to the large variability among the national methods and how they classify a common set of lakes. This large variability complicates the comparability of classification results from the different countries, and has posed a great challenge to the intercalibration of the good ecological status class boundaries of national methods, as required by the WFD (WFD CIS Intercalibration guidance, 2011). To facilitate the last phase of the WFD intercalibration process, there was therefore an urgent need for common metrics, including a common taxonomic composition metric for lake phytoplankton.

The objective of this paper is to present the taxonomic composition metric that was developed in the WISER EU FP7 project and how it has been used as a part of the common metric for intercalibration of phytoplankton in the Northern and Central-Baltic Geographical Intercalibration Groups (GIGs). The paper presents the huge pan-European dataset underlying the development of this metric, the relationship of the metric with eutrophication pressure and with other environmental variables, e.g. alkalinity, colour, lake size, depth and climate parameters, as well as the application of the metric in the intercalibration process.

Methods

Data

The analysis reported in this paper are based on phytoplankton composition and water chemistry data covering 1795 lakes from 20 countries (Table 1). These data were extracted from a pan-European database compiled for the Water Framework Directive intercalibration process and the EU Rebecca (Moe et al., 2008) and WISER Project (Moe et al., this issue; http://www.wiser.eu/). Phytoplankton data were from samples taken in July – September. Taxa records from each country were checked and allocated a unique code (REBECCA code, Moe et al., 2008) based on the reported taxonomic identity. Taxa recorded in less than three countries or from less than 10 samples were removed from the analysis as their species optima would be highly uncertain for application within a GIG or at a pan-European level. To reduce variability caused by different taxonomic traditions and counter ability, data were aggregated to genus level. Samples and stations in each water body were combined by averaging to create an annual mean bio-volume for each taxon, which was then converted to a fractional abundance, summing to a value of 1.0. The most recent annual fractional abundance value was then extracted from the data set for each lake so that the analysis was based on a single mean summer fractional abundance for each lake. Lakes where the raphidophyte *Gonyostomum* K.Diesing formed >50% of the total biomass were excluded from the analysis, as exploratory analysis revealed these lake were major outliers and could influence later ordinations used to establish taxa optima, yet it has been shown that species within this genus are known to form mass developments that are not clearly linked to anthropogenic nutrients (Cronberg et al., 1988). Water chemistry data were averaged to provide a mean for the period April – September, all data were screened and lakes where mean growing season total phosphorus was outside of the range of 1-1000µg L^{-1} were omitted. Long term average precipitation and monthly mean air temperature for each lake was taken from a gridded data set (Mitchell et al., 2004). Precipitation was averaged for the period over the sampling period (July – September). The monthly mean temperature values >0 ºC were summed for the period January – September to provide a metric (Degreedays**)** that summarised the temperature climate that would have influenced the seasonal algal succession to the end of the sampling period.

Lake Types

The intercalibration of phytoplankton assessment systems is based on the division of lakes into regions and lake types which are similar in their hydromorphological and geochemical attributes, referred to Geographic Intercalibration Regions (GIGs) (Poikane, 2009). Lakes were thus split into the Northern, Central Baltic, Mediterranean and Eastern Continental geographic regions and then sub-divided into three alkalinity types (low = alkalinity <0.2mEq L^{-1}, moderate = alkalinity 0.2 – 1.0 mEq L^{-1}, high = alkalinity >1.0 mEq L^{-1}) and three depth types (deep = mean depth >15m, shallow = mean depth 3-15m, very shallow = <3m). The low and moderate alkalinity lakes were further subdivided into clear (≤30 mg L^{-1}Pt) and humic (>30 mg L^{-1}Pt) , the distribution of types by country is shown in table 1. For some analysis lakes were also split into size (Large = area >10km^2, Moderate = area 1-10 km^2, Small = area 0.5-1.0 km^2 and Very Small = area <0.5 km^2) and altitude classes (High = elevation >800m, Moderate = elevation 200 – 800m, Low = elevation <200 m). Reference lakes were also identified by data providers using criteria agreed for the WFD intercalibration exercise (Poikane, 2009).

Statistical Methods

All statistical analysis was carried out using R (R Development Core Team, 2009), ordinations were done with routines from the *vegan* library (Oksanen et al., 2010). To determine the community structure of the phytoplankton an unconstrained non-metric multidimensional scaling was carried out using the *metaMDS* function on the full data set. Relationships with the key environmental variables, total phosphorus, total nitrogen, alkalinity, colour and mean depth were carried out using *envfit* function with significance testing using random permutation tests. All environmental data except latitude, longitude and degree months were log transformed. Prior to ordination analysis phytoplankton biovolumes were converted to a fraction of total biovolume and square root transformed to reduce the influence of rare taxa(Legendre & Gallagher, 2001).

Plankton Trophic Index (PTI)

Development of the plankton trophic index (PTI) metric was based on canonical correspondence analysis (CCA) using the *cca* function from the *vegan* library, with total phosphorus (mean of July – September samples) as a single constraining variable. When a single environmental variable is used CCA reduces to a weighted average ordination, an axis score of zero represents the global average of the constraining environmental variable, total phosphorus (Braak & Looman, 1986). Following a similar approach to that used by Dodkins et al. (2005) and Ptacnik et al. (2009) the 1st CCA ordination axis of the taxon scores were used to derive a trophic optimum (or indicator value) for each taxon. The sign of the ordination axis is arbitrary, but for the PTI negative scores reflect low phosphorus and positive scores high phosphorus. The CCA analysis was carried out on a random sub-set of the full data set, so that a separate training data set was used for metric development, leaving an independent set of lakes for validation. In addition to these "global" optima additional sets of optima were derived using sub-sets of data taken from lakes in NGIG and CBGIG, to support the intercalibration of lakes from these regions. In these regional analyses optima derived from species data were calculated and compared with those using genus-level data. Where the range of species optima within a single genus was large, the genus was split into species groups, and a group optimum derived using a weighted average of the species optima, where the weight was the number of records of each species in the group. In this way, species commonly occurring in many lakes get higher weight than rarely recorded species.

A PTI value was then calculated for each lake in the validation data set using a weighted average of the optima from the taxa present in the lake, with the proportion of total biovolume as weights (equation 1)

$$PTI = \frac{\sum_{j=1}^{n} a_j s_j}{\sum_{j=1}^{n} a_j} \text{ equation 1}$$

Where:

a_j = proportion of *j*th taxon in the sample

s_j= optimum of *j*th taxon in the sample

Relationships between the global PTI, and environmental variables were initially visualised using GAM models (Wood, 2006) and relationships investigated further using stepwise linear regression. For this analysis the full data set was used to maximise the number of lakes available. Data from all samples and all years were averaged to provide a single value for each lake to exclude any bias in the analysis of lakes with data from many sampling occasions. Linear models were developed using the *stepAIC* function from the R MASS library, which minimise the value of Aikaike's information criterion (AIC) to select the most parsimonious model (Venables & Rippey, 2002). In the analysis the multiple of the degrees of freedom used for the penalty were set to log (n), which increases the penalty associated with additional variables, often referred to as Bayes Information Criterion (BIC) (Schwarz, 1978). All lakes used in the analysis were assigned to European (intercalibaration) lake types by the data providers, but only a sub-set of lakes had sufficient data to determine mean values for a common set of environmental variables. Thus two sets of models were developed, one using mainly categorical variables on the full data set, while the other used a sub-set of data and co-variables.

Use of PTI to compare national phytoplankton assessment systems for intercalibration of good status boundaries

To enable the PTI scores to be used for the WFD the PTI values derived using the NGIG/CBGIG optima, were calculated for low and moderate alkalinity lakes in these GIGs and then converted to an Ecological Quality Ratio (EQR) using equation 2.

$$EQR_{PTI} = \left(\frac{PTI_{Obs} - PTI_{Max}}{PTI_{Ref} - PTI_{Max}} \right) \text{ .... Equation 2}$$

Where:

PTI_{Obs} = mean sample PTI for each lake year

PTI_{Max} = Maximum PTI score for type (1.3 for low alkalinity, 1.5 for moderate alkalinity)

PTI_{Ref} = Expected or reference PTI for type and country.

Reference values were derived using linear mixed models (Bates et al., 2011) with PTI as dependent variable, logTP as covariable and country as a random factor. Separate models were developed for low and moderate alkalinity lakes. The reference PTI for each country was calculated using the model coefficients and a reference TP value (median TP for all NGIG and CBGIG reference lakes for each alkalinity type, low alkalinity = 6μgL^{-1}, moderate alkalinity = 8μgL^{-1}). Further details of the models are given in (Lyche Solheim et al., 2012; Phillips et al., 2012).

The WFD requires an assessment of both taxonomic composition and abundance of phytoplankton. To provide an overall assessment of this biological quality element, the PTI EQR was averaged with an EQR for Chlorophyll a (equation 3). As the form of equation 3 results in a non-linear relationship with pressure the chlorophyll a EQRs were first transformed by linear interpolation so that the agreed class boundary EQRs (Poikane et al., 2010) fell on a linear scale of 0.2, 0.4, 0.6, 0.8.

$$EQR_{Chl} = \frac{Chl_{Ref}}{Chl} \quad \text{.............................. Equation 3}$$

Where:

Chl = observed mean chlorophyll a for the growing season (March – October)

Chl_{Ref}= reference chlorophyll a (values taken from Poikāne et al. (2010))

The resulting metric was used as a Common Metric (CM) (independent of national metrics) to compare phytoplankton assessment systems used by Finland, Norway, Sweden, Ireland and UK. Each national assessment method was applied to the data from lakes in all of these countries. For each lake type the resulting national EQRs were related to the CM using linear regression. Using the coefficients of these models, the boundaries defined by each country were expressed on the CM scale. The resulting CM boundary values for each country were then compared to the average for all countries to determine differences in boundaries set by each country. These average CM boundary values were then used to classify all the lakes in the data set to allow the overall performance of the CM to be assessed.

In this paper we report a summary of the result of this procedure for clear water moderate shallow alkalinity lakes in the NGIG. Other lake types and further details of the method can be found in Lyche Solheim et al. (2012) and Phillips et al. (2012).

Results

Environmental data

The data set analysed contained results from 20 countries, although the majority came from the northern and central region of Europe (Table 1). The environmental variables cover a wide range of conditions, but although country specific differences in lake types occurred, most countries had lakes covering a relatively wide spectrum of conditions (Fig 1). Given the source of the data it was not surprising that there were significant data gaps, few countries reported colour and many did not provide alkalinity data, although all placed the majority of their lakes into alkalinity and humic type categories. The results reflect distributions that might be expected, given the geology, topography and climate of Europe, with the lowest alkalinity lakes found in Scandinavia, and high alkalinity lakes in central Europe. The dominant depth type were shallow lakes (half of all the lakes), with relatively fewer very shallow lakes in Belgium, the Netherlands, Denmark, Latvia and Estonia, and deep lakes primarily in Norway, the UK and the reservoirs of the Mediterranean region. Clear and humic lakes were present in the data set, although there were relatively few humic lakes with very high colour (> 90mg L^{-1}Pt). The majority of lakes were between 0.5 and 4 km^2 (inter-quartile range), except in Finland where the median lake size range was 6-113 km^2, with several lakes >500 km^2. Finally the lakes covered a full range of trophic status, with very low TP and chlorophyll a in the north and the highest values in the lowland areas of central and eastern Europe.

Exploratory analysis of phytoplankton similarities and environmental factors affecting the taxonomic composition

The phytoplankton data were initially examined using non metric multidimensional scaling, an unconstrained ordination which uses a Bray Curtis dissimilarity matrix to create a 2-dimensional ordination. The projection was non-linear and the ordinations were unable to reach a convergent solution even after 40 iterations. The best solution had a stress value of 26.4, however the ordination shows a clear gradation of phytoplankton communities (Fig 2a), that are linked to geographic regions. Lakes from the Northern and Central-Baltic regions are separated from each other, but overlap with the Mediterranean and Eastern Continental regions. In the northern region, lakes from Norway, Sweden and Finland form distinct national clusters (Fig 2a), suggesting stronger country effects, than in other regions of Europe. All of the environmental variable vectors except mean depth are significantly ($p \leq 0.001$) related to the ordination (Fig 2b & Table 2). Chlorophyll *a* concentration has the strongest relationship, followed by alkalinity, air temperature and the pressure indicator variables total nitrogen (TN) and total phosphorus (TP). Surface area, altitude and latitude also have relatively strong relationships, while longitude, rainfall and colour explain less of the variation, although they were also significant. Considering the angles of the vectors (Fig 2b) it is clear that the 1st axis of the ordination represents a gradient of trophic status, with colder oligotrophic upland low alkalinity lakes at the left side and eutrophic lowland high alkalinity lakes at the right side. The 2nd axis represents the effects of rainfall and lake size, together with humic content. Smaller clear lakes experiencing higher rainfall at the upper end of the gradient and large humic lakes at the lower end. The geographic variables of latitude and longitude reflect the distribution of these lakes with smaller lower alkalinity lakes dominant in Norway and larger dystrophic lakes, with a longer retention time and higher humic content from Finland further to the east. In higher alkalinity lakes with higher levels of nutrients mainly found in the other regions of Europe, there is little evidence of country effects.

The phytoplankton typical of different parts of the NMDS axis 1 (corresponding to the trophic gradient) are taxa within the Chrysophyceae, Klebsomidiophyceae and Prasinophyceae having low axis 1 scores and are thus found mostly in oligotrophic low alkalinity lakes at higher altitudes and latitudes, while taxa within the Euglenophyceae and Cyanophyceae are found in high alkalinity and most nutrient enriched lakes (Fig 3). Phytoplankton classes with wide variation in NMDS axis 1 scores are Dinophyceae, Cryptophyceae, Conjugatophyceae, Chlorophyceae and Bacillariophyceae, illustrating the widely different nutrient preferences between genera and species within each of these classes.

Trophic optima for common phytoplankton genera

Given these strong relationships it was not surprising that the CCA ordination, constrained by log TP resulted in 27% of the variability of the global phytoplankton data set being explained by the 1st ordination axis; a relatively high proportion for a large taxonomic data set. Similar results were found for the NGIG/CBGIG data sets: 30% for genus data and 14% for species data. Thus the position of taxa along this axis should be a good indicator of their trophic affinity. The trophic optima and standard deviation of all genera occurring in more than 100 lakes in the global dataset are shown in Fig 4. The values are expressed as both the optima scores, derived from the 1st axis of the CCA, and the weighted average TP and standard deviation in lakes where each genus dominate

(Annex 1). The trophic optima and standard deviation can be used as indicator values and niche width respectively. The genera to the left in this figure are sensitive to eutrophication, as they are mainly occurring in oligotrophic lakes, while genera to the right are tolerant to eutrophication as they mainly occur in eutrophic lakes. A large proportion of the genera having low indicator values with optimum TP ranging from 5-10 μgL^{-1} belong to the classes with low scores in Fig 3, namely the Chrysophytes (*Chrysolykos* Mack, *Pseudokephyrion* Pascher, *Ochromonas* Wyssotzki, *Bitrichia* Woloszyńska, *Stichogloea* Chodat, *Chromulina* Cienkowski, *Spiniferomonas* Takahashi), the Klebsomidiophytes (*Elakatothrix* Wille and *Koliella* Hindák) and the Prasinophytes (*Scourfieldia* G.S.West). Genera in these classes are not found among the tolerant taxa with optimum TP indicator values above 20 μgL^{-1} TP. Conversely, most of the genera on the right side of this figure having TP optima above 30 μgL^{-1} belong to the classes with high scores in Figure 3, namely the Cyanobacteria (*Anabaena* Bory, *Planktothrix* Anagnostidis & Komárek, *Aphanizomenon* Morren, *Microcystis* Kützing) and the Euglenophytes (*Trachelomonas* Ehrenberg, *Phacus* Dujardin and *Euglena* Ehrenberg). With the exception of the Chroococcales genera *Merismopedia* Meyen, *Snowella* Elenkin and *Woronichinia* Elenkin, there are no genera among the Cyanobacteria and Euglenophytes having TP optima less than 20 μgL^{-1}. Genera with TP optima in the middle range from 10-30 μgL^{-1} in Figure 4 mainly belong to the classes with wide variability seen in the middle of Figure 3: Dinoflagellates, Cryptophytes, desmids (Conjugatophyceae), Chlorophytes and diatoms (Bacillariophyceae). The standard deviation appear quite similar across the trophic gradient, but as the figure 4 is on log-scale, the magnitude of the standard deviation increases from left to right, indicating a larger niche width for the more tolerant eutrophic genera than for the oligotrophic genera. The niche width is however also different among genera with quite similar optima, e.g. *Botryococcus* Kützing versus *Gymnodinium* Stein, both with a TP optimum close to 10 μgL^{-1}, but where *Botryococcus* has more narrow niche width than *Gymnodinium*. The same can be seen for some eutrophic genera, e.g. *Limnothrix* Meffert and *Scenedesmus* Meyen, both with optima close to 40 μgL^{-1}, but with considerably wider niche width for *Scenedesmus* than for *Limnothrix*. The wider niche width is mostly explained by the higher number of species within the genera *Gymnodinium* and *Scenedesmus* than within *Botryococcus* and *Limnothrix*.

The optima produced from this analysis were compared by correlation with other published values, taking the average value for the genus where species level values were available (Table 3). With the exception of the PTI from the Mediterranean region (Marchetto et al., 2009), all had significant correlations. The most similar were the PTI values from Norway (Ptacnik et al., 2009) and TPI from Sweden (Swedish EPA, 2010). The global and

NGIG/CBGIG optima were very similar (Table 3) and either could be used to derive the phytoplankton trophic index (PTI) and provide a good indicator of lake trophic status.

Relationship between PTI and environmental variables

Using an independent validation data set the PTI (based on the global optima) had a strong relationship with TP. A GAM model demonstrated that the relationship between PTI and TP was linear to approximately 75 μgL^{-1} levelling off at a TP concentration greater than 100 μgL^{-1} (GAM model adjusted R^2 = 0.60 $p<0.001$, linear model adjusted R^2 = 0.50 $p<0.001$) (Fig 5a). Using the full global data set improved the relationship (R^2 =0.66, $p<0.001$) and in contrast to mean depth and humic types, adding country or alkalinity type further improved the GAM model (R^2 = 0.71 with alkalinity type and 0.76 with country, (Fig 5b and Table 4) Thus for any particular level of TP there are likely to be small, but significant, differences in the PTI score for different countries, part of which may be explained by differences in the alkalinity of their lakes.

A stepwise application of linear models, applied to lakes with TP in the range of 5-100 μgL^{-1} confirmed that PTI had significant responses to TP and alkalinity type, but also showed that significant country effects remain even if alkalinity type is included in the model , with Norway having significantly lower PTI values than other countries (R^2 = 0.75 $p<0.001$), Table 5). To investigate the factors that might contribute to this country effect, the analysis was repeated excluding country from the models (Table 6). This reduced the overall significance of the relationship with TP (R^2 = 0.71 $p<0.001$), but in addition to alkalinity type the best model also contained significant type factors for surface area, together with the covariables for temperature (degree days? > 0ºC) and mean summer (July – September) precipitation.

PTI variability in Reference Lakes

Using a sub-set of reference lakes where sufficient data were available to apply typological co-variables rather than categorical factors, linear models showed similar results with alkalinity, and precipitation significant variables when country was included as a factor (R^2 = 0.80 $p<0.001$) (Table 7) and lake area and degree days? significant (R^2 = 0.61 $p<0.001$) when country was excluded (Table 8). When country was included TP was not a significant variable, which is not surprising given that these were reference lakes and thus had a relatively small TP range. However, when country was excluded TP was significant and together with alkalinity, surface area, temperature and precipitation contributed to the variability expressed as country. Thus higher alkalinity, higher temperature, larger lakes, and lower precipitation all combine to increase PTI values, in addition to the influence of TP.

Geographic variation of PTI in reference and impacted lakes across Europe

The range of values for PTI is illustrated geographically in Figure 6, showing that the PTI scores are generally lower in Scandinavia than in the rest of Europe, for both reference lakes and impacted lakes. This reflects climatic differences, the distribution of eutrophication in Europe and the dominance in northern Europe of low alkalinity lakes. The longitudinal gradient seen through Scandinavia going from Norway through Sweden to Finland with the lowest PTI scores in Norway, reflects these environmental gradients. For example, shorter retention time in Norwegian lakes due to a more humid climate, lower alkalinity due to geological conditions (mainly siliceous bedrock and thin soils, especially in Southern and Western Norway) and the dominance of large lakes and lower precipitation in Finland.

The lower PTI scores found in the Scandinavian reference lakes compared to reference lakes in the rest of Europe shown are also seen in box plots of PTI scores for the low and moderate alkalinity types (Figure 7). Norway and Sweden clearly have the lowest PTI scores in both low and moderate alkalinity reference lakes, while Portugal and Romania have the highest scores in reference lakes of the same alkalinity types. In high alkalinity lakes (> 1 $mEqL^{-1}$) there are no reference lakes from Scandinavia in the available dataset, and there are no clear differences among the other European countries. Thus, at least for low and moderate alkalinity lakes it is essential that country specific reference values for PTI are used for assessing lake status. An example of this approach, where mixed linear models with country as a random factor, were used to estimate PTI reference values for clear water moderate alkalinity shallow lakes in the Northern Geographical Intercalibration Group (NGIG) (Norway, Sweden, Finland, UK) is provided below.

Application of PTI as common taxonomic metric in intercalibration of national assessment methods in Northern Europe

The Common Metric (PTI combined with chlorophyll a) EQR has a strong linear relationship with TP (Fig 8 $R^2 = 0.77$, $p \leq 0.001$) and the EQRs produced by each of the NGIG countries, using their national assessment methods (Fig 9). The strongest relationships are with the assessment systems for UK and Norway ($R^2 = 0.89$

and 0.88 respectively p≤0.001), but all have R^2 values > 0.74, Using segmented regression it was found that the Finnish method had two linear relationships, with a shallower slope when the national EQR value was > 0.55. Both relationships were highly significant, converging close to the Good/Moderate boundary value of 0.60. The national boundary values on the common metric scale (derived from these models) were very similar, the greatest difference being found for Finland at ± 0.04 EQR units, well within the ±0.25 of a class (0.05 EQR units) required by the intercalibration guidance (WFD CIS Intercalibration guidance, 2011) . The average of the national method boundary EQRs expressed on the Common Metric scale were 0.89, 0.70 and 0.45 for the High/Good, Good/Moderate and Moderate/Poor boundaries.

Using these boundary values and assuming the Poor/Bad boundary was half of the Moderate/Poor value (0.23) the median and range were estimated for various eutrophication indicator metrics used by NGIG countries, TP, chlorophyll a, total bio-volume of phytoplankton and of cyanobacteria, the percentage of impact cyanobacteria (defined in (Swedish EPA, 2010) and PTI in this lake type for each of the five WFD classes (Fig 10). All of these metrics show a clear step wise transition from High to Bad, with relatively little overlap between classes, demonstrating the discriminatory power of the common metric. The PTI metric is particularly good in this respect with none of the upper and lower 25th quantiles of values overlapping between classes (Fig 10f).

Discussion

The trophic preferences found for different taxa in this pan-European study largely confirms previous findings on taxonomic changes of phytoplankton communities along the trophic gradient with Chrysophytes dominating in oligotrophic lakes and Cyanobacteria in eutrophic lakes (Taylor et al., 1979; Hörnström, 1981; Reynolds, 1984; Brettum, 1989; Brettum & Andersen, 2005; Mischke et al., 2008; Ptacnik et al., 2008; 2009; Järvinen et al., this issue). These changes are not only explained by nutrients, but also by other environmental factors. This was clearly demonstrated by the initial exploratory analysis using an unconstrained ordination showing the strong influence of alkalinity and temperature in addition to nutrient status in structuring the phytoplankton community. Similar results were found in other smaller regional studies, (Lepistö et al., 2004; Marchetto et al., 2009; Salmaso et al., 2006) that all found relationships with nutrients, alkalinity/conductivity along the 1st ordination axis. Thus, it is clear that the phytoplankton community composition changes significantly to these variables and thus has potential as a eutrophication indicator. Current indices, derived from the trophic preferences of different taxa, indicated by ordination or weighted averaging, are based on regional data sets, which have the advantage of minimising bio-geographic and climatic variation (Brettum, 1989; Mischke et al., 2008; Ptacnik et al., 2009; Salmaso et al., 2006; Swedish EPA, 2010). They are however, less applicable in other regions and subject to being over influenced by local conditions, a desirable property if the primary objective of the index was to estimate nutrient status, but less so if it was to assess overall status and compare across a large region such as Europe. For example (Marchetto et al., 2009) found poor relationships between observed TP and other trophic indices when these were applied to Sardinian reservoirs. In addition the trophic indices derived from these reservoirs also compared poorly with our values, in contrast to other optima that were derived from larger regional data (Table 3), probably because the Sardinian data used was from a very limited trophic range. Other optima had much higher correlations, including those covering central Europe (Mischke et al., 2008; UK in Lyche Solheim et al., 2012), although those from Scandinavia had the highest values (Ptacnik et al., 2009; Swedish EPA, 2010) as lakes from this area dominated our data set.

It is thus not surprising that we found significant country effects, similar results were obtained by Tolotti et al. (2009) in a study of phytoplankton assemblages in high altitude and high latitude lakes. They found distinct clusters for Finland, and the eastern Alps with geographic variables (latitude, longitude, altitude and ice cover) explaining variation along their 1st CCA axis and more local variables (TN, transparency, stratification and the presence of fish on the 2nd axis). Variables such as latitude and longitude are surrogates of others, particularly air temperature which we found to be highly correlated with latitude (R^2 = 0.88 $p<0.001$), as was alkalinity (R^2 = 0.45 $p<0.001$), reflecting the distribution of the major geological formations of Europe. Marchetto et al. (2009) point out that catchment geology distinguishes a 2nd phytoplankton composition gradient with reservoirs in siliceous areas having different taxa. Although alkalinity is often correlated with nutrients in ordinations (Salmaso et al., 2006) it also has an independent influence on the phytoplankton community composition. This should not be a surprise as alkalinity influences the availability of carbon (Stumm & Morgan, 1996).

In our study we found significant relationships between PTI and alkalinity that were independent of phosphorus. Thus, at a given phosphorus concentration the PTI was lower at lower alkalinities, and this difference may reflect availability of carbon, in particular the lack of bicarbonate in very low alkalinity waters. Reynolds (2006) points out that while the resource limitations of the major nutrients, nitrogen and phosphorus have been the focus of work on phytoplankton, linked no doubt to the need to control eutrophication, there is a wealth of evidence that the different abilities of planktonic algae to exploit soluble carbon will also influence composition. He illustrates this with reference to limestone upland areas in the UK where lakes deficient in nitrogen and phosphorus, but rich in bicarbonate are often dominated by green algae, dinoflagellates and species of *Anabaena*, *Gloeotrichia* J.C. Agardh and other cyanobacteria, all of which have high PTI optima, rather than genera more typical of low nutrients. Many of the Chrysophyceae, most of which have nutrient optima that fall at the oligotrophic end of the gradient, have little ability to use bicarbonate and their growth rates (or growth?) maybe more limited by availability of CO_2 than nutrients, particularly as most of the Chrysophytes are mixotrophic and can supplement their access to nutrients, in particular phosphorus, by consuming P-rich bacteria (Bird & Kalff, 1987). Thus at higher alkalinities it is not that surprising we see higher PTI scores as other taxa begin to gain a competitive advantage as small increases in nutrient supply reduce the available pool of dissolved carbon (Reynolds, 2006). It is interesting to note that the classic experiments of Talling (1976) produced evidence for an increasing tolerance to CO_2 depletion in the order (*Aulacoseira subartica → Asterionella formosa → Fragilaria crotonensis → Ceratium hirundinella*/*Microcystis aerugionsa*), which matches, with the exception of *Aulacoseira*, the order of increasing PTI optima of these genera (Fig 4).

Alkalinity is however not the only factor influencing the PTI. Our analysis shows a significant country effect, particularly for Norway, where PTI values are significantly lower than those of other countries, despite the inclusion of alkalinity in the model (Tables 5 & 7, Fig 7). Comparing models including and excluding country suggests that climate is another important factor (Tables 6 & 8). The duration of the ice free period has been shown to be related to the phytoplankton species richness and biomass in Sweden (Weyhenmeyer et al., 2012), partly because of correlations between nutrients, latitude and temperature (Weyhenmeyer, 2009), but also a direct result of a shorter growing season. When the ice free period increased above a threshold of 170 days the phytoplankton seasonal succession changed from one, to two peaks a year, with a consequent increase in species richness. Our temperature metric (degree days? > 0º C from January to the end of the sampling period used, September) cannot be directly related to the ice free period, but Norway experiences lower average temperatures than Finland and Sweden and the length of the growing season could be a factor influencing the summer phytoplankton community and thus the PTI metric. Comparing the taxa found in lakes from a narrow range of TP (5-10 μgL^{-1}) in NGIG countries (UK, NO, SE, FI) showed that NO had very few records (<5%) of several genera with higher PTI optima that were commonly found (>50% of samples) in other countries (eg *Pediastrum, Ankistrodesmus, Didymocystis, Tetrastrum, Aphanothece, Cyanodictyon, Trachelomonas, Aphanizomenon*). Thus, temperature is likely an important factor explaining the PTI variability among countries.

The other factor that was found to have a significant influence on PTI in our models was lake surface area and rainfall. The NMDS ordination suggested these two metrics influenced the phytoplankton community in opposite directions and in combination they may reflect flushing rates. Large lakes, found particularly in Finland, have typically greater volume, as lake area and depth are positively related (Nõges, 2009), and rainfall is significantly lower than in Norway (Fig 1). Thus, in comparison to other northern European countries, Norwegian lakes, not only experience lower temperatures, but may also have generally higher flushing rates, both factors that are likely to lead to fewer taxa and lower PTI scores, given the low nutrient regime. It should be stressed that this is just a hypothesis that could be tested on a dataset of Northern European lakes which had flushing rate data.

The relatively modest influence of colour (proxy for humic content) found in our analyses (Tables 2 and 4) is surprising, knowing the importance of this factor on both underwater light quantity and quality, on nutrient availability, as well as on pH and thereby bicarbonate availability (Keskitalo & Eloranta, 1999). Other studies have demonstrated the impact of humic content on phytoplankton taxonomic composition, in particular the dominance of the harmful and invasive algae *Gonyostomum semen* in humic lakes (Brucet et al., in press; Lepistö et al., 1994; 2004). A recent paper by Rengefors et al. (2012) demonstrates that *Gonyostomum* lakes have significantly higher DOC, higher nutrient levels, and lower pH than non-*Gonyostomum* lakes, based on a moderate Swedish dataset. Although the *Gonyostomum* appeared at the lowest end of the 2nd ordination axis in our exploratory analyses, and is thus positively correlated with colour, there are also other variables that are better correlated with this axis, e.g. lake size. This result may be caused by confounding factors in the dataset for Finnish lakes, as those are mostly much larger than the lakes from other countries, but together with the Swedish lakes also have generally higher colour than the Norwegian and UK lakes. The impact of colour on taxonomic composition may also be underestimated due to exclusion of lakes with high *Gonyostomum* biomass from all lakes, as that caused high TP and chlorophyll a (Järvinen et al., this issue)

The remaining unexplained variance of phytoplankton communities probably is related to variation in food web structure, including fish predation affecting zooplankton grazing and regeneration of nutrients (Carpenter & Kitchell, 1987; Pace, 1984). Furthermore, slight variation in sampling locations and frequency, as well as in phytoplankton analyses, also contribute to the variability, although many of the countries have harmonised their counting methods, so this variation is small, as confirmed by the WISER uncertainty analyses (Carvalho et al., this issue).

Thus, although our trophic index is less likely to perform as successfully in a particular country, due to the influence on the optima of phytoplankton populations drawn from a wide range of conditions, it has the advantage of a comprehensive set of taxa with robust scores, and provided country specific reference values are applied it can be used to assess lake status. The success of this is illustrated from its application to the intercalibration exercise of the Water Framework Directive (WFD) in both the Northern and Central Baltic GIGs, where it was used as part of a common metric to compare national assessment systems (Lyche Solheim et al., 2012; Phillips et al., 2012). The WFD requires a combination of metrics that assess both the taxonomic composition and the abundance of phytoplankton. Abundance is conveniently measured as chlorophyll a and after conversion to an EQR, using country specific reference values derived from mixed linear models that included country as a random factor, the PTI score provided an assessment of the change in taxonomic composition. Averaging these two metrics on the EQR scale produced a common metric that was highly significantly related to pressure (TP concentration) and national phytoplankton metrics and enabled the boundaries of the national assessment systems to be compared, and where necessary adjusted to ensure that the systems produced comparable classifications.

Using the average EQR value, from a set of independently developed national assessments systems that use a variety of approaches, expressed on the common metric scale is a powerful way of capturing in numeric form an agreed assessment of lake status. The example shown here, from clear water moderate alkalinity shallow lakes, demonstrates that the result is in line with ecological expectations. The upper quartile range for chlorophyll a at the High/Good and Good/Moderate boundaries were 3.5 and 7.5 $\mu g L^{-1}$ respectively, the latter is an appropriate value for a mesotrophic lake when compared to the widely accepted OECD trophic classification (OECD, 1982). Similarly the inter quartile ranges of the maximum biovolume of cyanobacteria show no overlap between classes. The lower quartile for Poor status had a value of 1.3 $mm^3 L^{-1}$ and the upper quartile for Moderate 1.2 $mm^3 L^{-1}$, values which are close to the low risk threshold for recreational waters (Carvalho et al., under review) defined by the WHO (WHO, 1999). This is consistent with guidance issued for eutrophication (WFD CIS Eutrophication guidance, 2009) which suggests that at Moderate status there should still be a low risk of undesirable disturbances, such as interference with recreational activity.

In conclusion we suggest that the taxonomic composition of the phytoplankton can be quantified using trophic optima derived from a pan-regional data set and provided that country specific reference values are used to account for climatic and bio-geographic differences and can be used to compare and assess the status of lakes across Europe.

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Central-Baltic GIG (Data manager: Ute Mischke, IGB)

Estonia: Estonian University of Life Sciences (EMU) gathered for the state monitoring programme supported by the Estonian Ministry of Environment.

Latvia: Latvian Environment, Geology and Meteorology Centre

Lithuania - EPA Lithuania

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Ireland: Environment Protection Agency (EPA)

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Tables

Table 1. Number of lakes used for analysis by country and core typology. BE = Belgium, CY = Cyprus, DE = Germany, DK = Denmark, EE = Estonia, ES = Spain, FI = Finland, FR = France, HU = Hungary, IE = Ireland, IT = Italy, LT = Lithuania, LV = Latvia, NL = Netherlands, NO = Norway, PL = Poland, RO = Romania, SE = Sweden, UK = United Kingdom

	Typology																
Alkalinity	High			Low						Moderate							
Mean Depth	Deep	Shallow	Very Shallow	Deep		Shallow		Very Shallow		Deep		Shallow		Very Shallow		Unable to type	
Colour	Not Split			Clear	Humic	Clear	Humic	Clear	Humic	Clear	Humic	Clear	Humic	Clear	Humic		
Country	HD	HS	HVS	LDC	LDH	LSC	LSH	LVSC	LVSH	MDC	MDH	MSC	MSH	MVSC	MVSH	U	Total
BE	0	5	4	0	0	0	0	0	0	0	0	0	0	0	0	0	9
CY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	7
DE	9	153	57	0	0	0	0	0	0	0	0	0	0	0	0	4	223
DK	0	25	59	0	0	1	0	4	0	0	0	5	0	11	0	2	107
EE	0	27	19	0	0	3	0	0	0	0	0	1	1	1	2	0	54
ES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	150	150
FI	0	0	0	3	3	24	63	2	9	1	3	16	21	0	9	5	159
FR	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	6	9
HU	0	3	17	0	0	0	0	0	0	0	0	0	0	0	0	0	20

IE	0	12	4	0	0	0	1	2	0	0	0	1	0	0	0	32	52
IT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	14
LT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	39
LV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	63	63
NL	2	14	33	0	0	0	0	0	0	0	0	0	0	0	0	0	49
NO	2	23	18	105	21	87	55	8	19	26	6	50	18	12	28	25	503
PL	1	44	5	0	0	0	0	0	0	0	0	0	0	0	0	0	50
PT	1	0	0	8	0	0	0	0	0	7	0	0	0	0	0	0	16
RO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	10
SE	0	3	3	0	0	13	52	0	12	1	1	0	9	0	4	6	104
UK	0	26	39	13	0	10	5	0	9	6	0	9	7	0	3	29	156
Totals	14	310	199	121	24	137	176	12	49	34	10	77	59	13	46	391	1795

Table 2 Environmental vectors fitted to NMDS ordination of phytoplankton data

p values based on 999 permutations.

	Directional cosine of vector for		Result random permutation tests	
	Axis 1	Axis 2	R^2	*p*
Log Chlorophyll a	1.000	-0.021	0.434	0.001
Log Alkalinity	0.881	0.474	0.394	0.001
Log Total N	0.955	0.295	0.294	0.001
Log Total P	0.981	0.193	0.282	0.001
Log Surface Area	0.158	-0.987	0.252	0.001
Log Altitude	-0.986	-0.166	0.195	0.001
Latitude	-0.990	-0.138	0.113	0.001
Longitude	-0.468	-0.884	0.074	0.001
Log Colour	-0.632	-0.775	0.055	0.001
Log Mean Depth	-0.450	-0.893	0.026	0.015

Table 3 Relationship between PTI optima and other reported optima used in similar metrics, values Spearman rank correlation coefficient $^{***}p<0.001$.

	PTI (N/CB GIG optima)	PTI UK[1]	Brettum[2]	PTI IT[3]	TAW DE[4]	ITS FR[5]	TPI SE[6]	PTI NO[7]	PTI Med[8]
PTI (global optima)	0.87^{***}	0.57^{***}	-0.49^{***}	-0.41^{***}	0.52^{***}	0.54^{***}	0.84^{***}	0.83^{***}	-0.19^{ns}
PTI (N/CB GIG optima)		0.58^{***}	-0.51^{***}	-0.34^{***}	0.51^{***}	0.49^{***}	0.81^{***}	0.85^{***}	-0.15^{ns}

[1] UK plankton trophic index (Phillips et al., 2012),[2] Brettum index (Brettum, 1989), [3] IT plankton trophic index (Buzzi et al., 2011), [4] DE trophic indicator score (Mischke et al., 2008), [5] FR indicator taxa score, [6] SE trophic plankton index (Swedish EPA, 2010), [7] NO plankton trophic index (Ptacnik et al., 2009), [8] PTI Med trophic value (Marchetto et al., 2009).

Table 4 Comparison of GAM regression models for relationships between PTI and TP showing inclusion of different lake type categories. Best model, from minimum value of BIC shown in bold

		Lake types					
GAM Model	log(TP)	Alkalinity	Mean depth	Humic	Country	adj R^2	BIC
0	X					0.66	2209.7
1	X	X				0.71	1931.7
2	X		X			0.66	2226.5
3	X			X		0.67	2152.9
4	**X**				**X**	**0.76**	**1675.5**

Table 5. Multiple linear regression predicting Plankton Trophic Index (PTI) as a function of TP , an index of air temperature and lake type, including country as a factor. Parameters were selected by stepwise method using the Bayesian information criteria BIC for model selection. Deselected parameters were, air temperature, mean precipitation, mean depth type, altitude type, surface area type and humic type. Significant variables shown in bold.

	Coefficient	Std. Error	t value	*p*	
Intercept	**-0.877**	**0.180**	**-4.882**	**0.000**	***
Log Total P	**0.865**	**0.038**	**22.807**	**<0.001**	***
Alkalinity type High	0.000				
Alkalinity type Low	**-0.264**	**0.039**	**-6.835**	**0.000**	***
Alkalinity type Moderate	0.005	0.038	0.145	0.884	
(Country BE)	0.000				
Country DE	0.258	0.168	1.533	0.126	
Country DK	-0.002	0.172	-0.010	0.992	
Country EE	-0.048	0.173	-0.279	0.780	
Country ES	-0.060	0.174	-0.343	0.732	
Country FI	-0.161	0.171	-0.942	0.346	
Country FR	-0.284	0.256	-1.109	0.268	
Country LT	0.219	0.176	1.240	0.215	
Country LV	0.195	0.173	1.125	0.261	

Country NL	0.189	0.183	1.032	0.302	
Country NO	**-0.437**	**0.171**	**-2.559**	**0.011**	*
Country PL	0.240	0.173	1.386	0.166	
Country PT	0.276	0.195	1.418	0.156	
Country RO	0.114	0.201	0.570	0.569	
Country SE	-0.134	0.171	-0.780	0.435	
Country UK	0.044	0.170	0.259	0.796	

adj R^2 = 0.747 p <0.001   F= 213 on 18 and 1275 DF

Table 6. Multiple linear regression predicting Plankton Trophic Index (PTI) as a function of TP, an index of air temperature and lake type(?), excluding country as a factor. Parameters were selected by stepwise method using the Bayesian information criteria BIC for model selection. Deselected parameters were, altitude type, and humic type. Significant variables shown in bold.7

	Coefficient	Std. Error	t value	*p*	
Intercept	-0.905	0.161	-5.613	**<0.001**	***
Log Total P	0.909	0.040	22.591	**<0.001**	***
Alkalinity type High	0.000				
Alkalinity type Low	-0.375	0.032	-11.590	**<0.001**	***
Alkalinity type Moderate	-0.147	0.032	-4.585	**<0.001**	***
Altitude type High	0.000				
Altitude type Low	0.104	0.081	1.290	0.197	
Altitude type Moderate	-0.030	0.083	-0.355	0.723	
Area type Large	0.000				
Area type Moderate	0.063	0.029	2.165	0.031	*
Area type Small	0.091	0.034	2.636	0.009	**
Area type Very Small	-0.062	0.033	-1.903	0.057	
Log Average precipitation July-Sept	-0.266	0.061	-4.329	**<0.001**	***

Degree months air temp					
>0ºC Jan-Sept	0.005	0.001	7.384	**<0.001**	***

adj R^2 = 0.711 p <0.001   F= 315.7 on 10 and 1283 DF

Table 7. Multiple linear regression predicting Plankton Trophic Index (PTI) as a function of environmental variables, including country as a factor in reference lakes. Parameters were selected by stepwise method using the Bayesian information criteria BIC for model selection. Deselected parameters were, TP, mean depth, surface area and air temperature. Significant variables shown in bold.

	Coefficient	Std. Error	t value	*p*	
Intercept	-0.399	0.212	-1.882	0.062	.
Log Alkalinity	**0.526**	**0.080**	**6.607**	**<0.001**	***
Log Average precipitation July-Sept	**0.321**	**0.108**	**2.970**	**0.004**	**
Country ES	0.000				
Country FI	-0.116	0.102	-1.138	0.257	
Country NO	**-0.797**	**0.105**	**-7.590**	**<0.001**	***
Country PL	-0.105	0.226	-0.465	0.642	
Country PT	**0.291**	**0.138**	**2.108**	**0.037**	*
Country RO	**0.412**	**0.169**	**2.442**	**0.016**	*
Country SE	**-0.299**	**0.115**	**-2.606**	**0.010**	*
Country UK	-0.083	0.105	-0.788	0.432	

adj R^2 = 0.804 p <0.001 F= 68.8 on 9 and 140 DF

Table 8. Multiple linear regression predicting Plankton Trophic Index (PTI) as a function of environmental variables, excluding country as a factor in reference lakes. Parameters were selected by stepwise method using the Bayesian information criteria BIC for model selection. Deselected parameters were, mean depth, altitude and mean summer preciptiation. Significant variables shown in bold.

	Coefficient	Std. Error	t value	*p*	
Intercept	**-1.026**	**0.210**	**-4.892**	**<0.001**	***
Log Total P	**0.567**	**0.151**	**3.767**	**<0.001**	***
Log Alkalinity	**0.548**	**0.108**	**5.053**	**<0.001**	***
Log Area	**0.097**	**0.028**	**3.462**	**0.001**	***
Degree months air temp >0ºC Jan-Sept	**0.004**	**0.001**	**3.008**	**0.003**	**

adj R^2 = 0.605 p <0.001   F= 58.1 on 4 and 145 DF

Figure legends

Fig. 1 Range of environmental variables by country. a) mean growing season total phosphorus (µgL^{-1}), b) mean growing season chlorophyll a (µgL^{-1}), c) alkalinity (mEqL^{-1}), d) altitude (m), e) mean depth (m), f) surface area (km^2), g) colour (mg L^{-1}Pt), h) air temperature as degree days? >0ºC for January – September, i) mean precipitation July-September (mm).

Fig. 2 Non Metric Multidimensional Scaling site ordination showing a) distribution by country, b) relationship with environmental variables (Alk = alkalinity, TN = total nitrogen, TP = total phosphorus, Chl = chlorophyll a, Col= colour, Lat = latitude, Long = Longitude, Area= Surface Area, AvgPPT = mean precipitation July-September, Temp = degree days? > 0ºC January-September. Ellipses show location of GIG regions (standard deviation)(?) N = Northern, CB = Central Baltic, M = Mediterranean, EC = Eastern Continental.

Fig. 3 Distribution of Non Metric Multidimensional Scaling axis taxon scores for phytoplankton class, box width proportional to number of records in each class.

Fig 4 Weighted average (WA) total phosphorus concentrations and corresponding CCA axis 1 optima for phytoplankton genera recorded in >100 lakes, bars represent ± 1SD of WA mean (tolerance), dotted line marks the 0.00 value for CCA axis 1, equivalent to the mean TP for the data set (20µg L^{-1}).

Fig 5 Relationship between PTI site scores for validation data set and growing season mean total phosphorus concentration a) validation data only, b) full data set split by alkalinity types. Lines in a) are GAM and linear models, linear models for TP in range of 5-100 µg L^{-1} in b), low alkalinity = black, moderate alkalinity = blue, high alkalinity = purple.

Fig 6 Geographic distribution of mean plankton trophic index (PTI) for summer (July – September) samples in a) reference lakes, b) non-reference lakes. Colours show range of PTI value for each lake.

Fig 7 Range of PTI in reference lakes split by country and alkalinity type.

Fig 8 Relationship between common metric for moderate alkalinity shallow lakes and mean growing season total phosphorus from NGIG and CBGIG countries (Denmark, Estonia, Finland, Ireland, Norway and UK).

Fig 9 Relationship between national standardised EQR and common metric EQR. a) SE, b) FI, d) NO, e) UK, f) IE for moderate alkalinity shallow lakes showing linear models fitted to data. Relationship for FI was split into 2 segments at point marked by horizontal line (±SE) using segmented regression. Dotted lines are High/Good and Good/Moderate national EQR boundaries on national and standardised national scales, solid horizontal lines are GIG mean value of national EQR boundaries on common metric scale.

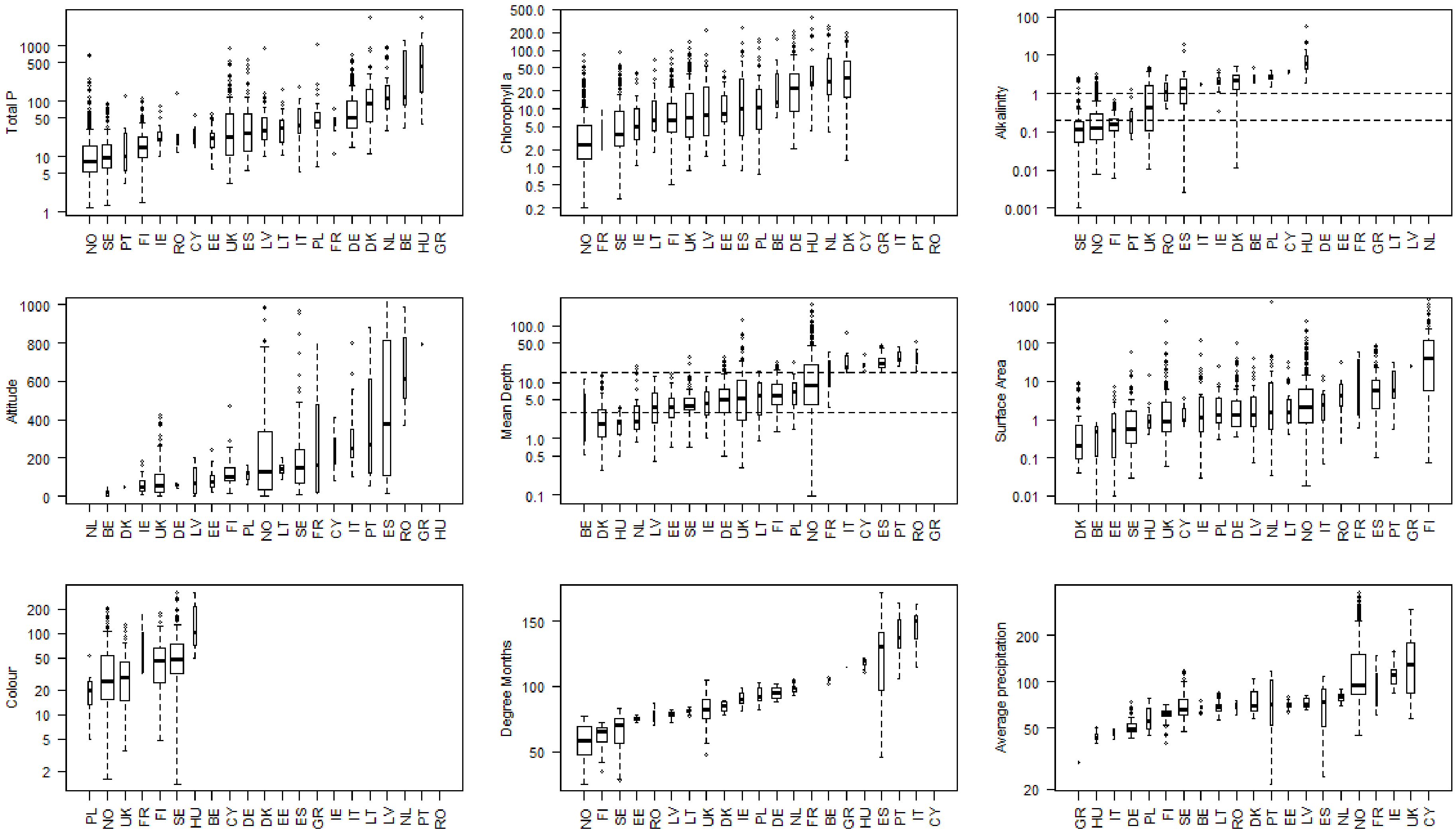

Total P
NO SE PT FI IE RO CY EE UK ES LV LT IT PL FR DE DK NL BE HU GR
Chlorophyll a
NO FR SE IE LT FI UK LV EE ES PL BE DE HU NL DK CY GR IT PT RO
Alkalinity
SE NO FI PT UK RO ES IT IE DK BE PL CY HU DE EE FR GR LT LV NL
Altitude
NL BE DK IE UK DE LV EE FI PL NO LT SE FR CY IT PT ES RO GR HU
Mean Depth
BE DK HU NL LV EE SE IE DE UK LT FI PL NO FR IT CY ES PT RO GR
Surface Area
DK BE EE SE HU UK CY IE PL DE LV NL LT NO IT RO FR ES PT GR FI
Colour
PL NO UK FR FI SE HU BE CY DE DK EE ES GR IE IT LT LV NL PT RO
Degree Months
NO FI SE EE RO LV LT UK DK IE PL DE NL FR BE GR HU ES PT IT CY
Average precipitation
GR HU IT DE PL FI SE BE LT RO DK PT EE LV ES NL NO FR IE UK CY

Fig 1

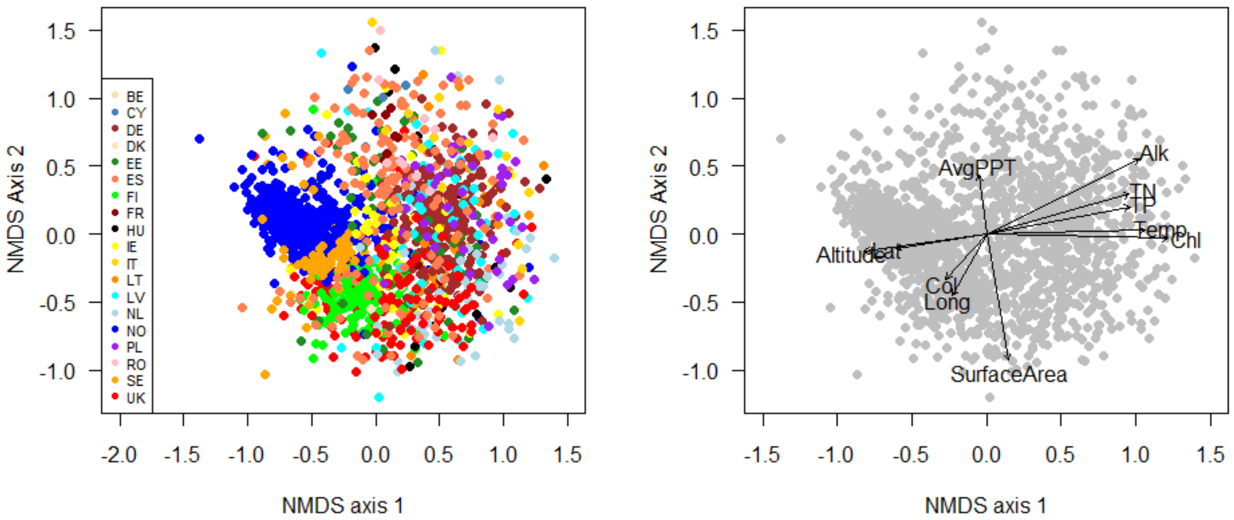

NMDS Axis 2
NMDS axis 1
BE
CY
DE
DK
EE
ES
FI
FR
HU
IE
IT
LT
LV
NL
NO
PL
RO
SE
UK
AvgPPT
Alk
TN
TP
Temp
Chl
Altitude
Lat
Col
Long
SurfaceArea

Fig 2

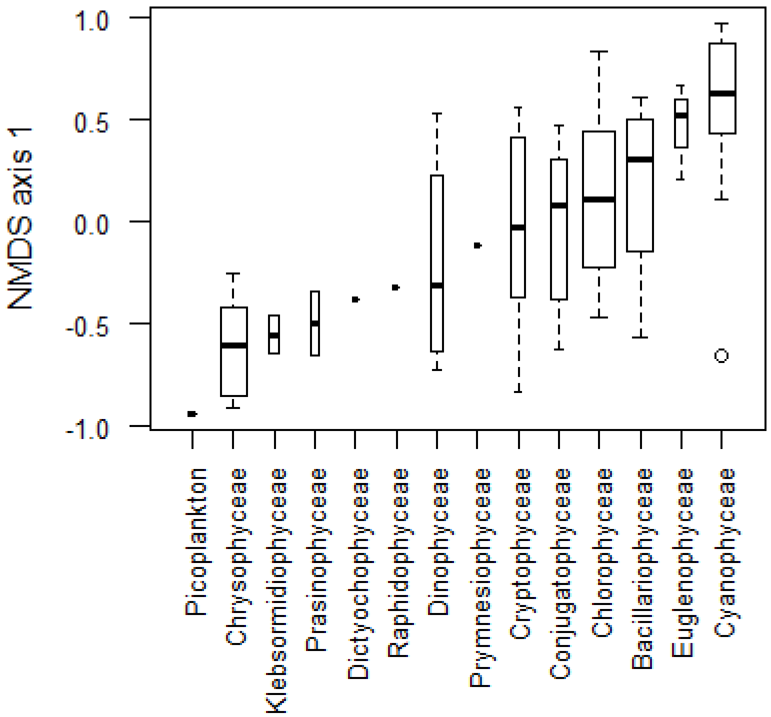
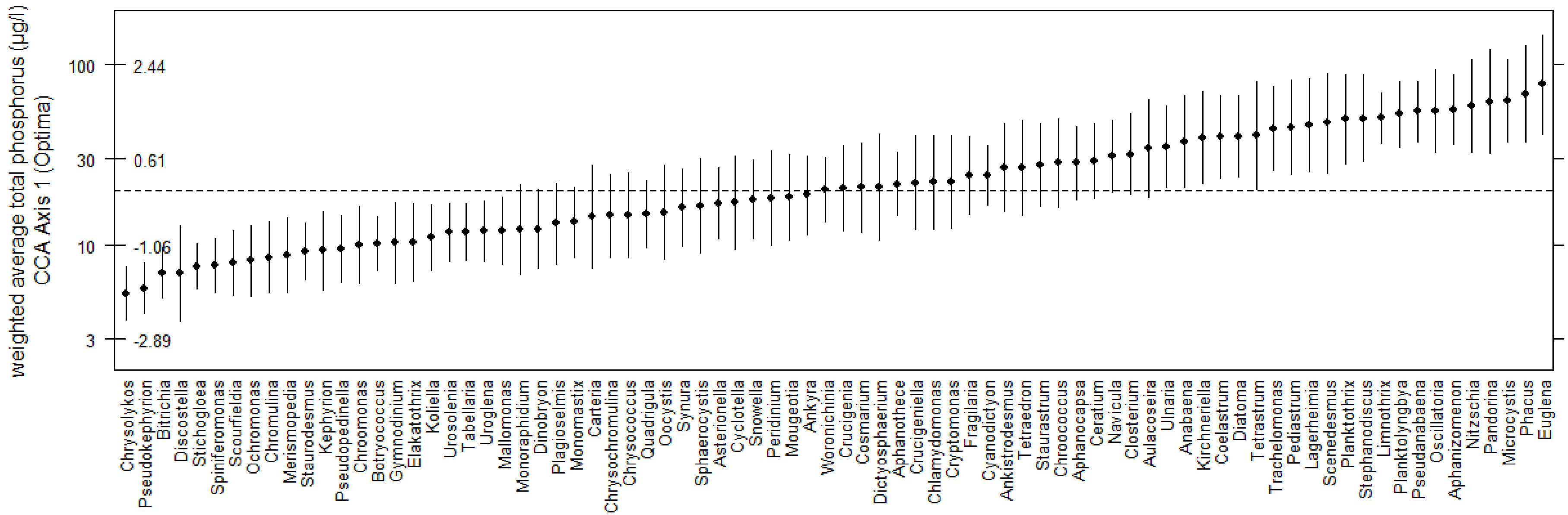

NMDS axis 1
1.0
0.5
0.0
-0.5
-1.0
Picoplankton
Chrysophyceae
Klebsormidiophyceae
Prasinophyceae
Dictyochophyceae
Raphidophyceae
Dinophyceae
Prymnesiophyceae
Cryptophyceae
Conjugatophyceae
Chlorophyceae
Bacillariophyceae
Euglenophyceae
Cyanophyceae

Fig 3



weighted average total phosphorus (µg/l)
CCA Axis 1 (Optima)
100
30
10
3
2.44
0.61
-1.06
-2.89
Chrysolykos
Pseudokephyrion
Bitrichia
Discostella
Stichogloea
Spiniferomonas
Scourfieldia
Ochromonas
Chromulina
Merismopedia
Staurodesmus
Kephyrion
Pseudopedinella
Chroomonas
Botryococcus
Gymnodinium
Elakatothrix
Koliella
Urosolenia
Tabellaria
Uroglena
Mallomonas
Monoraphidium
Dinobryon
Plagioselmis
Monomastix
Carteria
Chrysochromulina
Chrysococcus
Quadrigula
Oocystis
Synura
Sphaerocystis
Asterionella
Cyclotella
Snowella
Peridinium
Mougeotia
Ankyra
Woronichinia
Crucigenia
Cosmarium
Dictyosphaerium
Aphanothece
Crucigeniella
Chlamydomonas
Cryptomonas
Fragilaria
Cyanodictyon
Ankistrodesmus
Tetraedron
Staurastrum
Chroococcus
Aphanocapsa
Ceratium
Navicula
Closterium
Aulacoseira
Ulnaria
Anabaena
Kirchneriella
Coelastrum
Diatoma
Tetrastrum
Trachelomonas
Pediastrum
Lagerheimia
Scenedesmus
Planktothrix
Stephanodiscus
Limnothrix
Planktolyngbya
Pseudanabaena
Oscillatoria
Aphanizomenon
Nitzschia
Pandorina
Microcystis
Phacus
Euglena

Fig 4

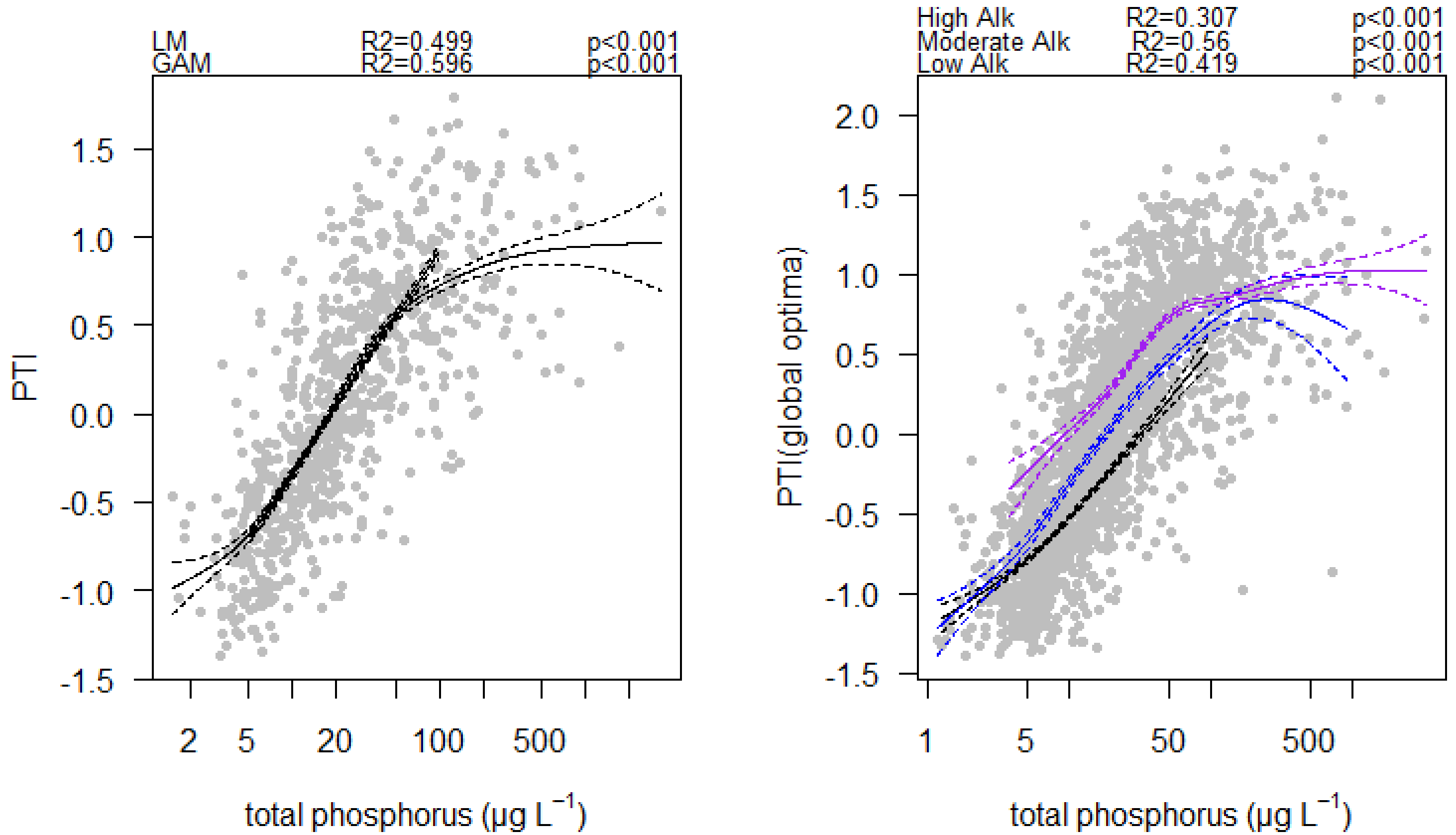

LM R2=0.499 p<0.001
GAM R2=0.596 p<0.001
PTI
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
2
5
20
100
500
total phosphorus (µg L^{-1})
High Alk R2=0.307 p<0.001
Moderate Alk R2=0.56 p<0.001
Low Alk R2=0.419 p<0.001
PTI(global optima)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
1
5
50
500
total phosphorus (µg L^{-1})

Fig 5

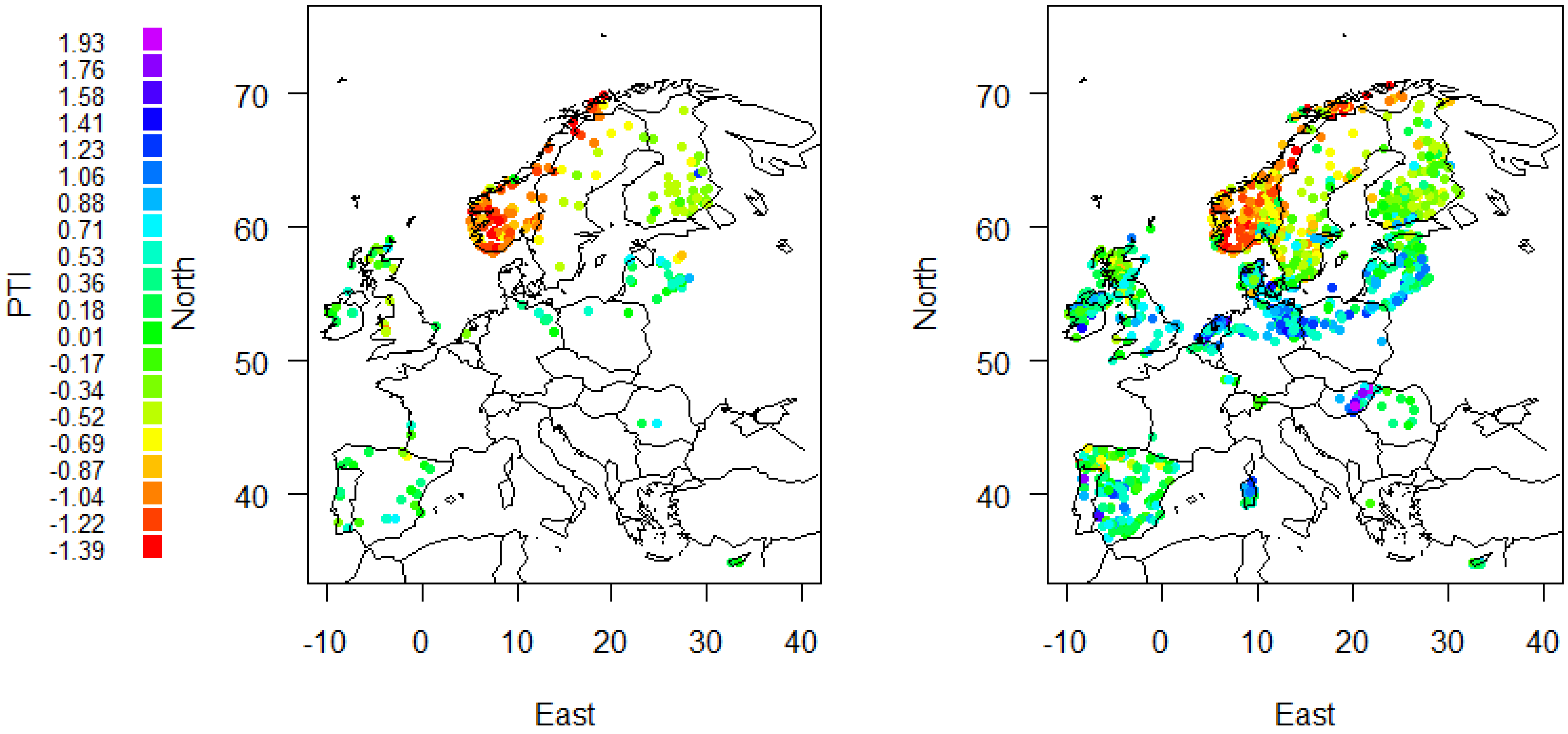

PTI
1.93
1.76
1.58
1.41
1.23
1.06
0.88
0.71
0.53
0.36
0.18
0.01
-0.17
-0.34
-0.52
-0.69
-0.87
-1.04
-1.22
-1.39
North
70
60
50
40
-10
0
10
20
30
40
East
North
70
60
50
40
-10
0
10
20
30
40
East

Fig 6

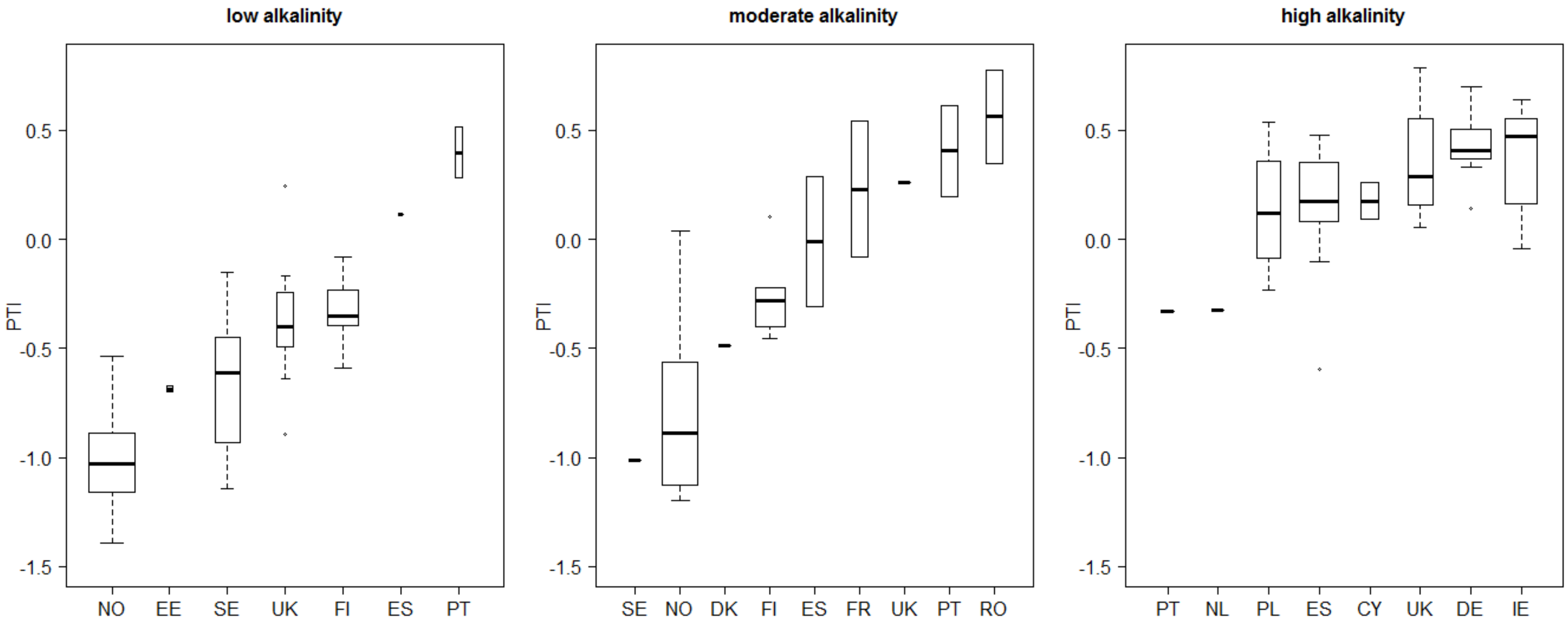

low alkalinity
moderate alkalinity
high alkalinity
PTI
0.5
0.0
-0.5
-1.0
-1.5
NO EE SE UK FI ES PT
SE NO DK FI ES FR UK PT RO
PT NL PL ES CY UK DE IE

Fig 7

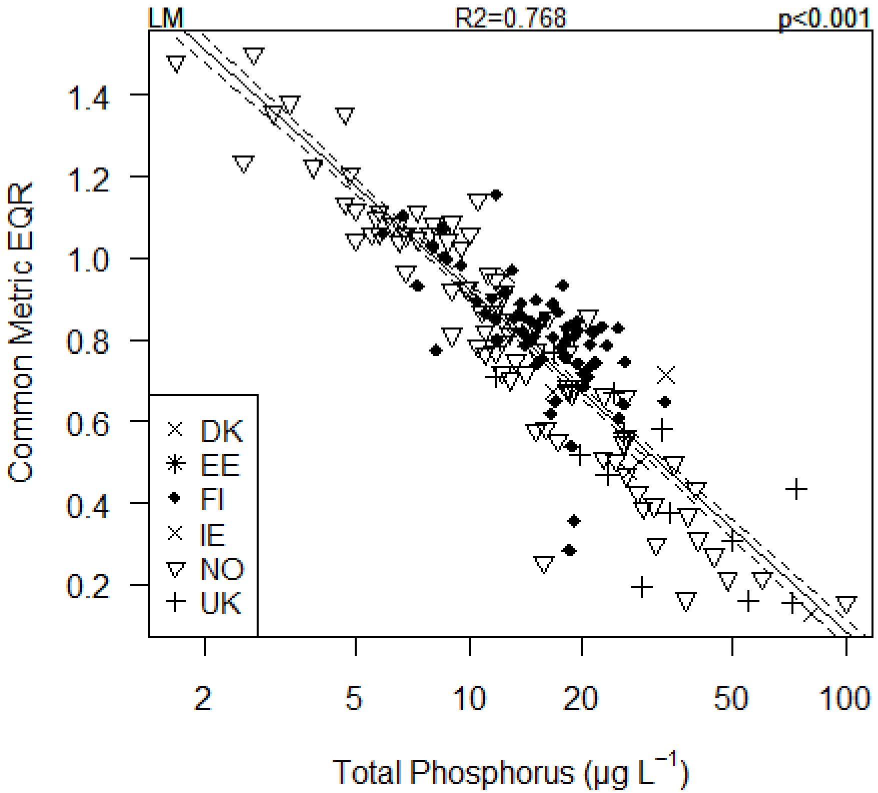

LM
R2=0.768
p<0.001
Common Metric EQR
1.4
1.2
1.0
0.8
0.6
0.4
0.2
DK
EE
FI
IE
NO
UK
2
5
10
20
50
100
Total Phosphorus (µg L⁻¹)

Fig 8

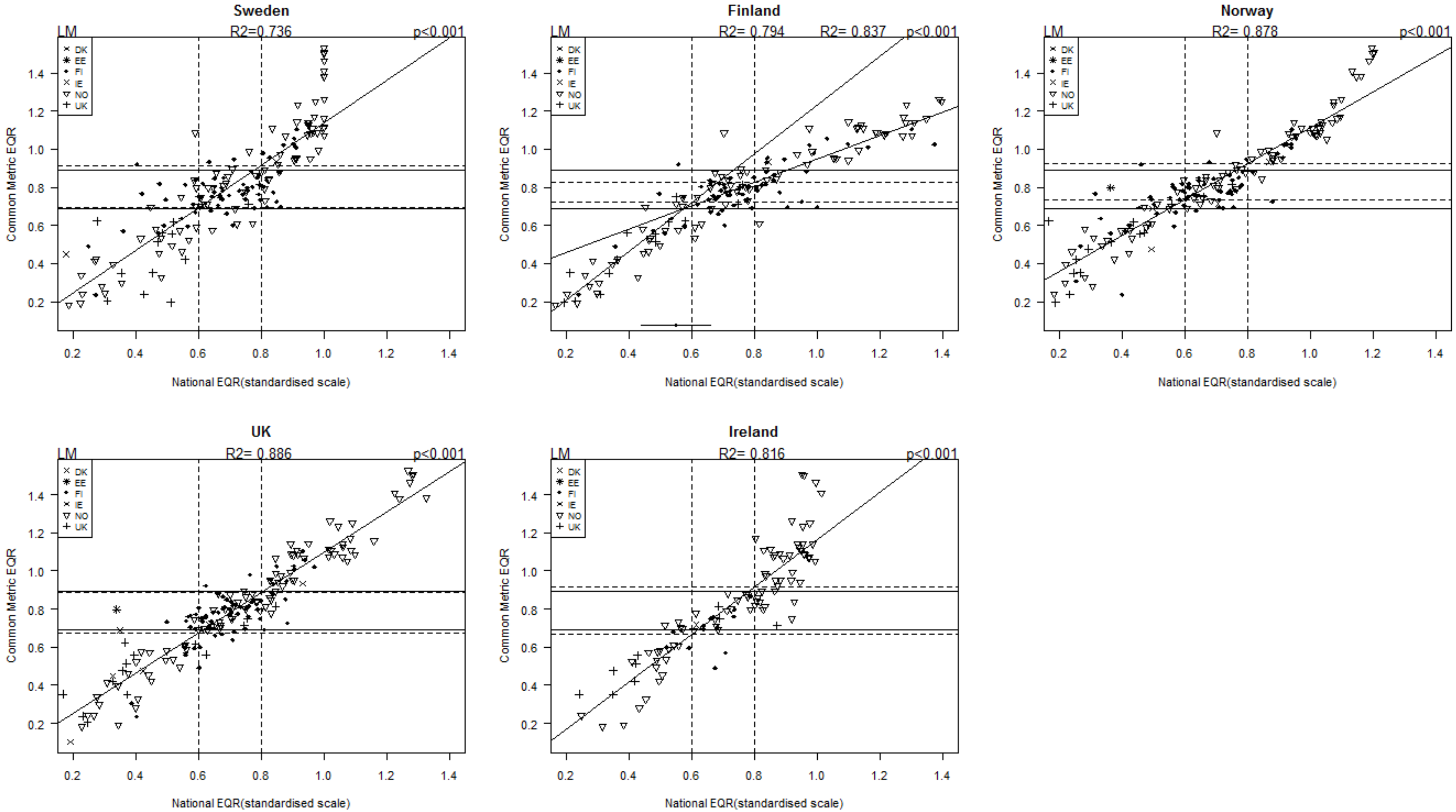

Sweden
LM
R2=0.736
p<0.001
Finland
LM
R2= 0.794
R2= 0.837
p<0.001
Norway
LM
R2= 0.878
p<0.001
UK
LM
R2= 0.886
p<0.001
Ireland
LM
R2= 0.816
p<0.001
DK
EE
FI
IE
NO
UK
Common Metric EQR
National EQR(standardised scale)

Fig 9

Annex 1. List of phytoplankton PTI optima for global data set

Genus	Order	Class	CCA Axis 1 Optima	N lakes with taxa	Weighted Average TP µg L^{-1}	Weighted Average Tolerance lower TP µg L^{-1}	Weighted Average Tolerance upper TP µg L^{-1}
Acanthoceras	Centrales	Bacillariophyceae	0.561	88	29	20	40
Achnanthes	Pennales	Bacillariophyceae	-0.504	77	14	8	26
Achnanthidium	Pennales	Bacillariophyceae	0.100	16	21	13	33
Achroonema		Cyanobacteria	1.364	28	48	28	83
Actinastrum	Chlorococcales	Chlorophyceae	2.608	84	109	70	172
Actinocyclus	Centrales	Bacillariophyceae	3.430	36	187	142	247
Amphidinium	Gymnodiniales	Dinophyceae	-0.140	2	18	14	23
Amphora	Pennales	Bacillariophyceae	0.463	29	27	13	54
Anabaena	Nostocales	Cyanobacteria	0.984	612	38	21	67
Anabaenopsis	Nostocales	Cyanobacteria	3.311	28	173	113	266
Ankistrodesmus	Chlorococcales	Chlorophyceae	0.470	144	27	15	47
Ankyra	Chlorococcales	Chlorophyceae	-0.071	240	19	11	31
Aphanizomenon	Nostocales	Cyanobacteria	1.595	409	56	36	88
Aphanocapsa	Chroococcales	Cyanobacteria	0.562	250	29	18	46
Aphanothece	Chroococcales	Cyanobacteria	0.154	212	22	15	33
Asterionella	Pennales	Bacillariophyceae	-0.227	561	17	11	27
Aulacoseira	Centrales	Bacillariophyceae	0.847	565	34	18	65
Bitrichia	Stylococcales	Chrysophyceae	-1.586	389	7	5	10
Botryococcus	Chlorococcales	Chlorophyceae	-1.008	331	10	7	14
Carteria	Volvocales	Chlorophyceae	-0.480	100	14	7	28
Centritractus	Mischococcales	Xanthophyceae	0.992	20	38	19	75
Ceratium	Peridiniales	Dinophyceae	0.583	564	29	18	47
Chlamydocapsa	Tetrasporales	Chlorophyceae	-0.139	5	18	13	25

Chlamydomonas	Volvocales	Chlorophyceae	0.182	539	22	12	41
Chlorella	Chlorellales	Trebouxiophyceae	1.373	57	49	27	87
Chlorogonium	Volvocales	Chlorophyceae	2.624	10	110	78	155
Chlorotetraedron	Chlorococcales	Chlorophyceae	1.367	13	48	24	99
Chromulina	Chromulinales	Chrysophyceae	-1.280	301	9	5	14
Chroococcus	Chroococcales	Cyanobacteria	0.559	278	29	16	50
Chroomonas	Cryptomonadales	Cryptophyceae	-1.042	370	10	6	16
Chrysamoeba	Chromulinales	Chrysophyceae	-0.151	5	18	11	30
Chrysidiastrum	Chromulinales	Chrysophyceae	-1.320	64	8	6	12
Chrysochromulina	Prymnesiales	Prymnesiophyceae	-0.472	457	15	8	25
Chrysococcus	Chromulinales	Chrysophyceae	-0.468	154	15	8	25
Chrysolykos	Chromulinales	Chrysophyceae	-1.992	212	5	4	8
Chrysosphaerella	Chromulinales	Chrysophyceae	-0.590	19	13	9	21
Chrysostephanosphaera	Chromulinales	Chrysophyceae	-1.583	9	7	6	9
Closteriopsis	Chlorococcales	Chlorophyceae	1.595	42	56	35	91
Closterium	Desmidiales	Conjugatophyceae	0.732	435	32	19	54
Cocconeis	Pennales	Bacillariophyceae	1.148	45	42	25	69
Coelastrum	Chlorococcales	Chlorophyceae	1.078	250	40	24	68
Coelosphaerium	Chroococcales	Cyanobacteria	0.827	83	34	20	59
Coenochloris	Chlorococcales	Chlorophyceae	0.372	57	25	16	40
Coenococcus	Chlorococcales	Chlorophyceae	-0.919	7	11	8	14
Coenocystis	Chlorococcales	Chlorophyceae	0.980	4	38	12	121
Colacium	Euglenales	Euglenophyceae	0.098	6	21	11	39
Cosmarium	Desmidiales	Conjugatophyceae	0.081	361	21	12	37
Crucigenia	Chlorococcales	Chlorophyceae	0.056	298	21	12	35
Crucigeniella	Chlorococcales	Chlorophyceae	0.170	137	22	12	40
Cryptomonas	Cryptomonadales	Cryptophyceae	0.189	1094	22	12	41
Cyanodictyon	Chroococcales	Cyanobacteria	0.318	100	24	17	35
Cyanonephron	Chroococcales	Cyanobacteria	1.289	3	46	26	81

Cyclostephanos	Centrales	Bacillariophyceae	2.223	63	85	50	143
Cyclotella	Centrales	Bacillariophyceae	-0.209	544	17	9	31
Cylindrospermopsis	Nostocales	Cyanobacteria	2.121	42	79	53	119
Cylindrotheca	Pennales	Bacillariophyceae	2.132	19	80	51	125
Cymatopleura	Pennales	Bacillariophyceae	1.577	10	56	40	77
Cymbella	Pennales	Bacillariophyceae	0.353	37	25	15	42
Diatoma	Pennales	Bacillariophyceae	1.082	116	40	24	67
Dictyosphaerium	Chlorococcales	Chlorophyceae	0.094	298	21	11	41
Didymocystis	Chlorococcales	Chlorophyceae	0.637	93	30	18	49
Dinobryon	Chromulinales	Chrysophyceae	-0.727	785	12	7	20
Diplochloris	Chlorococcales	Chlorophyceae	3.853	17	247	123	498
Discostella	Centrales	Bacillariophyceae	-1.582	153	7	4	13
Elakatothrix	Klebsormidiales	Klebsormidiophyceae	-0.995	525	10	6	17
Epipyxis	Chromulinales	Chrysophyceae	-1.250	68	9	6	13
Erkenia	Chromulinales	Chrysophyceae	0.797	17	33	26	43
*Euastrum	Desmidiales	Conjugatophyceae	-0.492	38	14	8	24
Eudorina	Volvocales	Chlorophyceae	0.694	66	31	18	55
Euglena	Euglenales	Euglenophyceae	2.095	180	78	42	147
Eunotia	Pennales	Bacillariophyceae	-0.318	58	16	10	26
Eutetramorus	Chlorococcales	Chlorophyceae	2.048	15	76	58	99
Fragilaria	Pennales	Bacillariophyceae	0.317	589	24	15	40
Franceia	Chlorococcales	Chlorophyceae	0.504	13	28	16	48
Frustulia	Pennales	Bacillariophyceae	-1.392	10	8	6	11
Geitlerinema	Oscillatoriales	Cyanobacteria	2.695	7	116	73	184
Glenodinium	Peridiniales	Dinophyceae	0.192	26	22	13	40
Gloeocapsa	Chroococcales	Cyanobacteria	0.559	6	29	21	38
Gloeocystis	Chlorococcales	Chlorophyceae	-1.636	34	7	5	10
Gloeotila	Ulotrichales	Ulvophyceae	-1.251	50	9	5	14
Gloeotrichia	Nostocales	Cyanobacteria	1.232	4	44	40	50

Golenkinia	Chlorococcales	Chlorophyceae	1.053	25	39	22	71
Golenkiniopsis	Chlorococcales	Chlorophyceae	1.752	8	62	34	113
Gomphonema	Pennales	Bacillariophyceae	0.903	22	36	20	63
Gomphosphaeria	Chroococcales	Cyanobacteria	1.363	38	48	25	92
Goniochloris	Mischococcales	Xanthophyceae	1.984	40	73	39	135
Gonium	Volvocales	Chlorophyceae	0.671	6	31	22	43
Gonyostomum	Chattonellales	Raphidophyceae	-0.069	99	19	14	26
Gymnodinium	Gymnodiniales	Dinophyceae	-1.000	682	10	6	17
Gyrosigma	Pennales	Bacillariophyceae	0.490	14	27	15	49
Isthmochloron	Mischococcales	Xanthophyceae	-2.022	14	5	4	7
Katodinium	Gymnodiniales	Dinophyceae	0.343	12	25	13	49
Kephyrion	Chromulinales	Chrysophyceae	-1.143	265	9	6	15
Keratococcus	Chlorococcales	Chlorophyceae	0.579	9	29	21	40
Kirchneriella	Chlorococcales	Chlorophyceae	1.056	152	40	22	71
Koliella	Klebsormidiales	Klebsormidiophyceae	-0.898	160	11	7	17
Lagerheimia	Chlorococcales	Chlorophyceae	1.306	119	47	26	84
Lepocinclis	Euglenales	Euglenophyceae	1.951	12	71	30	170
Limnothrix	Oscillatoriales	Cyanobacteria	1.441	143	51	37	71
Lyngbya	Oscillatoriales	Cyanobacteria	1.345	10	48	36	63
Mallomonas	Synurales	Chrysophyceae	-0.766	595	12	8	18
Melosira	Centrales	Bacillariophyceae	1.711	30	61	36	103
Merismopedia	Chroococcales	Cyanobacteria	-1.242	356	9	5	14
Micractinium	Chlorococcales	Chlorophyceae	1.444	46	51	30	87
Microcystis	Chroococcales	Cyanobacteria	1.788	310	64	38	108
Monochrysis	Chromulinales	Chrysophyceae	-1.242	85	9	7	12
Monomastix	Mamiellales	Prasinophyceae	-0.596	104	13	8	21
Monomorphina	Euglenales	Euglenophyceae	2.296	33	89	51	155
Monoraphidium	Chlorococcales	Chlorophyceae	-0.744	805	12	7	22
Mougeotia	Zygnematales	Conjugatophyceae	-0.112	174	18	11	32

Navicula	Pennales	Bacillariophyceae	0.687	132	31	20	49
Nephrochlamys	Chlorococcales	Chlorophyceae	3.322	20	175	82	371
Nephrocytium	Chlorococcales	Chlorophyceae	-0.652	62	13	8	21
Nephroselmis	Polyblepharidales	Prasinophyceae	1.363	17	48	30	78
Nitzschia	Pennales	Bacillariophyceae	1.674	331	59	33	107
Ochromonas	Chromulinales	Chrysophyceae	-1.350	445	8	5	13
Oocystis	Chlorococcales	Chlorophyceae	-0.405	764	15	8	28
Ophiocytium	Mischococcales	Xanthophyceae	0.582	13	29	21	40
Oscillatoria	Oscillatoriales	Cyanobacteria	1.575	119	56	33	93
Pandorina	Volvocales	Chlorophyceae	1.763	103	63	32	122
Paulschulzia	Tetrasporales	Chlorophyceae	0.121	40	21	14	34
Pediastrum	Chlorococcales	Chlorophyceae	1.260	399	45	25	83
Peridiniopsis	Peridiniales	Dinophyceae	-0.057	64	19	11	32
Peridinium	Peridiniales	Dinophyceae	-0.125	774	18	10	33
Phacotus	Volvocales	Chlorophyceae	1.134	57	42	29	60
Phacus	Euglenales	Euglenophyceae	1.912	104	69	37	128
Phormidium	Oscillatoriales	Cyanobacteria	1.666	11	59	43	82
Pinnularia	Pennales	Bacillariophyceae	-0.290	14	16	9	31
Plagioselmis	Cryptomonadales	Cryptophyceae	-0.618	734	13	8	22
Planctonema	Ulotrichales	Ulvophyceae	0.730	42	32	18	57
Planktolyngbya	Oscillatoriales	Cyanobacteria	1.513	170	53	35	81
Planktosphaeria	Chlorococcales	Chlorophyceae	0.755	26	32	16	67
Planktothrix	Oscillatoriales	Cyanobacteria	1.416	327	50	28	88
Pseudanabaena	Oscillatoriales	Cyanobacteria	1.570	234	55	38	81
Pseudodictyosphaerium	Chlorococcales	Chlorophyceae	2.870	4	130	92	184
Pseudogoniochloris	Mischococcales	Xanthophyceae	0.985	18	38	24	58
Pseudokephyrion	Chromulinales	Chrysophyceae	-1.884	260	6	4	8
Pseudopedinella	Pedinellales	Dictyochophyceae	-1.104	304	10	6	15
Pseudosphaerocystis	Tetrasporales	Chlorophyceae	0.027	32	20	15	27
Pseudostaurastrum	Mischococcales	Xanthophyceae	1.095	28	41	25	66

Pteromonas	Volvocales	Chlorophyceae	2.053	31	76	40	144
Puncticulata	Centrales	Bacillariophyceae	-0.163	20	18	13	25
Quadricoccus	Chlorococcales	Chlorophyceae	2.519	15	103	43	246
Quadrigula	Chlorococcales	Chlorophyceae	-0.436	187	15	10	23
Radiocystis	Chroococcales	Cyanobacteria	-0.331	54	16	9	27
Raphidocelis	Chlorococcales	Chlorophyceae	0.008	93	20	11	37
Rhabdoderma	Chroococcales	Cyanobacteria	-0.448	18	15	9	24
Rhabdogloea	Chroococcales	Cyanobacteria	-1.908	13	6	3	10
Rhodomonas	Cryptomonadales	Cryptophyceae	0.632	77	30	16	57
Romeria	Oscillatoriales	Cyanobacteria	3.035	8	145	85	246
Scenedesmus	Chlorococcales	Chlorophyceae	1.340	606	48	25	90
Schroederia	Chlorococcales	Chlorophyceae	1.477	78	52	29	93
Scourfieldia	Scourfieldiales	Prasinophyceae	-1.400	160	8	5	12
Siderocelis	Chlorococcales	Chlorophyceae	1.787	20	64	32	126
Skeletonema	Centrales	Bacillariophyceae	2.853	36	128	98	168
Snowella	Chroococcales	Cyanobacteria	-0.157	343	18	11	30
Spermatozopsis	Volvocales	Chlorophyceae	2.214	19	84	51	139
Sphaerocystis	Chlorococcales	Chlorophyceae	-0.277	226	17	9	30
Spiniferomonas	Chromulinales	Chrysophyceae	-1.435	265	8	5	11
Spirulina	Oscillatoriales	Cyanobacteria	2.954	9	137	82	229
Spondylosium	Desmidiales	Conjugatophyceae	-0.480	72	14	9	24
Staurastrum	Desmidiales	Conjugatophyceae	0.526	426	28	16	47
Staurodesmus	Desmidiales	Conjugatophyceae	-1.155	183	9	6	13
Stauroneis	Pennales	Bacillariophyceae	2.554	9	105	73	153
Staurosira	Pennales	Bacillariophyceae	1.801	39	64	36	116
Stephanodiscus	Centrales	Bacillariophyceae	1.427	236	50	29	88
Stichococcus	Prasiolales	Trebouxiophyceae	1.708	13	61	39	94
Stichogloea	Chromulinales	Chrysophyceae	-1.460	126	8	6	10
Strombomonas	Euglenales	Euglenophyceae	3.715	7	226	101	503
Surirella	Pennales	Bacillariophyceae	1.626	19	57	29	113

Syncrypta	Chromulinales	Chrysophyceae	1.195	9	43	25	75
Synechococcus	Chroococcales	Cyanobacteria	1.167	34	43	28	63
Synechocystis	Chroococcales	Cyanobacteria	0.920	10	36	21	62
Synura	Synurales	Chrysophyceae	-0.316	177	16	10	26
Tabellaria	Pennales	Bacillariophyceae	-0.790	368	12	8	17
Teilingia	Desmidiales	Conjugatophyceae	-0.715	28	12	9	18
Tetrachlorella	Chlorococcales	Chlorophyceae	0.832	21	34	20	58
Tetraëdriella	Mischococcales	Xanthophyceae	-0.604	27	13	8	21
Tetraedron	Chlorococcales	Chlorophyceae	0.476	419	27	15	50
Tetraselmis	Volvocales	Chlorophyceae	1.015	24	38	20	74
Tetrastrum	Chlorococcales	Chlorophyceae	1.100	124	41	20	82
Thalassiosira	Centrales	Bacillariophyceae	3.035	9	145	96	218
Trachelomonas	Euglenales	Euglenophyceae	1.227	274	44	26	76
Treubaria	Chlorococcales	Chlorophyceae	1.054	63	39	21	73
Tribonema	Tribonematales	Xanthophyceae	1.124	20	41	32	53
Trichormus	Nostocales	Cyanobacteria	1.248	43	45	30	68
Ulnaria	Pennales	Bacillariophyceae	0.881	392	35	21	60
Ulothrix	Ulotrichales	Ulvophyceae	1.430	6	51	34	76
Uroglena	Chromulinales	Chrysophyceae	-0.772	247	12	8	18
Urosolenia	Centrales	Bacillariophyceae	-0.799	272	12	8	17
Volvox	Volvocales	Chlorophyceae	1.032	11	39	25	60
Westella	Chlorococcales	Chlorophyceae	0.503	5	28	23	33
Willea	Chlorococcales	Chlorophyceae	-0.941	41	11	6	18
Woronichinia	Chroococcales	Cyanobacteria	0.043	223	20	13	31
Xanthidium	Desmidiales	Conjugatophyceae	-0.055	32	19	12	31
	Centrales	Bacillariophyceae	1.063	288	40	21	77
	Chlorococcales	Chlorophyceae	-0.436	429	15	9	25
	Cryptomonadales	Cryptophyceae	1.055	17	40	24	65
	Ochromonadales	Chrysophyceae	-1.772	186	6	4	9
	Oscillatoriales	Cyanobacteria	1.600	42	56	36	88

	Pennales	Bacillariophyceae	0.577	100	29	16	51
	Volvocales	Chlorophyceae	0.930	83	36	16	82
		Chlorophyceae	1.336	87	47	29	77
		Chrysophyceae	-1.468	525	8	5	12
		Cryptophyceae	1.562	47	55	32	93
		Cyanobacteria	1.455	115	51	26	101
		Dinophyceae	-1.319	315	8	5	14
		Euglenophyceae	1.689	15	60	34	105
		Xanthophyceae	0.998	15	38	26	55
Picoplankton			-1.475	414	8	5	12