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SEVENTH FRAMEWORK PROGRAMME

WISER

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

DELIVERABLE

Deliverable D3.1-1: Report on lake phytoplankton composition metrics, including a common metric approach for use in intercalibration by all GIGs

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PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
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Content

Chapter 1 Introduction

Responsible: Anne Lyche Solheim

Background

Annex V of the WFD requires that ecological status of phytoplankton in lakes should be assessed using biomass, composition and bloom metrics. In many countries of Europe, the national assessment systems for phytoplankton in lakes, however, still lack metrics for phytoplankton composition (and blooms) (Poikane 2009, Birk et al. 2010). Thus, to facilitate efficient development of WFD-compliant national assessment systems across Europe, there is an urgent need for metrics for phytoplankton composition, including common metrics that can be used as a tool for intercalibration of existing national metrics (IC guidance 2010). The new metrics should be based on Guidelines for Indicator Development given by WISER D.2.2.2 (Hering et al. 2010).

Objective

The objective of this report is to present new candidate metrics for phytoplankton composition and suggest common metrics for use in intercalibration of phytoplankton phase 2 in close dialogue with Geographical Intercalibration Groups (GIGs).

Chapter 2 WISER phytoplankton database and data analyses

Responsible: Birger Skjelbred and Jannicke Moe (contributions from Ute Mischke)

2.1. Database structure, taxa list, data content and compilation

The database structure is shown in figure 2-1 (based on the WISER common database structure, see also Moe et al. 2008). In each database, the data were organised into eight main tables (Fig. 2-1): dataset information, waterbody information, environmental waterbody information, station information, physico-chemical sample information, biology sample information, physico-chemical values (incl. pressure variables such as phosphorus) and biology values (such as biomass or abundance per taxon). Chlorophyll values were stored in the physico-chemical table, even though it represents a biological element, because it is usually measured together with chemical parameters, and it does contain any taxonomic information. Separate tables for identifying physico-chemical samples and biology samples were necessary because the biology samples did not always have corresponding physico-chemical samples from exactly the same station and date. More physico-chemical samples than biology samples were provided.

The common taxalist used for the WISER project is an update of the REBECCA code list. There are now altogether 2299 phytoplankton taxa in the updated WISER_REBECCA codelist. Overview of the taxa used in the analyses is given in Chapter 3 and Appendix 1 below.

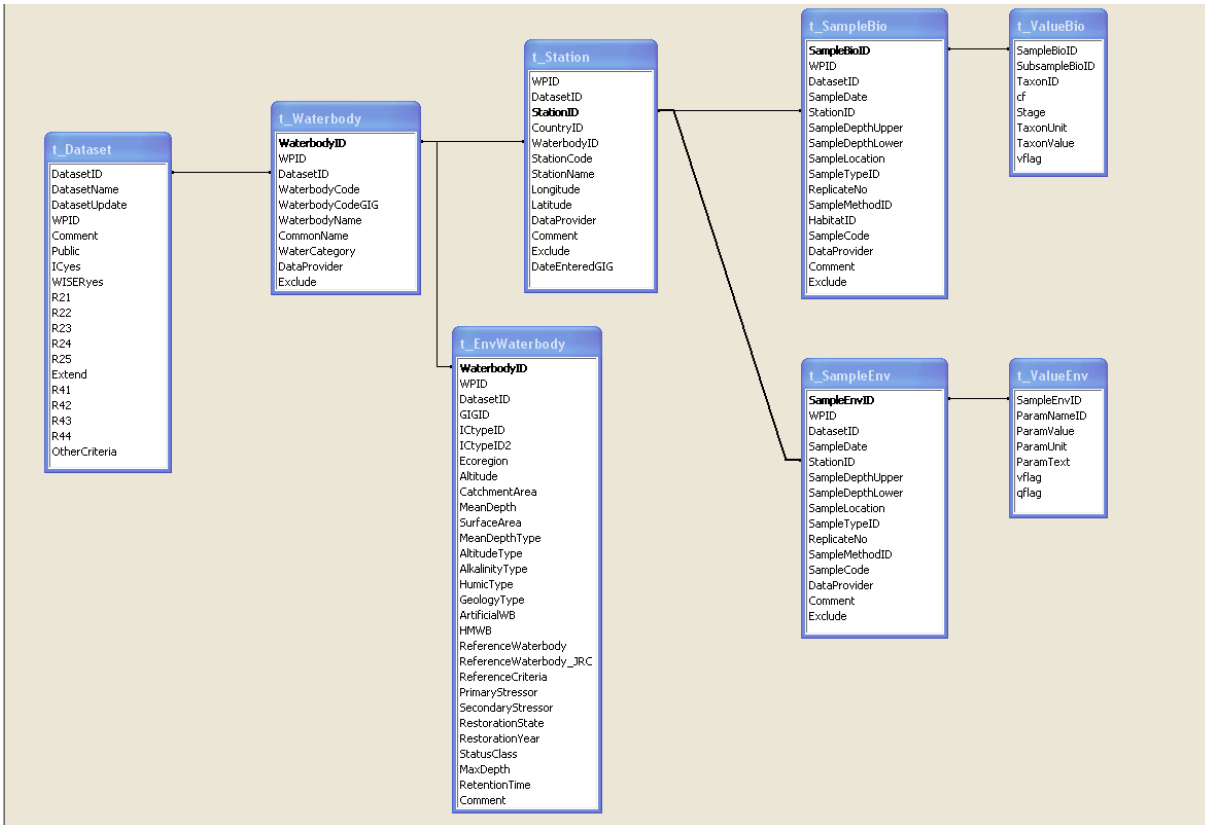

Figure 2-1. Illustration of database structure; main tables and relationships between fields within tables.

Data on phytoplankton and physico-chemical parameters were compiled and are managed by the Norwegian Institute for Water Research (NIVA). An overview of the number of lakes from 21 countries and the intercalibrated lake types (IC-types) are given in table 2-1. Lakes belonging to the same IC-type are assumed to have similar ecological reference conditions. L-CBX, L-MX and L-NX are lakes with typology data, but with no defined IC-type yet; L-CBU, L-MU, and L-NU are lakes with missing typology data (for one or more typology factors, e.g. colour, alkalinity etc.).

1759 of the 6925 waterbodies have both biology and physico-chemical data, 1892 waterbodies have biology data only and 4685 waterbodies have chlorophyll data (table 2-2). The 10633 stations in the database contain data from 123077 physico-chemical samples and 16576 biology samples (table 2-3).

Data were provided upon request by key contacts in the Geographical Intercalibration Groups (GIGs): Central-Baltic GIG (CB-GIG), Mediterranean GIG (M-GIG), Northern GIG (N-GIG) and Eastern Continental GIG (EC-GIG) in addition to internal data from the REBECCA database. No data from Alpine GIG has been provided. New data from the WISER field campaign in 2009 are not yet included.

Data were added and adjusted to the main databases by a series of so-called append queries and update queries.

Table 2-1. An overview of the number of lakes from 21 countries and the IC-types.

Ictype / Country	FR	BE	CY	DE	DK	EE	ES	FI	GR	HU	IE	IT	LT	LV	NL	NO	PL	PT	RO	SE	UK	**Sum**
L-CB1		6		163	25	32					32		27	33	17		45				34	**41**
L-CB2		6		57	58	22					13		13	23	33		5				46	**27**
L-CB3	3				21	12					1			9							2	**4**
L-CBU				3	4								1	1								
L-CBX																					3	
L-EC1										24												**2**
L-EC2										6												
L-M1							1															
L-M5							4											6				**1**
L-M7							9		1									11	5			**2**
L-M8	4		7				39					2						1	5			**5**
L-MU	2						90					13										**10**
L-MX							25															**2**
L-N1								113								45				1	11	**17**
L-N10								2								1						
L-N11								400												9	2	**41**
L-N2a								248			4					52				22	27	**35**
L-N2b								7								76					24	**10**
L-N3																3						
L-N3a								462			4					26				64	1	**55**
L-N3b								191												19	1	**21**
L-N5								17								39				20	7	**8**
L-N6a								49								21				56	4	**13**
L-N6b								7								7				7		**2**
L-N7																1				8		
L-N8																6						
L-N8a								203			2					13				16	7	**24**
L-N8b								25												5		**3**
L-N9								1								24				3	6	**3**
L-NU								2227			4					68				7	5	**231**
L-NX								1006			2					149				68	15	**124**
Sum	**9**	**12**	**7**	**223**	**108**	**66**	**168**	**4958**	**1**	**30**	**62**	**15**	**41**	**66**	**50**	**531**	**50**	**18**	**10**	**305**	**195**	**692**

Table 2-2. Left panel: An overview of lakes from the providing countries with physico-chemical and biology data, biology data only and chlorophyll data only. Right panel: An overview of samples with chlorophyll and biology data, total phosphorus and chlorophyll.

	Waterbodies			Samples		
Country	Physico-chemical and biology	Biology	Chlorophyll	Biology and chlorophyll	Total P	Chlorophyll
BE	11	11	11	243	204	243
CY	7	7	0	0	35	0
DE	223	223	223	2021	1981	2020
DK	1	108	1	19	19	19
EE	66	66	66	152	129	153
ES	162	162	43	612	1110	151
FI	80	80	3131	1137	52424	54559
FR	9	9	3	12	56	12
GR	1	1	0	0	0	0
HU	29	29	29	117	143	150
IE	54	54	62	141	1081	1129
IT	15	15	0	0	202	0
LT	41	41	41	216	211	220
LV	65	65	64	176	188	177
NL	50	50	50	257	598	509
NO	516	516	474	3597	5812	3699
PL	50	50	50	211	214	214
PT	16	16	0	0	64	0
RO	10	10	0	0	135	0
SE	209	218	242	1170	6209	3723
UK	144	161	195	380	4040	4770
Sum	**1759**	**1892**	**4685**	**10461**	**74855**	**71748**

Table 2-3. An overview of stations in the database and samples with biology and physico-chemical parameters from the providing countries.

Country	Station	Biology samples	Physico-chemical samples
BE	12	371	365
CY	7	36	36
DE	243	2045	2063
DK	108	715	19
EE	66	154	153
ES	169	1172	11214
FI	8418	1329	83992
FR	9	33	457
GR	1	6	5
HU	44	120	146
IE	187	90	1334
IT	15	162	201
LT	41	217	230
LV	70	193	192
NL	52	343	658
NO	540	6945	8716
PL	72	211	426
PT	18	64	64
RO	10	162	139
SE	305	1428	6326
UK	246	780	6341
Sum	**10633**	**16576**	**123077**

2.2. Data Quality Assurance (QA):

While compiling the data into the WP3.1 database queries were run to check and correct data. Some problems were caused by erroneous unit for total P and mixing of the coordinates for longitude and latitude. In some cases, data providers were contacted to check and correct data.

To exclude duplicates and synonyms in the WISER taxa list, author of description were added and each taxon checked against literature and databases (www.algaebase.org/, www.algaterra.org/and www.eol.org/).

Chapter 3: Phytoplankton trophic index for taxonomic composition

Responsible: Geoff Phillips, contributions by Anne Lyche Solheim, Tom Andersen, Birger Skjelbred, Laurence Carvalho, Ute Mischke

3.1. Introduction

Taxonomic composition is one of the phytoplankton community characteristics needed to describe the ecological status of lake phytoplankton (WFD Annex V). Existing national metrics used to quantify taxonomic composition range from simple metrics, such as proportions of Cyanobacteria or Chrysophytes, to more sophisticated trophic indices based on trophic scores of taxa along the eutrophication gradient (e.g. Salamaso et al. 2006, Mischke et al. 2008, Ptacnik et al. 2009).

The objective of this chapter is to present a new pan-European phytoplankton taxonomic index (PTI) that can be used as a common metric for several GIGs and/or as a national metric for the 2nd phase of intercalibration for those countries that still lack such a metric in their national assessment system. If successfully applied, this index may contribute to the harmonisation of assessment systems across Europe in the years to come.

As for many existing national metrics, the new PTI index is developed from trophic scores of phytoplankton taxa along the eutrophication gradient. The index is based on the data presented in chapter 2 above. The chapter first describes the methods used to develop the index, including the data cleaning procedure, the statistical methods used, the formula used to calculate the index, the calculation of EQRs and the designation of sensitive and tolerant taxa that can be used to set class boundaries. The results includes the indicator values or taxa optima for the different taxa, the designation of sensitive and tolerant taxa, the relationship of the metric with pressure for lakes in various GIGs, lake types and countries, the assessment of reference conditions (reference value), and different ways to set class boundaries, showing examples for one lake type (moderate alkalinity clearwater lakes). The latter should be expanded to include all the major lake types and be further discussed in the GIGs over the coming months.

3.2. Methods

3.2.1. Dataset used

Data were extracted from the WISER Access database which contained data from 21 European countries, these data were compiled from previous data sets (REBECCA), but were updated with more recent data provided by GIGs which will be used for the 2nd round of intercalibration (see chapter 2). Two data extracts were made, the first was used to create the PTI metric and the second, larger extract was used to test the metric and illustrate how it can be used for intercalibration.

To develop the metric sample records of phytoplankton biovolume were extracted for the late summer period, defined as July to September. All samples, except those in Spitsbergen, with matching

environmental data were extracted and the mean late summer biovolume for each taxa was calculated. To reduce differences caused by different taxonomic traditions and counter ability all taxa were aggregated at genus level or higher taxonomic categories where genus was not available. The modified REBECCA code was used to achieve this. The greatest taxonomic differences were expected from samples in central Europe and a considerable amount of time was spent harmonising taxonomic names for data collected from the Central Baltic GIG (CBGIG). Ute Mischke, IGB/FVB was responsible for this taxonomic harmonisation in dialogue with Birger Skjelbred at NIVA. Samples and stations in each water body were combined and only the latest year of matching biological and environmental data were used. Thus the data set used to develop the metric was based on a single mean late summer value for each water body.

The biological data were converted to a proportion of biomass, summing to a value of 1. Data were initially extracted using the data extraction tool (Dudley 2010), this ensured a consistent approach to averaging and matching environmental and biological data.

3.2.2. Description of data cleaning procedure:

Taxa recorded in less than 3 countries, or from less than 10 samples were removed from the analysis, this reduced the taxa list from 395 entries to 216 (Appendix 1). The environmental data were screened and water bodies with total phosphorus concentrations outside of the range of 1-1000µg l^{-1} were omitted. A plot of total phosphorus v chlorophyll a revealed that the resulting data contained very few outliers (fig 1) and no further water bodies were excluded from the analysis.

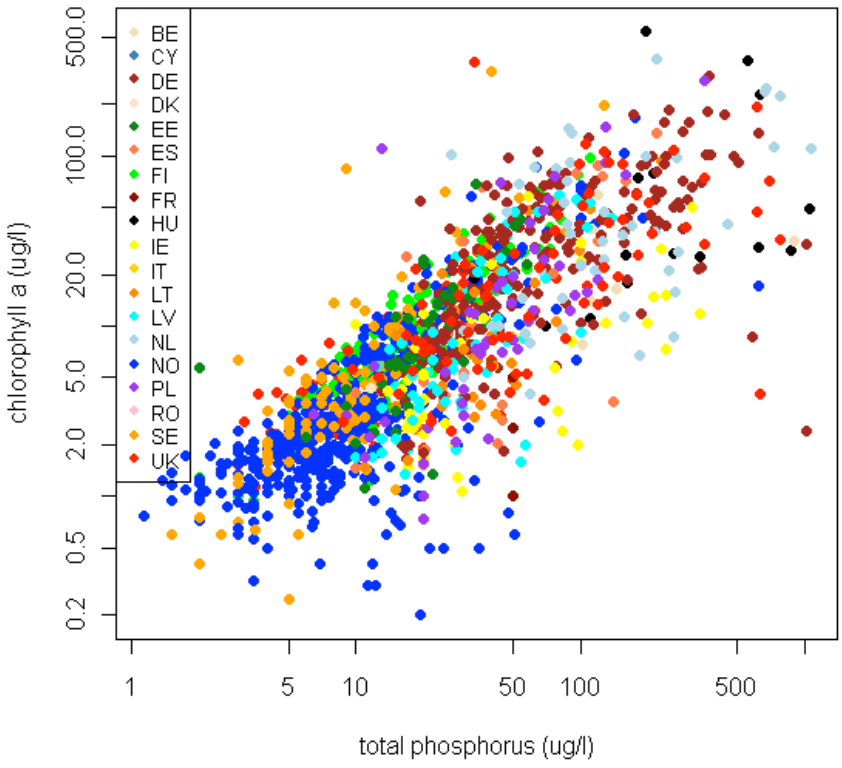

Fig 1 Plot of mean late summer (July – September) total phosphorus and chlorophyll a from lakes used to develop and validate PTI metric, each point represents a single lake water body year.

This produced a data set of 1656 lakes from 19 countries. Initial analysis demonstrated that lakes containing more than 50% biomass of *Gonyostomum* were outliers in ordinations and these 29 lakes were also excluded from analysis. For Northern and Central European lakes reference lakes were checked with GIG contacts. During this process it was noted that different data sources had resulted in duplicate lakes being entered into the database, these were identified and removed during data extraction. A summary of the final data set is provided in table 1 and locations are shown in fig 2.

Table 1 Number of lake water bodies by country and type, used to develop and test PTI metric

	Total	EC-1	EC-2	L-CB1	L-CB2	L-CB3	L-CBU	L-MU	L-N1	L-N10	L-N11	L-N2a	L-N2b	L-N3a	L-N3b	L-N5	L-N6a	L-N6b	L-N7	L-N8	L-N8a	L-N8b	L-N9	L-NU	L-NX
BE	7	0	0	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CY	7	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DE	223	0	0	163	57	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DK	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EE	54	0	0	27	19	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ES	150	0	0	0	0	0	0	150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FI	152	0	0	0	0	0	0	0	14	0	1	22	2	45	7	2	9	0	0	0	18	2	1	4	25
FR	9	0	0	0	0	3	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HU	15	13	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IE	52	0	0	30	13	1	0	0	0	0	0	2	0	2	0	0	0	0	0	0	1	0	0	2	1
IT	14	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LT	39	0	0	25	13	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LV	61	0	0	32	21	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NL	48	0	0	16	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NO	493	0	0	0	0	0	0	0	39	1	0	47	76	26	0	39	20	7	1	3	13	0	24	52	145
PL	49	0	0	45	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RO	10	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SE	88	0	0	0	0	0	0	0	0	0	0	6	0	19	3	4	19	0	3	0	6	1	1	6	20
UK	155	0	0	24	40	2	0	0	11	0	2	19	20	1	0	7	4	0	0	0	7	0	6	2	10
Total	1627	13	2	367	201	23	4	187	64	1	3	96	98	93	10	52	52	7	4	3	45	3	32	66	201

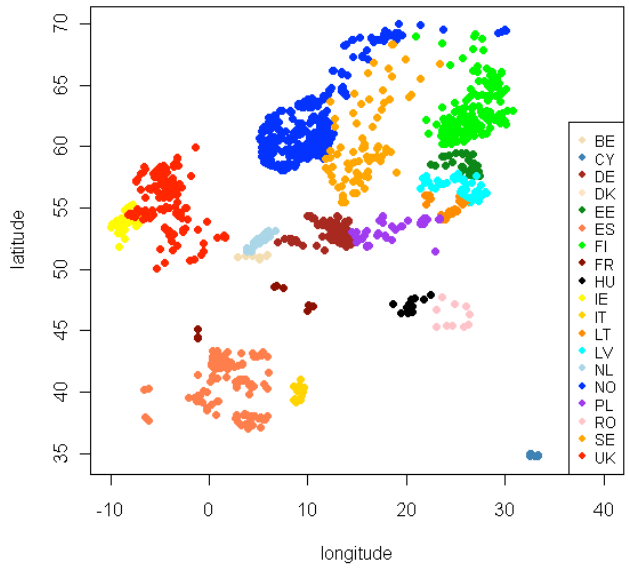

Fig 2. Location of lakes used for development of PTI metric

3.2.3. Statistical methods

Statistical analysis was carried out using R (R Development Core Team 2009) ordinations were done with the vegan package (Oksanen *et al.* 2010). An unconstrained non-metric multidimensional scaling was carried out using the metaMDS function on the full extracted data set and on reference lakes. Relationships with the key environmental variables, total phosphorus, total nitrogen, alkalinity, colour and mean depth were carried out using the envfit function with significance tested using random permutation tests. Metric development was based on Canonical Correspondence Analysis using the vegan cca function. All environmental data were log transformed, biological data were square root transformed. For CCA analysis 70% of the data were allocated to a training data set, the remainder retained for validation. Data were allocated randomly by Country and GIG types to ensure that both data sets reflected the general conditions. A final check was carried out to ensure all taxa were represented in each data set. Relationships between biological metrics were explored using GAM models (Wood 2006) and quantile regression described by Koenker (2010).

3.2.4. Definition and calculation of the Phytoplankton Trophic index (PTI)

The proposed metric, Phytoplankton Trophic Index (PTI) was derived from a CCA ordination constrained by a single environmental variable, total phosphorus. This variable was found to be the most significantly related to the 1st axis of all the unconstrained ordinations tested and was selected as it reflects the main pressure of concern in lake management, eutrophication. 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, in this case total phosphorus (Braak and

Looman 1986). Sites and taxa are then arranged along the 1st ordination axis with increasing or decreasing relationship to phosphorus. The sign of the ordination axis is arbitrary, but for the purpose of metric development negative scores reflect lower than the average phosphorus and positive scores higher than the average phosphorus, ordination scores were transformed by multiplication by -1 as necessary to achieve this.

Ordinations were carried out on the following groups of lakes to determine five sets of taxon optima, corresponding to indicator values, for each taxon included in the cleaned dataset:

1. All lakes in the data set
2. Clear water lakes from Northern GIG (NGIG), and clear water, low and moderate alkalinity lakes from Central Baltic GIG (CBGIG)
3. Humic water lakes from NGIG, with humic water low and moderate alkalinity lakes from CB GIG
4. All lakes from CBGIG
5. All lakes from Mediterranean GIG (MGIG)

Taxon optima were obtained from the CCA taxon axis 1 scores from each of these ordinations, values are shown in Appendix table A1, and are equivalent to the TP concentration for the mean occurrence for each taxon within each of the five groups of lakes.

Site scores produced by CCA represent the weighted average of taxon optima present in each waterbody, the weighting being the square root transformed proportional biomass. An initial validation of the ordinations was done by examining the relationship between the site scores and total phosphorus and chlorophyll a using lakes from the validation data set. All ordinations were found to have significant relationships with both total phosphorus and chlorophyll (table 2) and subsequent analysis was carried out by calculating the PTI for all samples in the database. Although square root transformations were used to reduce the influence of dominant taxa in the ordinations (Ref?) the environmental status of lakes is better reflected by the dominant taxa and thus the PTI site scores were calculated using an untransformed proportional biomass as a weight. The method uses the same approach as the Trophic Diatom Index (Kelly *et al.* 2008) using equation 1 below

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

Where:
a_j = proportion of jth taxon in the sample
s_j= optimum of jth taxon in the sample

Table 2 Proportion of variance explained by 1st ordination axis of CCA carried out on training sub-set of lakes from different GIGs

GIG	Proportion variance explained by 1st

	ordination axis
All lakes (global data)	0.27
NGIG clear and low/moderate alkalinity clear lakes CBGIG	0.30
NGIG humic and low/moderate alkalinity humic lakes CBGIG	0.26
CBGIG lakes	0.12
MGIG lakes	0.16

Access queries have been written which allow each set of optima to be applied to all samples collected from July to September in the WISER database. The resulting PTI values have been combined by date (to cover multiple sample depths), month, station and water body for each year of data available. Environmental data have also been extracted and combined in a similar way and then matched by year and water body to provide a summary table for analysis. An additional table has been produced which combines years to provide an average value for each waterbody. Both tables also contain classifications for chlorophyll based on the phase 1 intercalibration type boundaries, where ranges were agreed, and the mean boundary values have been used. These summary data can be made available to GIGs and could form the basis of the intercalibration work using a common metric. Some relationships have been explored and are presented below.

3.2.5. Calculation of EQR for the PTI

The PTI metric can be used directly as a common metric, allowing status of water bodies to be compared between countries. However, if the metric were to be used directly to determine water body status it would need to be converted to an EQR with a scale from 0 to 1. As the PTI metric is a trophic index with site scores that can range from -3 to +3, with high scores implying higher nutrient conditions the metric needs to be converted. This is done using equation 2 below

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

Where:

PTI_{Obs} = Sample PTI

PTI_{Max} = Maximum PTI score for type, the lower (worst) anchor.

PTI_{Ref} = Expected or reference PTI for type, the upper (best) anchor

PTI_{Ref} values still need to be determined for each lake type used (an example for a single lake type is shown below to illustrate how the metric might be used).

The value of PTI_{Max} will also need to be determined for each lake type used, it needs to be fixed and must be at least as high as the worst likely sample for a type. The value of PTI_{Max} will determine the range of the EQR scores and needs to be appropriate for the lake type.

3.2.6. Identification of Sensitive and Tolerant taxa for PTI boundary setting

The relative proportions of taxa known to be sensitive or tolerant of nutrient enrichment may be a useful approach to boundary setting for the water framework directive (Phillips *et al.* 2003) and a number of national biological classification systems propose the use of sensitive and tolerant taxa (Schaumburg *et al.* 2004). To facilitate boundary setting taxa have thus been split into different response groups using their PTI optima as a guide. However the optima are essentially a continua, referenced to the nutrient pressure gradient and thus the placement of PTI optima boundaries of sensitivity is essentially arbitrary. None the less the approach is potentially useful and two approaches are being trialled. Expert judgement has been used to identify categories of sensitivity (very sensitive, sensitive, tolerant and very tolerant) a similar approach to that used by (Ptacnik *et al.* 2008). A problem with this approach is that it would be difficult to apply to each lake type, and at the type level the data sets are unlikely to cover the full range of pressure. To overcome this an approach developed by Willby et al (2010) has been used. Type specific ordinations, constrained by the site PTI score derived from the global data set were carried out. This approach reduces the bias of the smaller data sets, by recalibrating the taxa along the full pressure-response gradient. For each lake type taxa were split into two groups, Sensitive and Tolerant around the zero point of the type specific ordination axis. It would be possible to further sub-divide each of these groups, but this has not yet been done. If the sample size and environmental gradients were similar in each of the lake types this approach might be sufficient to identify the appropriate PTI optima from the full (global or GIG specific) data set, however this is not the case. To standardise the PTI optima which delimit the taxon response groups (very sensitive, sensitive, tolerant and very tolerant taxa) the median global PTI optima for each of these (type specific taxon response groups) was calculated and regressed against the $\log_{10}$ median type specific morpho-edaphic index (MEI), an index which has been shown to reflect the natural trophic gradient(Cardoso *et al.* 2007). MEI was calculated as alkalinity (meq/l)/mean depth (m). The resulting regressions were used to predict the global PTI optima which represent the mid-point of the type specific Sensitive and Tolerant Taxon groups. The average of these two points was used to identify the boundary between Sensitive and Tolerant, the mid points of each were used as the boundary between Very Sensitive and Very Tolerant.

3.3. Results

3.3.1. Exploratory analyses of dataset

The 1656 lakes, from 19 countries cover the full range of European lakes. The majority are relatively small (75% < 7km^2 surface area), shallow (75% <14m mean depth) and found in the lowlands (75% < 250m altitude). However, it is notable that lakes in the data set from Finland are larger, with some very large lakes (>400 km^2) (fig 3a), that some of those from Norway are much deeper (>50m mean depth) and those from Romania from a much higher altitude (>400m). They span a range of alkalinities, with lakes from Hungary having much higher values which may make them less comparable to those from other countries.

The GIG typology aims to categorise lakes into broad types that are directly comparable. The range of total phosphorus concentrations, the most convenient and available pressure variable differs between lake types (fig 4) with the lowest values in the NGIG low alkalinity (<0.2 mEq l^{-1}) deep or mid to high altitude lakes and highest in the Eastern Continental GIG, with the Central Baltic GIG high alkalinity shallow and very shallow lakes having slightly lower values. The range of total phosphorus values in the moderate alkalinity clear and humic water lakes in both Northern and Central Baltic GIGs are similar, and intermediate between those of the low and high alkalinity lakes. Log transformed Alkalinity and total phosphorus are significantly correlated (Pearson Corr = 0.59 $p<0.001$), as was the MEI (Pearson Corr = 0.67 $p<0.001$). This emphasises the difficulties of separating natural from anthropogenic elevation of total phosphorus across the alkalinity gradient and emphasises the need for type specific approaches.

The biological data was initially examined using Non Metric Multi Dimensional Scaling, a simple and unconstrained ordination which uses a Bray Curtis dissimilarity matrix to project onto a 2 dimensional ordination. The projection is non-linear (see Shepard plot, fig 1a in Appendix) and the ordinations were unable to reach convergent solutions even after 40 iterations. Ordinations were carried out on both the full data set and on those lakes marked as reference sites. The best solution found for the full data set had a stress value 26.4 and for the reference data set 20.2. However the ordination for all lakes (fig 5) shows a clear gradation of phytoplankton communities along the 1st ordination axis which is significantly related to pressure gradients of total phosphorus, chlorophyll and total nitrogen and to typological gradients of altitude and alkalinity . Mean depth was not significantly related to axis 1, but an examination of the GAM contour plot (fig 5a) clearly shows a gradation of mean depth decreasing along the same gradient of alkalinity. The deeper lakes from Norway can be clearly seen. Variation on the 2nd axis is less easy to explain, significant vectors that were orientated close to the 2nd ordination axis were colour and lake area. The contour plots for these variables, (fig 5c) clearly shows that the cluster of Finish lakes are associated with larger surface area and higher colour. This suggests that further consideration of both lake depth and lake area may be needed in the current GIG typology for NGIG. As expected reference lakes are not evenly spread across the ordination (fig 5d), reference lakes are more often identified in the Northern parts of Europe.

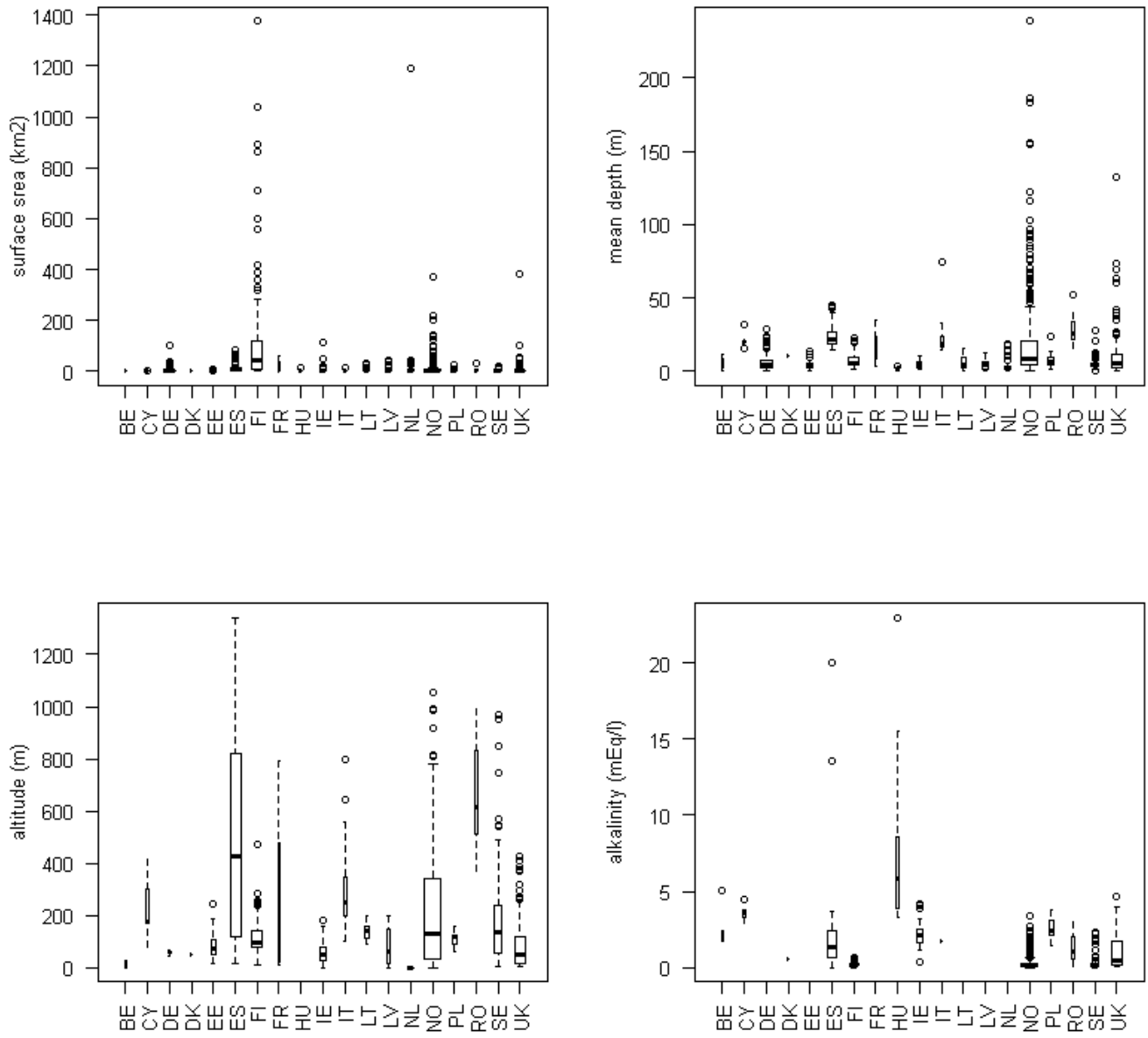

Fig 3Distribution of typological variables, surface area, mean depth, altitude and alkalinity

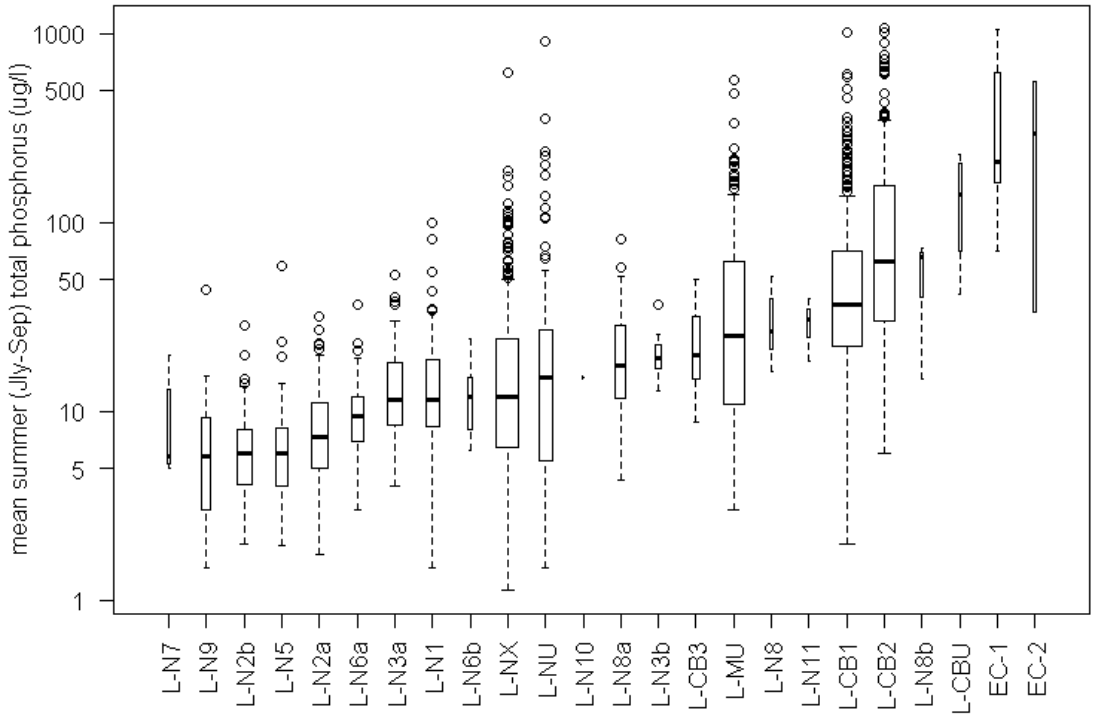

Fig 4. Range of mean late summer total phosphorus concentration by lake type. Boxes represent upper and lower quartiles, whiskers are either the maximum value or 1.5 x the interquartile range. Types ending in U or X are lakes that fall outside of the current GIG type definitions or there are insufficient information to place the lake into a GIG type.

There are significant relationships between the axis and the pressure variables total nitrogen and total phosphorus and the response variable chlorophyll a (fig 6a-c). There is an indication that the response with phosphorus is reduced at concentrations greater than 50ug/l, but this should be interpreted with care due the non linearity of the NMDS axis and taxon dissimilarity. As for phosphorus the range of communities is related to GIG type. The order of the types in fig 6d, might give some indication of where GIG types can be combined or divided. What should be noted is the relative low range of axis 1 scores in the CBGIG, despite the large number of countries and large geographic range of the GIG. This does not support previous suggestions of the need to split this GIG and is in marked contrast to the clear clustering of lakes from the Nordic countries (blue = NO, orange = S, green = FI) . However it can also be noted that LCB3 lakes are positioned close to LN1 and LN8 lakes from NGIG. These are the moderate alkalinity lakes, many from Finland and further consideration should be given to making a broader comparison across the CB and NGIGs for these lake types, particularly as many of the LCB3 lakes are humic, a factor not recognised in the CBGIG typology.

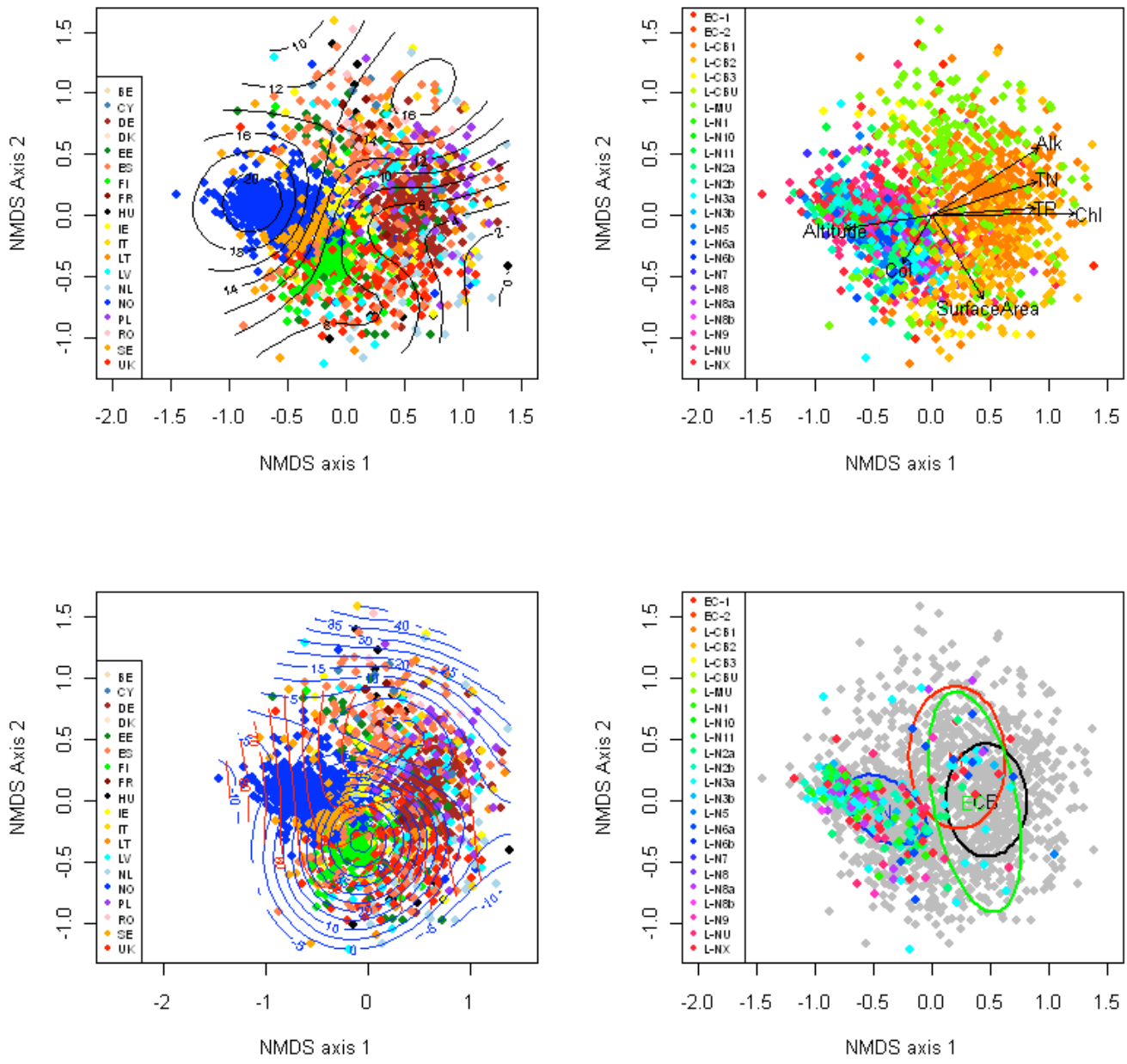

Fig 5 Non Metric Multidimensional Scaling ordination for all water bodies. a) by country, contours show mean depth b) by GIG type, vectors show significant (p <0.001) correlations with environmental variables , alkalinity, total phosphorus, total nitrogen, chlorophyll a, surface area and altitude; c)with an overlay of contours of lake surface area (blue) and colour (red), d) location of reference lakes by GIG type, ellipses show location of GIGs.

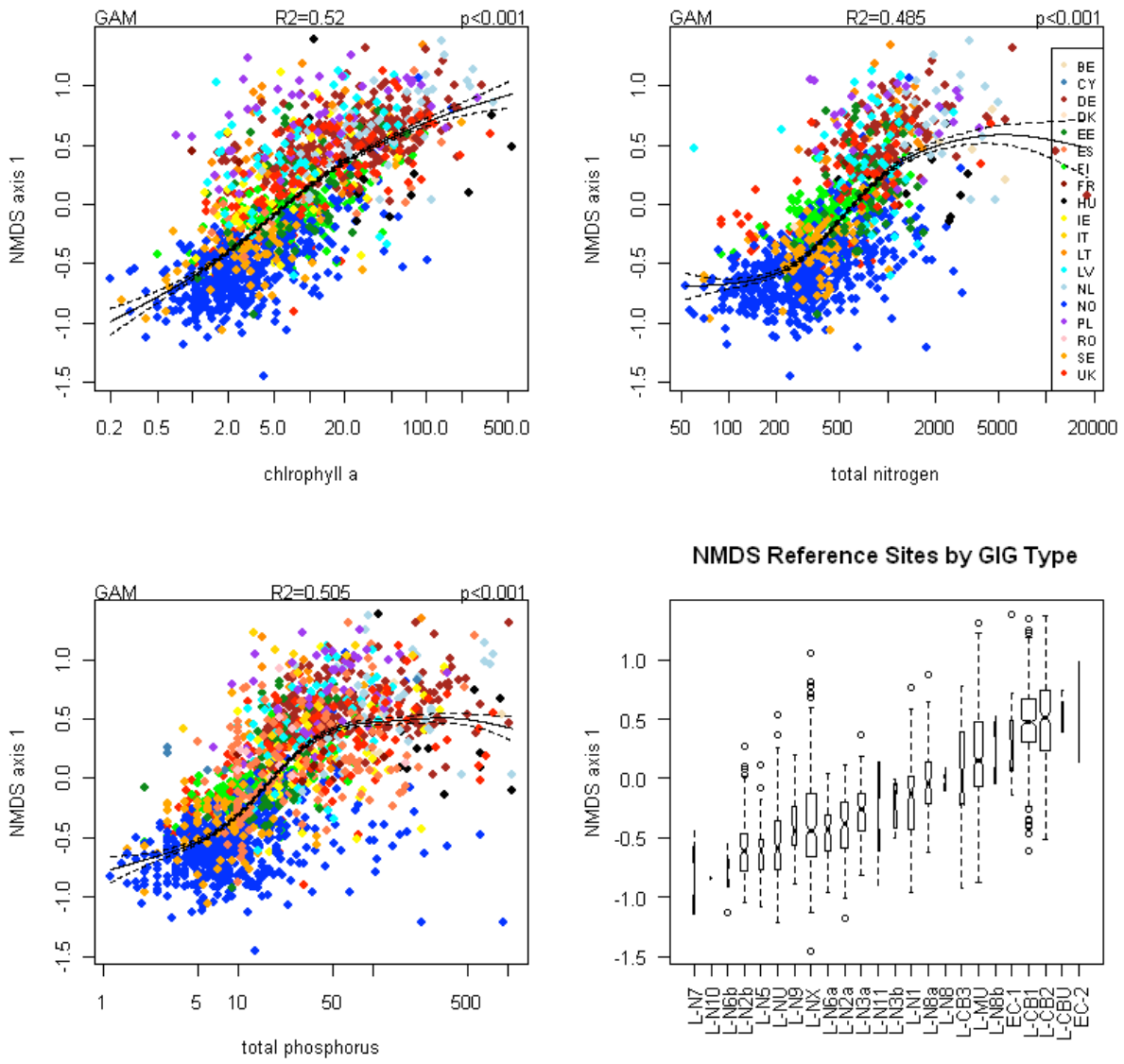

Fig 6 a-c) Relationship between NMDS ordination axis 1 site scores and mean late summer chlorophyll a, total nitrogen and total phosphorus. d) range of MDS ordination axis 1 site scores by GIG type.

More insight should be available from the ordination of reference lakes, as these are not influenced by anthropogenic pressures. A very similar pattern to that shown in the all lake ordinations is seen for reference lakes (fig 7). The overlapping, but broad distribution of the CBGIG contrasts to the much tighter clusters of the NGIG. Ranges of the pressure and response variables are shown in fig 8 and follow what would be expected, with higher values found in the high alkalinity lakes of CBGIG. The range of values suggests that a primary factor used by countries for the identification of reference sites was the chlorophyll concentration, and it is clear that there are some reference lakes with relatively high total phosphorus concentrations, particularly in LCB1 lakes.

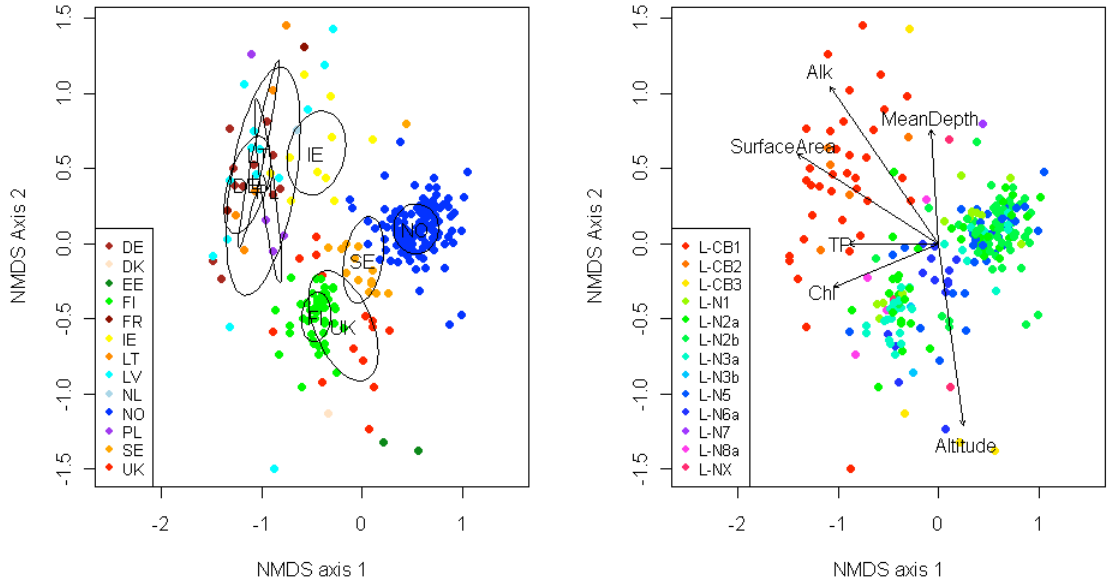

Fig 7 Non Metric Multidimensional Scaling ordination for reference water bodies. a) by country, b) by GIG type, vectors show correlations with environmental variables , alkalinity (p<0.001), total phosphorus (p<0.01), chlorophyll a (p<0.001), surface area(p<0.001), mean depth(p<0.001), and altitude(p<0.001).

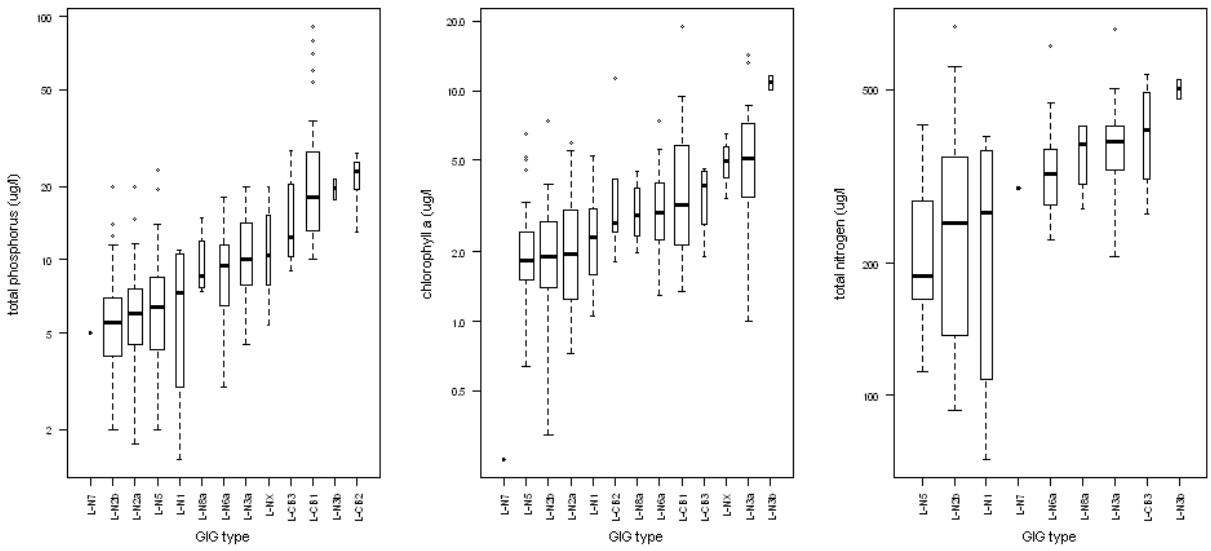

Fig 8 range of total phosphorus, chlorophyll a and total nitrogen for reference sites by GIG type

3.3.2. PTI Taxa optima (indicator values)

Based on this initial evaluation of the biological data it was clear that nutrients and the co-varying environmental factors such as alkalinity, mean depth and altitude strongly structure the phytoplankton community. Tests with a variety of environmental variables and different types of ordination were carried out (DCA, RDA, CCA), but it was decided that an ordination constrained by log total phosphorus was the best approach for producing a phytoplankton trophic index (PTI). With a single constraint the ordination reduces to a simple weighted average, with the zero point of the 1st ordination axis representing the average total phosphorus concentration of the data set. Thus the position of taxa and sites along this axis should be a good indicator of trophic status, as used by Ptacnik *et al* (2009). Ordinations were carried out on a training sub-set of all lakes and five major groups of lakes. The results from each group were similar and as would be expected from such large data sets the variability explained by the constrained axis (1) ranged from 12% to 30% (table 2). The lowest values were from CBGIG and MGIG, where the lakes have the shortest environmental gradients, being dominated by high alkalinity and most impacted lakes, the highest values were from the NGIG clear water lakes. The ordination of all lakes, however explained almost as much variability (27%) as the NGIG clear water lakes and the axis 1 site scores for this ordination were shown to be significantly related to both total phosphorus and chlorophyll a in a separate validation data set (fig 9).

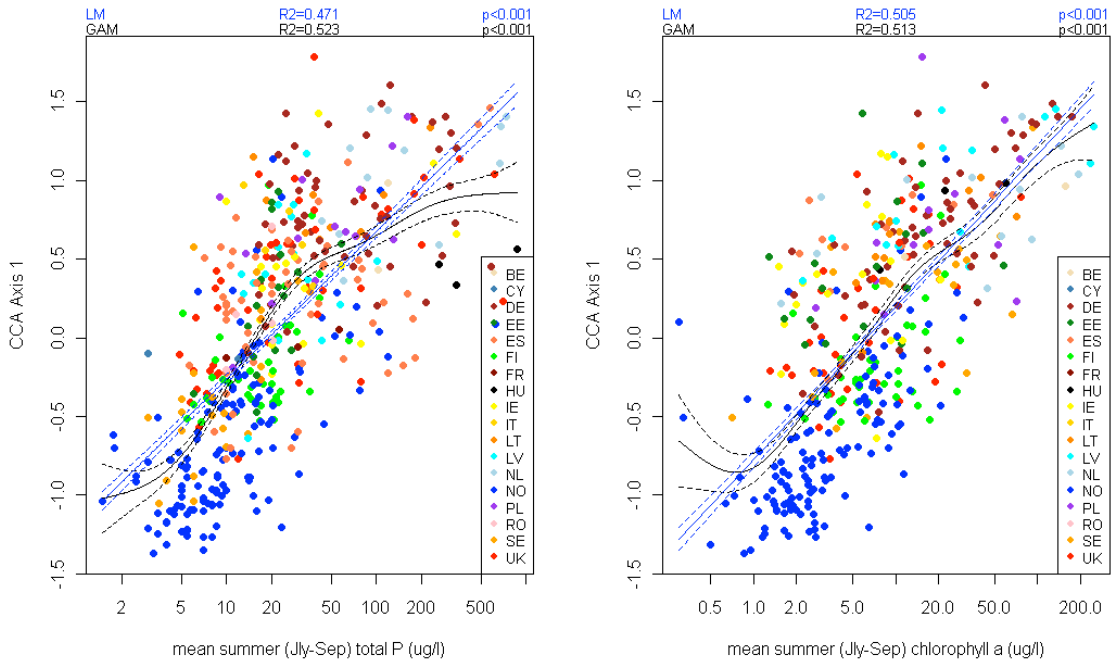

Fig 9 Relationship between PTI site scores and mean summer total phosphorus and chlorophyll a concentrations (sqrt transformed weights applied when calculating site scores) using separate validation data set. Points represent summer mean values from a single lake year.

With a weighted average approach the smallest scatter would be expected close to the 0 value of the CCA axis score (PTI). There is some evidence of this, but in general there tends to be more scatter at higher concentrations of phosphorus and chlorophyll. The relationship is slightly better with chlorophyll than total phosphorus, but both are highly significant ($p<0.001$). A GAM model was used to detect non-

linearity, there is some evidence for a flattening of the response at moderate total phosphorus levels, but the confidence intervals of the fit widen at this point due the paucity of data and increased scatter. In general a linear fit seems appropriate.

PTI optima for all taxa from each of these ordinations are shown in table A1 of the appendix. An initial examination of the relationships between the different sites scores derived from the different global and type specific optima suggests that the GIG specific optima produce slightly better relationships. However, these are not thought to be significantly different and while type specific differences in reference conditions are clearly expected, the taxa optima are dependent on the environmental gradients available to the CCA model. For this reason the all lake (global) optima are thought to be more representative and more attention has been given to the PTI values they produce in this report.

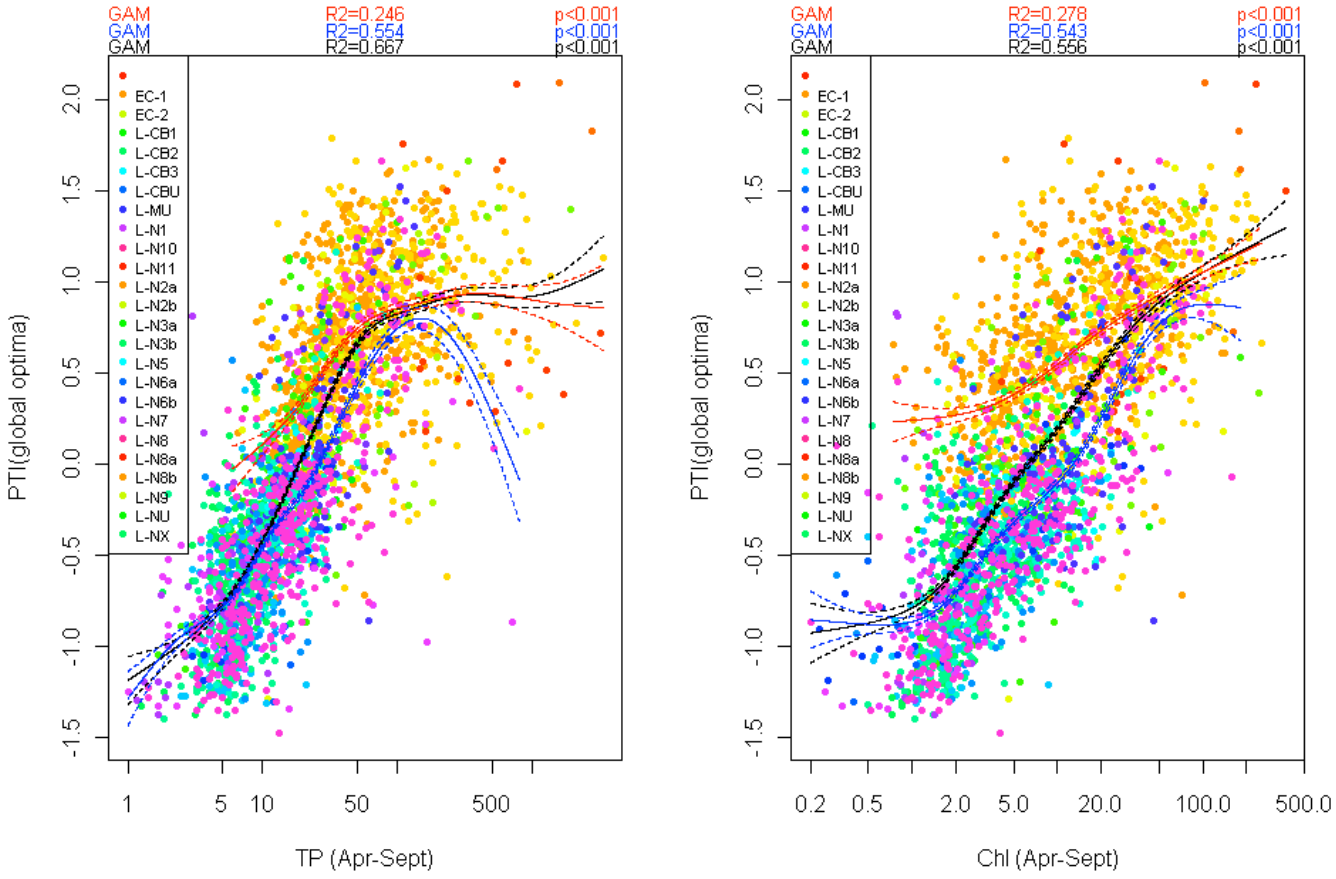

Fig 10 Relationship between PTI site scores (derived from global all lake optima) and growing season total phosphorus and chlorophyll a concentration. Lines are GAM models, black = all lakes, blue = NGIG, red=CBGIG. Points represent lake years and are coloured by GIG type.

The relationship between PTI and mean growing season total phosphorus and chlorophyll a for all lake years, calculated from the average of all samples collected from a lake during the late summer, are shown in figs 10-12. GAM models were fitted to the data to visually identify non-linearity and categorical variables for GIG, Alkalinity type and Colour type were also fitted. This demonstrated that there were

significant GIG, Alkalinity type and Colour effects and specific GAM models were fitted and plotted to each sub-category to illustrate the differences. For GIG specific differences, CBGIG lakes had significantly higher PTI values than NGIG, but MGIG lakes were not significantly different from the average of the other 2 GIGs (Fig 10), there were too few lakes to check ECGIG lakes. These GIG specific effects may be partly explained by the shorter alkalinity/nutrient gradients that the NGIG and CBGIG cover and to explore this, the effect of alkalinity type was also tested (Fig 11). High alkalinity lakes had higher PTI optima than moderate alkalinity, which was higher than low alkalinity. Colour was also a significant factor (Fig 12) with poly-humic lakes having higher optima than clear water lakes. There has not been time to consider interaction effects of factors and a more careful analysis is needed before firm conclusions can be drawn. The approach however illustrates how the PTI can be used and the analysis needed to identify typological differences.

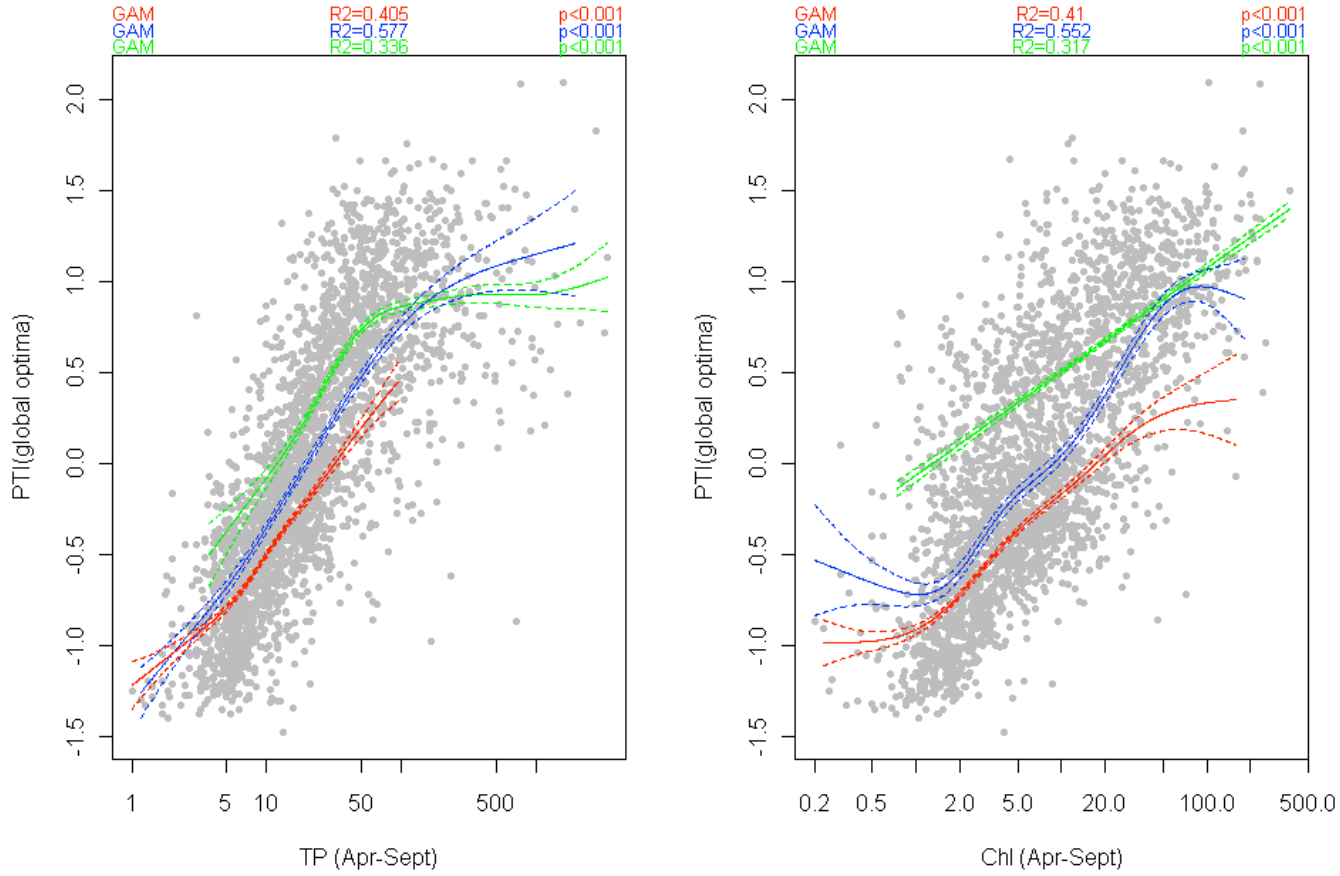

Fig 11 Relationship between PTI site scores (derived from global all lake optima) and growing season total phosphorus and chlorophyll a concentration, showing alkalinity type differences. Lines are GAM models, green = high alkalinity, blue = moderate alkalinity, red=low alkalinity. Points represent lake years

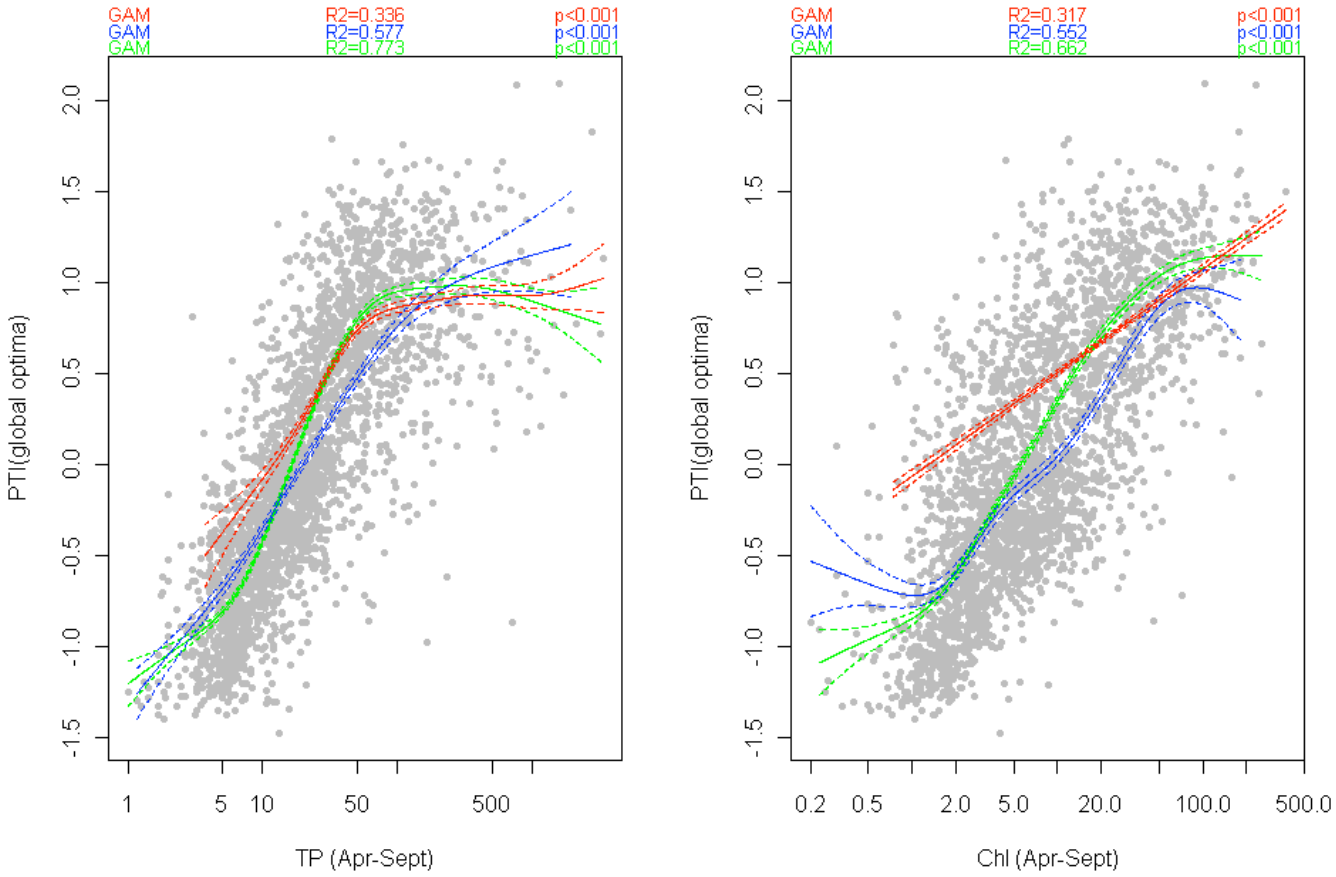

Fig 12 Relationship between PTI site scores (derived from global all lake optima) and growing season total phosphorus and chlorophyll a concentration, showing humic type differences. Lines are GAM models, green = clear, blue = moderate colour (humic), red=high colour (polyhumic). Points represent lake years

3.3.3. Sensitive and Tolerant Taxa

A series of types specific CCA ordinations, constrained by the PTI site scores (derived from the global all lake PTI optima) were run to determine type specific sensitivity of taxa according to the ranking of their type specific PTI optima. Taxa were ordered by their type specific axis 1 scores and split into Sensitive and Tolerant groups around the zero point on axis 1. The intention of splitting groups in this way was to identify potential boundaries for the site PTI scores. These could use any of the PTI optima (eg all lakes, or NGIG clear water etc) and to facilitate this the appropriate PTI optima for each taxa were identified. To allow for differences in the length of the environmental gradients of the type specific data sets the resulting PTI boundaries between sensitive and tolerant taxa were modelled using the median type specific PTI value of Sensitive and Tolerant taxa, against the median type specific MEI value. Although in theory the same approach could be used with the GIG specific PTI optima, the environmental gradients would be to short to justify the approach and it is suggested that the use of the optima derived from the global data set are the most appropriate. The median PTI taxon optima for each of the response groups (Tolerant, Sensitive) for a range of lake types had a linear relationship with the median MEI value of the type (fig 13). Models for both the tolerant and sensitive taxa groups are highly

significant (R2 0.80 & 0.85, p<0.001) and the slopes of the relationships are very similar (0.35, 0.37). These models were then used to identify the median PTI (global optima) values for Sensitive and Tolerant taxa for each lake type, overcoming the limitation of the type specific gradients. Time has precluded applying the method to all lake types, the single humic type (NGIG L-N3a) followed the same relationship as the other clear lakes and it seems likely that the relationship is independent of colour, this needs to be confirmed. As the median PTI values identify the type specific mid point PTI of each response group, the average of these values was taken as the boundary PTI between the sensitive and tolerant taxa. The median values themselves were taken as the boundaries of very sensitive and very tolerant response groups. The results for the 216 taxa used are shown in table A2 of the appendix .

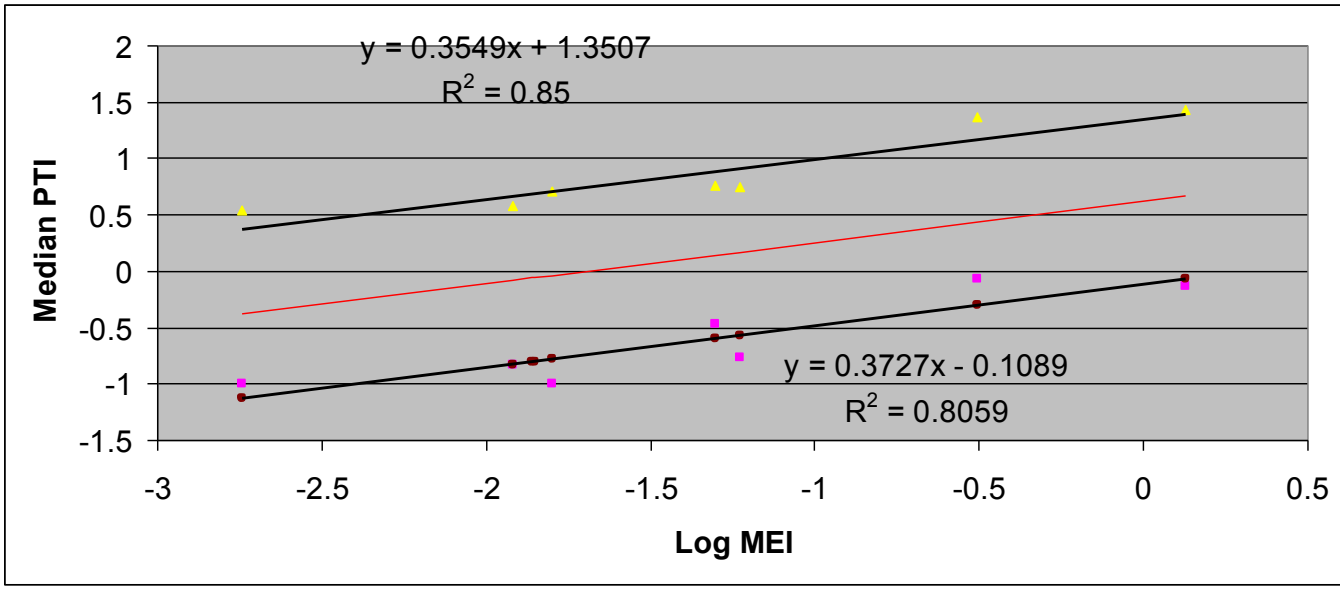

Fig 13 Models used to standardise threshold values of PTI delimiting different response groups in different lake types. The observed values of PTI are the thresholds determined from the axis 1 scores of a CCA analysis of all lakes (global) data set. MEI is the median value for the lake types LN1, LN2a, LN2b, LN3a, LCB3, LCB1, LCB2. Upper (yellow) points are Tolerant taxa, lower (purple) points are Sensitive taxa, red line is the average of the fitted lines for Tolerant and Sensitive taxa.

The relationship of these groups of sensitive and tolerant taxa against the PTI for moderate alkalinity shallow lakes are explored below as an illustration of boundary setting protocols.

3.3.4. Example of use of PTI in a single lake type – Moderate Alkalinity Lakes.

Potential for Cross N-GIG and CB-GIGcomparisons

There are a number of lake types in NGIG which include moderate alkalinity lakes. Moderate alkalinity shallow clear water lakes (LN1), moderate alkalinity shallow humic (LN8a) and moderate alkalinity poly-humic lakes (LN8b). Many of these lakes are in Finland, geographically close to similar lakes in Estonia and Latvia, which are within the CBGIG (fig 14). In CBGIG the lake type LCB3 (Lobelia lakes) are mostly moderate alkalinity, many are humic, particularly in Estonia. This raises the question of

whether it would be more appropriate to include these lakes within a cross-gig intercalibration. An advantage of this approach would be the increased number of reference sites from the NGIG and longer environmental gradients by inclusion of the CBGIG lakes.

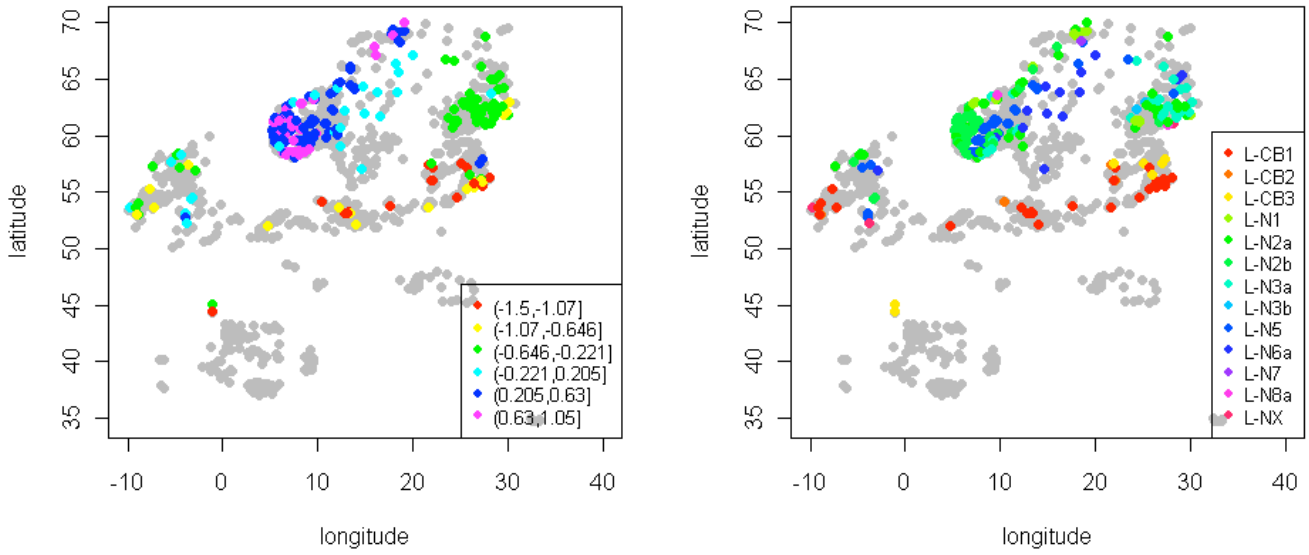

Fig 14 Location of reference lakes; a) showing NMDS axis 1 scores, b)showing GIG type.

The relationship between PTI and total phosphorus and PTI and chlorophyll for these lake types is shown in fig 15. The GAM models clearly overlay each other and adding type and country as factors within the model demonstrate there they have no significant effect for the relationship with phosphorus and only slightly significant ($p<0.01$) differences for the LN-8b type and for country with chlorophyll. This suggests that the types are not substantially different and might be combined. Further evidence of this is suggested by the reference NMDS scores for these lakes, which show that some of the LCB3 lakes have similar scores to those in Finland (fig 15b).

The distribution of the PTI metrics for the reference sites for this lake type is shown in Fig 16. Both the PTI global metric (fig 16a) and the NGIG clear lake metric (Fig16b) show country specific differences. With the exception of lakes in Latvia, the global PTI metric has the greatest similarity between countries and this metric will be explored further to illustrate possible boundary setting. (A similar method could be followed for the NGIG clear water PTI metric and for other types).

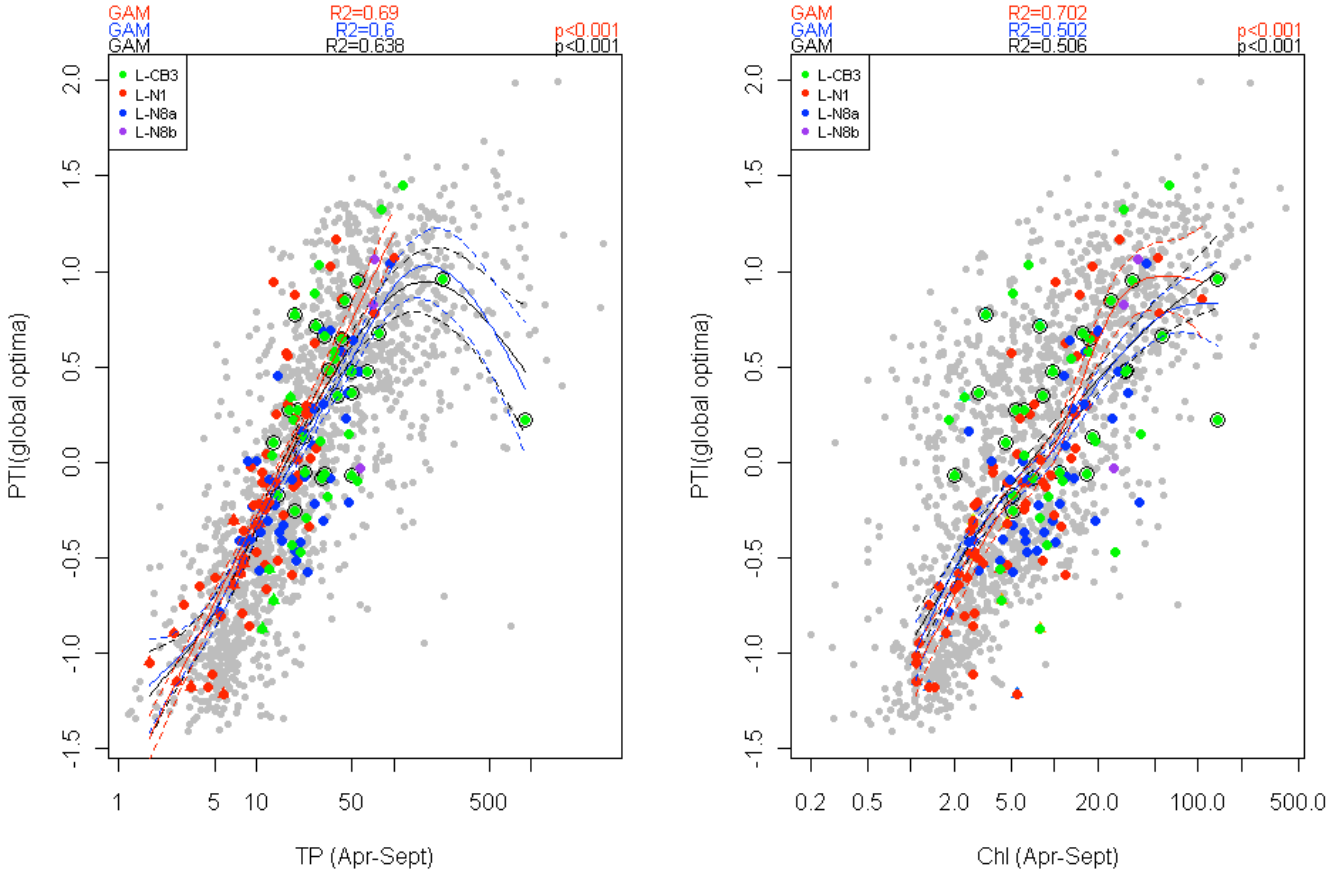

Fig 15. Relationship between PTI site scores (derived from global all lake optima) and growing season total phosphorus and chlorophyll a concentration for moderate alkalinity lakes. Points represent mean values for all years for a waterbody, grey points all lakes, green points LCB3 lakes, green points with black outlines LCB3 lakes which are humic or polyhumic, red points are LN1 (clear water shallow), blue points are LN8a (humic shallow), purple points LN8b (polyhumic shallow). GAM models shown, black line = all LCB3 and LN1 lakes, blue line = all lakes, red line = LN1 lakes

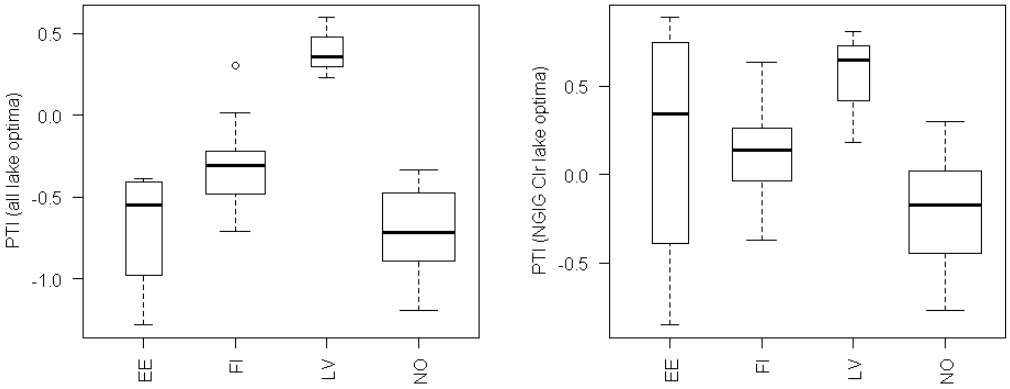

Fig 16 Range of PTI scores a)derived from all lake global optima, b)derived from NGIG clear water optima, for clear water moderate alkalinity lakes from NGIG and CBGIG by county.

Check of Reference Conditions

The range of pressure variables (total phosphorus and total nitrogen) impact variable (chlorophyll a) and pressure metrics (population density and % natural and semi-natural land) were examined for reference lakes in each country for lakes that were from lake type L-N1 or L-CB3$_{clear}$ (Fig 17).

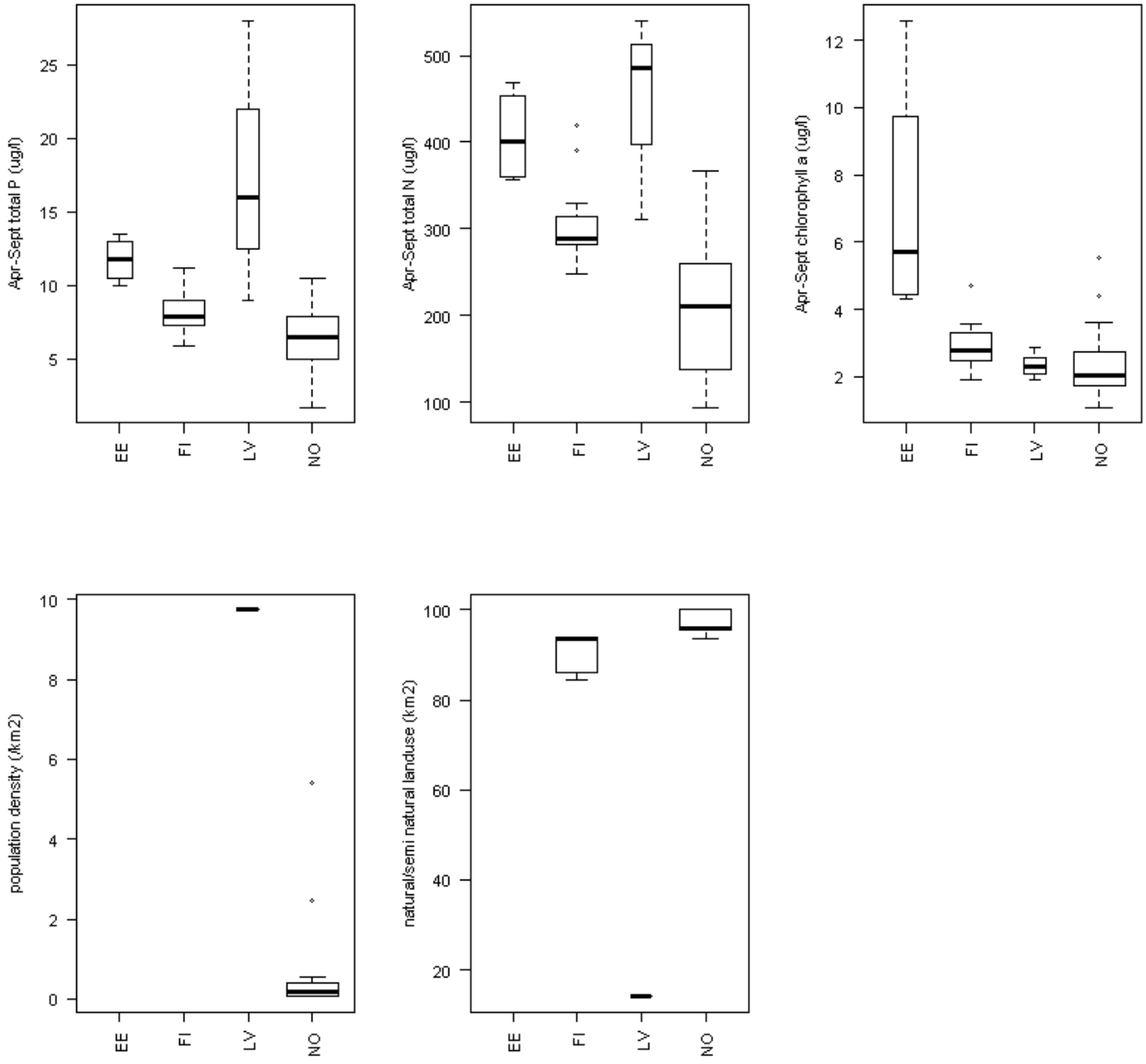

Fig 17. Range of pressure variables total phosphorus and total nitrogen, response variable chlorophyll a and pressure indicators population density and percentage of natural and semi-natural land use by country.

Reference lakes from Estonia and Latvia had higher total phosphorus and total nitrogen concentrations in their reference sites than those in Finland and Norway. Chlorophyll a values were higher for Estonia, but similar for Latvia. Comparing chlorophyll a and total phosphorus values (fig18a) suggests some of the Estonian chlorophyll values may be higher than expected given their TP concentraions, while for the Latvia some of the chlorophyll values were lower than expected.

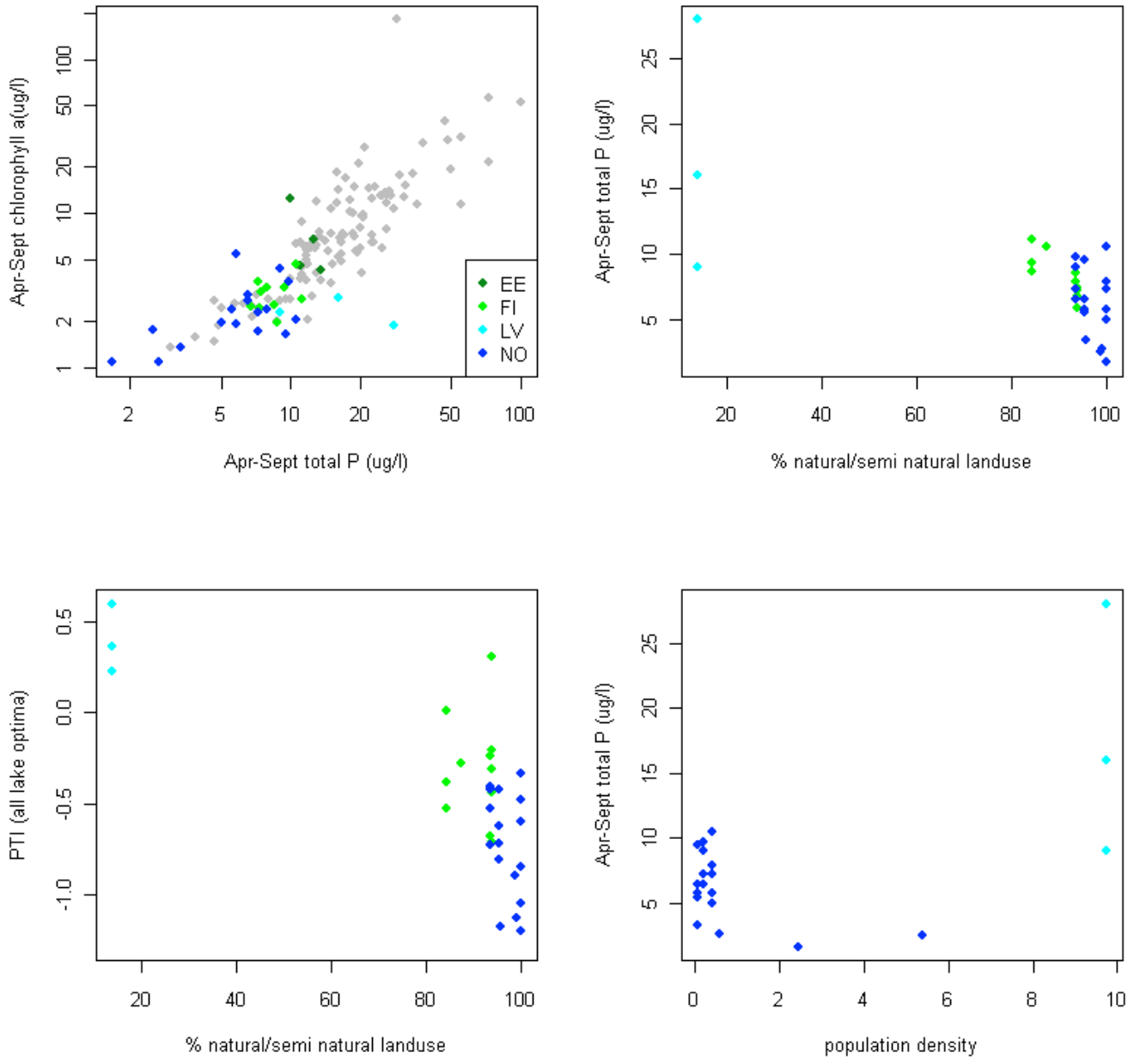

Fig 18 Relationship between a) total phosphorus and chlorophyll a for moderate alkalinity lakes, reference lakes identified by colours

No pressure data for reference sites were available for Estonia, but the Latvian reference sites had much lower percentage of natural and semi-natural land than those from Finland and Norway (fig 17e, fig 18c).

Finland also had slightly lower percentage of natural and semi-natural land and higher total phosphorus than Norway, which may account for the slightly higher reference PTI values from Finland in comparison to Norway (fig 16a). Further discussion is needed to agree which of all these sites should be considered reference, land-use information from Estonia would be particularly useful. However, to take forward an example of the calculation of EQRs and boundary setting it was decided to exclude the Latvian reference sites, but include all others and use them to calculate EQR values.

Calculation of EQR

The distribution of the PTI metric for reference sites for this lake type are shown in table 3. It is proposed that the median value is taken as the reference PTI for this lake type, a value of -0.528.

The EQR values were calculated for all samples for lakes of this type in the WISER database were calculated, using equation 2, where

PTI_{Obs} = Sample PTI

PTI_{Max} = Maximum PTI score for type, the lower (worst) anchor = 1.5 (arbitrary value, can be reduced in the future).

PTI_{Ref} = Expected or reference PTI for type, the upper (best) anchor = -0.528

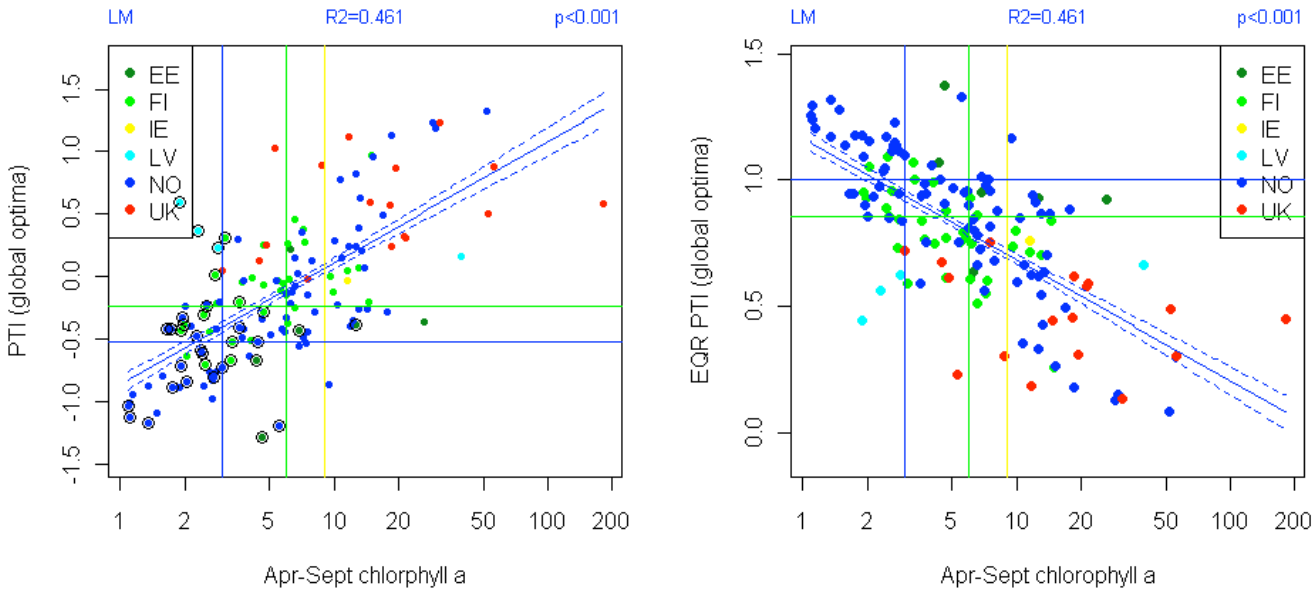

Fig 19 Relationship between a)PTI and chlorophyll for moderate alkalinity lakes, points represent lake years, colours identify country and black outlines show reference lakes. b)EQR for PTI and chlorophyll. Vertical lines mark IC phase 1 boundaries for chlorophyll, horizontal lines mark a) reference PTI for lake type = blue and 90th percentile of reference PTI values = green, b) reference EQR = blue, EQR value calculated from 90th percentile of reference PTI values = green.

The resulting values are shown plotted against chlorophyll a in fig 19b and various options considered for boundary setting.

Options for boundary setting

a) Distribution of PTI metric in reference sites

The first and simplest option is to use the distribution of PTI values from reference sites. This was one of the approaches used to determine the H/G boundary for chlorophyll a in phase 1 of intercalibration.

The distribution of PTI values for reference sites is shown in table 3. It is suggested that the 90th percentile of the reference site PTI could be considered a H/G boundary value, a value of -0.241.

Table 3 Distribution of PTI metric (global optima) and EQR values for reference and classes defined by chlorophyll a for shallow moderate alkalinity clear water lakes from Estonia, Finland and Norway .

	Min	10%	25%	50%	75%	90%	Max
PTI Ref Lakes	-1.283	-1.11625	-0.74661	-0.52827	-0.3851	-0.24073	0.303
PTI Lakes classified as High by chl a		-0.964	-0.877	-0.764	-0.425	-0.140	
PTI Lakes classified as Good by chl a		-0.474	-0.435	-0.326	-0.083	0.213	
PTI Lakes classified as Moderate by chl a		-0.335	-0.261	-0.059	0.266	0.789	
EQR PTI Lakes classified as High by chl a	0.23		0.81	0.95	1.12		1.37
EQRPTI Lakes classified as Good by chl a	0.30		0.63	0.78	0.90		1.01
EQRPTI Lakes classified as Moderate by chl	0.09		0.35	0.61	0.77		1.17

b) Comparison with chlorophyll a boundaries or status classifications

Another approach would be to compare with chlorophyll a, and use the boundaries already established during phase 1 intercalibration. The PTI metric and its EQR equivalent value for the shallow moderate alkalinity lakes were plotted against chlorophyll a (Fig 19). The relationship was linear and a linear model was fitted (R^2 = 0.46) (table 4). Taking the chlorophyll boundaries for L-N1 of HG=6 and GM=9 gives potential PTI boundaries of H/G = -0.111 and G/M = 0.060.

An alternative approach is to classify all these lakes by chlorophyll and examine the distribution of the PTI metric in each resulting group of lakes (fig 20). Boundaries could then be derived from the overlap of the distributions of High and Good status sites. For example an average of the upper and lower quartiles of lake classified as High and Good for the HG boundary and those classified as Good and Moderate for the GM boundary. For the example lake type these values would be H/G = -0.233 and G/M = 0.077, the approach is illustrated in fig 20.

Table 4 Linear model for relationship between PTI and ln (chlorophyll a)

Call:

lm(formula = y ~ x)

```
Residuals:
     Min       1Q   Median       3Q      Max
-1.05695 -0.29116 -0.05033  0.24726  1.19670

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.86928    0.07531  -11.54   <2e-16 ***
x            0.42301    0.03815   11.09   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.4201 on 144 degrees of freedom
  (42 observations deleted due to missingness)
Multiple R-squared: 0.4606,     Adjusted R-squared: 0.4569
F-statistic:   123 on 1 and 144 DF,  p-value: < 2.2e-16
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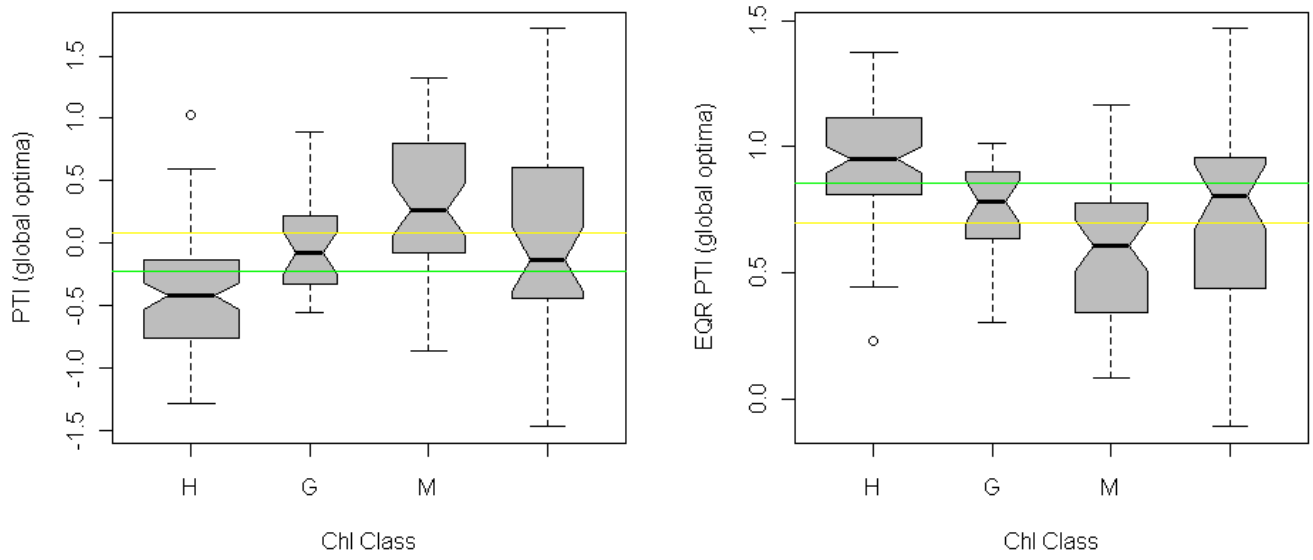

Fig 20 Range of a) PTI scores, b) EQR PTI for moderate alkalinity lakes classified by chlorophyll a. If notches do not overlap categories are significantly different. The box plot to the right in each panel are unclassified lakes. Horizontal lines are potential HG and GM boundaries based on mean of upper and

lower quartiles of categories classified by chlorophyll a for High & Good (green), Good & Moderate (yellow)

The HG value is close to the value derived from the 90th percentile PTI of reference sites (-0.241), so we may conclude that a possible HG PTI boundary for this lake type is between -0.241 and -0.111. Possible GM boundaries lie between 0.060 and 0.077.

These PTI boundary values for the H/G boundary can be converted to the EQR scale as follows:

EQR = (-0.241 – 1.5)/)-(0.528 – 1.5) = 0.85 – using the 90th percentile PTI from reference sites

EQR = (-0.233 – 1.5)/)-(0.528 – 1.5) = 0.86 – using the average of the chlorophyll classes

EQR = (-0.111 – 1.5)/)-(0.528 – 1.5) = 0.79 – using the average of the chlorophyll classes

The first two values are very similar to the EQR value calculated by taking the average of the upper and lower quartiles of the PTI EQR distributions for lakes classified as High and Good by chlorophyll a (table 3), a value of 0.85.

The potential G/M boundaries on the EQR scale would be:

EQR = (0.077– 1.5)/)-(0.528 – 1.5) = 0.70 – using the average of the chlorophyll classes

EQR = (0.06– 1.5)/)-(0.528 – 1.5) = 0.71 – using relationship between chlorophyll and PTI

c) Relationship of PTI site scores with sensitive and tolerant taxa.

Another, slightly more independent approach would be to examine the relationship between tolerant and sensitive taxa. The most independent would be to use taxonomic groups known to have different sensitivities to eutrophication. Ptacnik *et al* (2008) suggested the the proportions of Chrysophytes and Cyanobacteria can be used and intial tests on this data set confirm these views. Time has precluded presenting the information here, but the approach has been extended to include all taxa, split into tolerance groups using their relative position defined by their trophic optima.

The distribution of very sensitive, sensitive + very sensitive, tolerant + very tolerant and very tolerant taxa (see table A2 in appendix for identification of taxa) in reference and impacted moderate alkalinity shallow lakes are shown in fig 21. Their distribution in lakes classified by chlorophyll a are shown in fig 22.

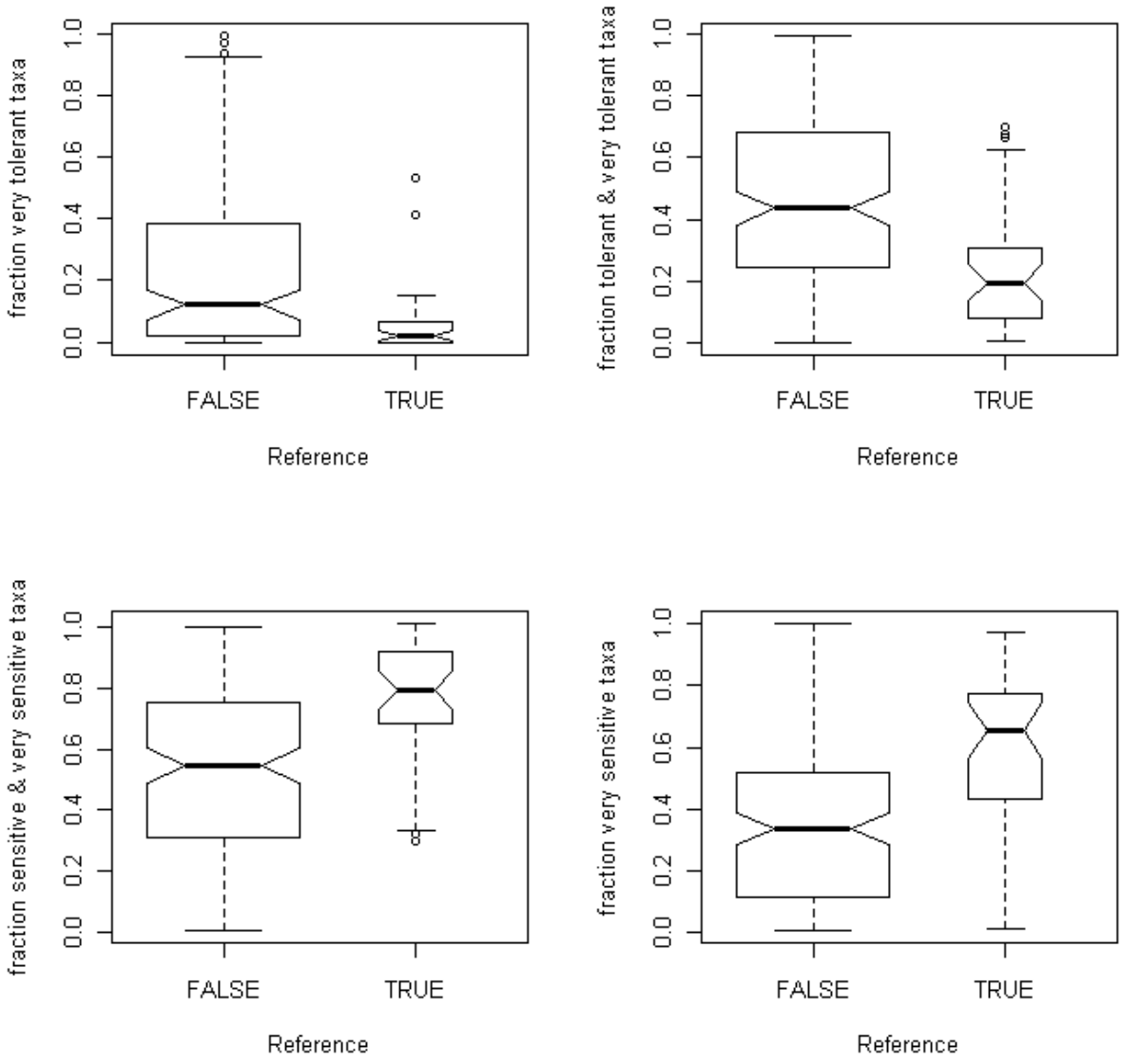

Fig 21 Range of sensitive and very sensitive taxon groups for reference (TRUE) and non-reference (FALSE) shallow moderate alkalinity lakes (lake years, clear water LN1 & LCB3)

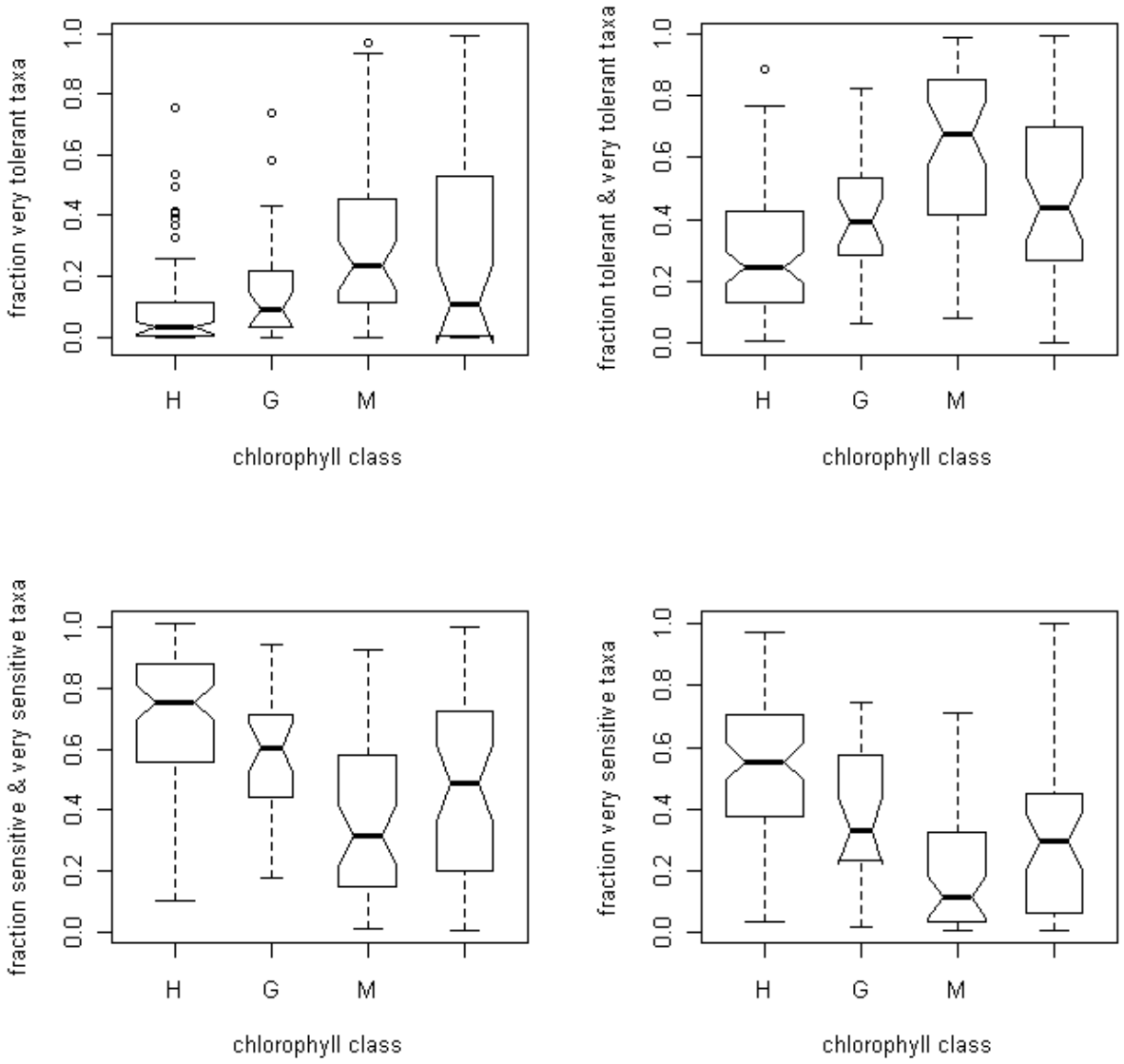

Fig 22 Range of sensitive and very sensitive taxon groups for moderate alkalinity lakes (lake years, clear water LN1 & LCB3), classified by growing season chlorophyll a. The right box plot in each panel are unclassified lakes.

The relationship between these groups and PTI is shown in fig 23, together with the proposed reference PTI value (-0.528 blue line) and boundaries for HG (-0.23 or -0.24 green line) and GM (0.06 yellow line). The reference PTI value is lower than the cross-over between very sensitive and tolerant + very tolerant taxa. Thus the reference lakes are likely to have >50% of very sensitive taxa (see GAM blue line fig 23a), only 10% would have less than 40% and 90% have at least 70% of VS taxa (see 10th and 90th quantiles fig 23b). Based on these observations the proposed PTI value of -0.528 seems a reasonable value for Reference in this lake type.

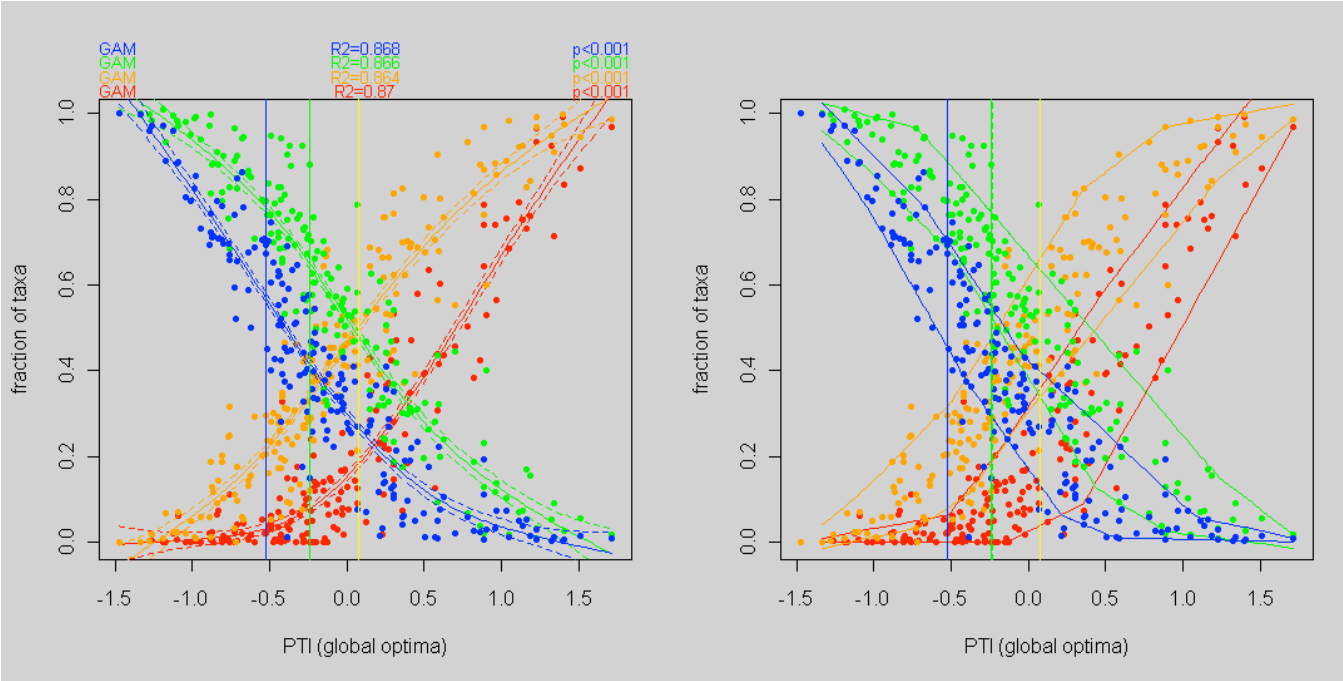

Fig 23 Relationship between fraction of very sensitive (blue), sensitive and very sensitive (green), tolerant and very tolerant (orange) and very tolerant (red) taxa with PTI scores in shallow moderate alkalinity lakes. fitted models a)GAM, b)quantile regressions showing upper and lower 10th, 90th quantiles. Vertical lines median PTI reference sites (blue), 90th percentile PTI reference sites & average of upper and lower quartiles of sites classified as High & Good by chlorophyll a (green), average of upper and lower quartiles of sites classified as Good & Moderate by chlorophyll a (yellow)

The potential -0.23 PTI boundary for HG (green line fig 23) is lower than the cross over of the GAMs for Very sensitive and tolerant + very tolerant and slight lower than the lower 10th quantile of VS and upper 90th quantile for VT. In addition at this point on the PTI gradient the GAM for VT has a value of <0.1. So at this point we still have dominance by very sensitive taxa (ca. 50%), a dominance of sensitive and very sensitive (ca. 70%) and only a small chance of very tolerant taxa (10% of sites with > 20%), a reasonable position for the HG boundary.

The potential 0.06-0.07 PTI boundary (yellow line fig 23) for GM is close to the cross-over point for the GAMs for VS+S and T+VT groups. It is also close to the cross-over of the lower 90th quantile of the S and upper 10th quantile of VT taxa, slightly lower than the lower 10th quantiles for the same groups. At this PTI value the community has similar proportions of T+VT than S+VS. The VS taxa still represent >20% of the community and although the VT taxa are clearly making an appearance (<10% of sites would have <5%) they are most likely to represent <20% (GAM model). This seems a reasonable position for the GM boundary.

3.4. Discussion

This will be elaborated for the final version.

Remaining work

a) Relationships between optima defined by MS and the Common metric optima eg Germany, Sweden and Norway.

b) Testing the relationship between the common metric and national metrics. This is a GIG exercise, but could be done on the WISER dataset, at least for NGIG and CBGIG

c) Completion of the MAS lakes example. We could calculate the chlorophyll EQR values, normalise them and propose a combination rule, perhaps also including bloom metrics.

e) Test the type differences, comparing the linear slopes of PTI vs. TP for different types.

f) Robustness test for the PTI metric

3.5. Conclusions and recommendations for use in Intercalibration

To be written for the final draft, once the remaining work has been done.

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Chapter 4: Phytoplankton size structure and morpho-functional groups

Responsible: Giuseppe Morabito, contributions by Laurence Carvalho

Rationale

The importance of morphological and functional traits in shaping phytoplanktonic assemblages was pointed out more than 20 years ago by Margalef (1978) and Reynolds (1984). They suggested that morphology and functions of a phytoplankton cell are strictly related in determining the role of the organisms in the environment and that a trait-based approach can explain the species distribution across environmental gradients. Trait-based approaches are being increasingly used in ecology (see the review by Litchman et al., 2010), because they allow a simple, mechanistic classification of phytoplankton, strongly connected with the functioning of the community.

Among the functional traits, phytoplankton cell size is a key feature in the ecological relationships, being related to the efficiency of many eco-physiological processes (nutrient assimilation, photosynthetic efficiency, respiration, buoyancy), most of which affected by trophic changes (see i.e. Capblanq & Catalan, 1994; Litchman et al., 2010).

Following the dimensional approach, a phytoplankton assemblage can be described in terms of size spectra: the use of size spectra in describing the response of a phytoplankton assemblage to environmental gradients has been proven to be a valid instrument (Kamenir et al., 2008; Kamenir & Morabito, 2009).

Even the use of simple morphological traits has been proven to be successful in describing a phytoplankton succession in environments with different characteristics: Salmaso & Padisak (2007) analysed the phytoplankton composition in terms of Morpho-Functional Groups, finding a very good relationship with the changes in the species composition during a seasonal cycle.

One of the main advantages of both classification systems is that they are almost completely a-taxonomic, allowing facing the problems due to correct taxa identification. Therefore, a trait-based approach could be useful when using phytoplankton as ecological indicator, reducing the risk of incorrect species taxonomic classification and the possible wrong attribution of a lake to a quality class.

A proposal for using size classes and Morpho-Functional Groups as classification systems towards the development of a trait-based phytoplankton trophic index is presented in this chapter.

Approach

In the case of size spectra, the classification is done by dividing the cells in a certain number of size classes, created by doubling the cell volume, i.e., by standard increments of the cell size logarithm. In the following example, the size classes are ≤0.5 μm^3, followed by 0.5-1, 1-2, 2-4 μm^3, etc. Each of them is indicated by the notation VX, where V means Volume and X is the upper limit of the size class expressed as logV. A total number of 19 size classes were obtained, from V-0.3 to V5.1 (Kamenir & Morabito, 2009). An example is given in Table 1.

In the case of Morpho-Functional Groups, the organisms are grouped according to their morphology, following the classification proposed by Salmaso & Padisak (2007): the features of the groups are reported in Table 2.

It was possible to assign about 60% of the species included in the WISER/REBECCA list to one of the size classes as well as to a MF Group. The species allocation was done on the basis of available data on species volumes and using the literature. This represented approximately 70-80% of the total phytoplankton biomass per lake.

In order to test the response of Size Spectra and MF-Groups to trophic changes, two trophic indexes were developed. This was done by weighted averaging, following the same calculation steps described in Ptacnik et al. (2009) and Marchetto et al. (2009), but using, instead of species biomass, the average biomass of each size class and MFG respectively. The average values were transformed in percentage and then as double square root, in order to decrease the importance of the most dominant size classes/MF Groups.

The two indices presented in this chapter are called Size Phytoplankton Index (SPI), based on size classes and the Morpho-Functional Group Index, based on MF-Groups (MFGI). The final calculation of the indices needs two parameters, calculated on a calibration dataset: a trophic score, indicating the trophic position of a size class of MFG across the trophic spectrum and an indicator value, estimating the "power" of each size class/MFG as biotic indicator.

The trophic scores were estimates as follows:

Where:

Y_{ik} = transformed biomass of size class/MFG k in lake i.

Y_{+k} = transformed biomass of size class/MFG k in all the calibration lakes.

TP_i= Total phosphorus concentration in lake i.

The indicator values were estimated as follows:

Where T_k = tolerance of size class/MFG (see ter Braak et al., 1995).

The indicator values (IV) were estimated from the ratio between T_k and TS_k: this ratio may vary between 0.14 and 0.38 for size classes and between 0.18 and 0.30 for MF Groups. The range of values has been divided in 6 equal width classes (Table 3), assigning to each class a code from 1 to 6. A low value indicates a high tolerance and, therefore, a poor indicator value. The opposite is true for a high indicator value.

The value of the two trophic indices (SPI and MFGI) is finally calculated as:

Where TI indicates SPI or MFGI and BV_k the transformed biovolume of each size class or MF Group. Trophic scores and indicator values of size classes and Morpho Functional Groups are reported in Appendix 1.

Table 1. Examples of Operational Taxonomic Units (OTU) ranked by their typical cell volume (V, µm3). Size classes defined by the upper border of cell volume. From Kamenir and Morabito (2009).

Taxonomic group	Operational Taxonomic Unit (OTU)	**Cell Volume (V, µm^3)**	LogV	**Size Class: The volume range (upper border log)**
CYA	Aphanothece clathrata (01-02)	0.4	-0.40	<=0.5 (-0.3)
CYA	Aphanothece smithii (03-05)	0.4	-0.40	
CYA	Cfr. Cyanobium sp.	0.5	-0.30	
CYA	Aphanocapsa incerta	0.6	-0.22	>0.5-1 (0.0)
CYA	Aphanothece cf. floccosa (03-05)	0.8	-0.10	
CYA	Microcystis incerta	1.0	0.00	
CYA	Aphanothece clathrata (98-00)	1.1	0.04	>1-2 (0.3)
CHLO	Hyaloraphidium contortum	1.2	0.08	
CYA	Aphanothece clathrata (86-90)	1.3	0.11	
CHLO	Lyngbya limnetica	2.7	0.43	>2-4 (0.6)
CHLO	Choricystis coccoides	3.3	0.52	
CYA	Cyanodictyon planctonicum	3.3	0.52	
CHLO	Lobocystis sp.(05)	4.1	0.61	>4-8 (0.9)
CHLO	Dictyosphaerium sp.	6.2	0.79	
CYA	Microcystis aeruginosa (92-94)	6.4	0.81	

One of the problems in using a classification based on size spectra is given by the colonies. In fact, some colony-forming species can occupy a different position in the size spectrum, depending on the number of cells building the colony. Because the functional approach consider the colony as a single individual, the choice of a certain size class for the colony-forming species can be crucial. The classification system used in this analysis is based on size ranges, thus buffering, to a certain extent, the variability of the organism individual volume. The effect of colony size was, anyway, tested: the test was carried out on the calibration dataset, by assigning some species to different size classes and re-calculating the trophic scores. Differences in the trophic scores and in the SPI metric per lake were statistically evaluated with Friedman rank sum test.

Because of the lack of information on colony size in the WISER database, the results reported in Olenina et al. (2006) were taken into account for evaluating the variability of the colony size. The species assigned to different size classes were: *Aphanocapsa conferta*, *Aphanocapsa delicatissima*, *Aphanocapsa elachista*, *Aphanocapsa holsatica*, *Aphanocapsa incerta*, *Aphanocapsa planctonica*, *Aphanothece bachmannii*, *Aphanothece clathrata*, *Aphanothece minutissima* (all species assigned to classes 0, 0.3, 0.9, 1.5, 2.1), *Merismopedia tenuissima* (classes 0.3, 1.5, 1.8, 2.1, 2.4), *Snowella lacustris* (classes 1.5, 2.4, 2.7, 3.0), *Woronichinia tenera* (classes 1.5, 2.4, 2.7, 3.3), *Microcystis aeruginosa* (classes 1.8, 3.3, 3.6, 3.9), *Pandorina* sp. (classes 2.4, 3.3, 3.6).

Table 2. Morpho-Functional Groups. From Salmaso & Padisak (2007).

Flagellates	Potential mixotrophs	1 Large (colonial or unicellular)	1a Large Chrysophytes/Haptophytes
			1b Large Dinophytes
			1c Large Euglenophytes
		2 Small (unicellular)	2a Small Chrysophytes/Haptophytes
			2b Small Dinophytes
			2c Small Euglenophytes
			2d Cryptophytes
	Mostly autotrophs	3 Phytomonadina	3a Unicellular Phytomonadina
			3b Colonial Phytomonadina
Without flagella	Cyanobacteria	4 Unicellular	4 Unicellular cyanobacteria
		5 Colonies	5a Thin filaments (Oscillatoriales)
			5b Large vacuolated Chroococcales
			5c Other large colonies, mostly non-vacuolated Chroococcales
			5d Small colonies, Chroococcales
			5e Nostocales
	Diatoms	6 Large	6a Large Centrics
			6b Large Pennates
		7 Small	7a Small Centrics
			7b Small Pennates
	Others—Unicellular	8 Large	8a Large unicells-Unicellular Conjugatophytes/Chlorophytes
			8b Large unicells—Other groups
		9 Small	9a Small unicells – Conjugatophytes
			9b Small unicells—Chlorococcales
			9c Small Chrysophytes
			9d Small unicells—Other groups
	Others—Colonial	10 Filaments	10a Filaments—Chlorophytes
			10b Filaments—Conjugatophytes
			10c Filaments—Xanthophytes
		11 Non filamentous colonies	11a Chlorococcales—Naked colonies
			11b Chlorococcales—Gelatinous colonies
			11c Other colonies

Table 3.Class widths of T_k/TS_k ratio and corresponding Indicator Values for the two functional metrics.

Size classes		Morpho Functional Groups	
T_k/TS_k classes	Indicator Values	T_k/TS_k classes	Indicator Values
<0.15	6	<0.2	6
0.15-0.20	5	0.20-0.22	5
0.20-0.25	4	0.22-0.24	4
0.25-0.30	3	0.24-0.26	3
0.30-0.35	2	0.26-0.28	2
>0.35	1	>0.28	1

Exploratory analyses of dataset

Initial analysis has focused on the most common lake types across GIGs: lowland and shallow or very shallow lakes. Lakes with only one sample in the growing period were discarded. After applying the above criteria, 228 lakes were selected, distributed as follows: CBGIG – 122, NGIG – 77, MGIG – 29.

A first exploratory analysis has been carried out at evaluating the changes in the importance of different size classes and Morpho-Functional Groups across the trophic range: the lakes were divided in TP classes and the shift of the phytoplankton assemblages in terms of functional traits was analysed using a CCA approach, as detailed below.

In a second step, the trophic indices were calculated, as explained before, and their response across trophic gradient was evaluated using regression analysis.

The calibration dataset used for calculating the trophic scores was composed of 78 lakes, belonging to CB and N GIGs: the lakes selected provide a good covering of the trophic spectrum.

The biomass data from different months of the same year were averaged in each lake and in some cases the average was calculated among samples collected in two consecutive years to reduce the dataset to a single lake year per lake.

In a third step the effect of colony size was tested on the calibration dataset and, finally, the robustness of the metrics was evaluated. In the test of robustness the analysis has been carried out on single samples, instead of yearly average.

Size classes and morpho-functional groups along TP gradient and validation of metric

The response of single size classes and MF Groups to trophic gradient has been evaluated using PCA ordination analysis. This analysis has been carried out on absolute data (not percentage), after double square root transformation, to reduce the weight of the dominant classes. Size classes and MF Groups has been considered as species in the analysis. Lakes were divided in 5 groups, according to their TP concentration: 0-10, 10-20, 20-40, 40-80 and over 80 µg TP l^{-1}. The results are shown in Figs. 1 and 2.

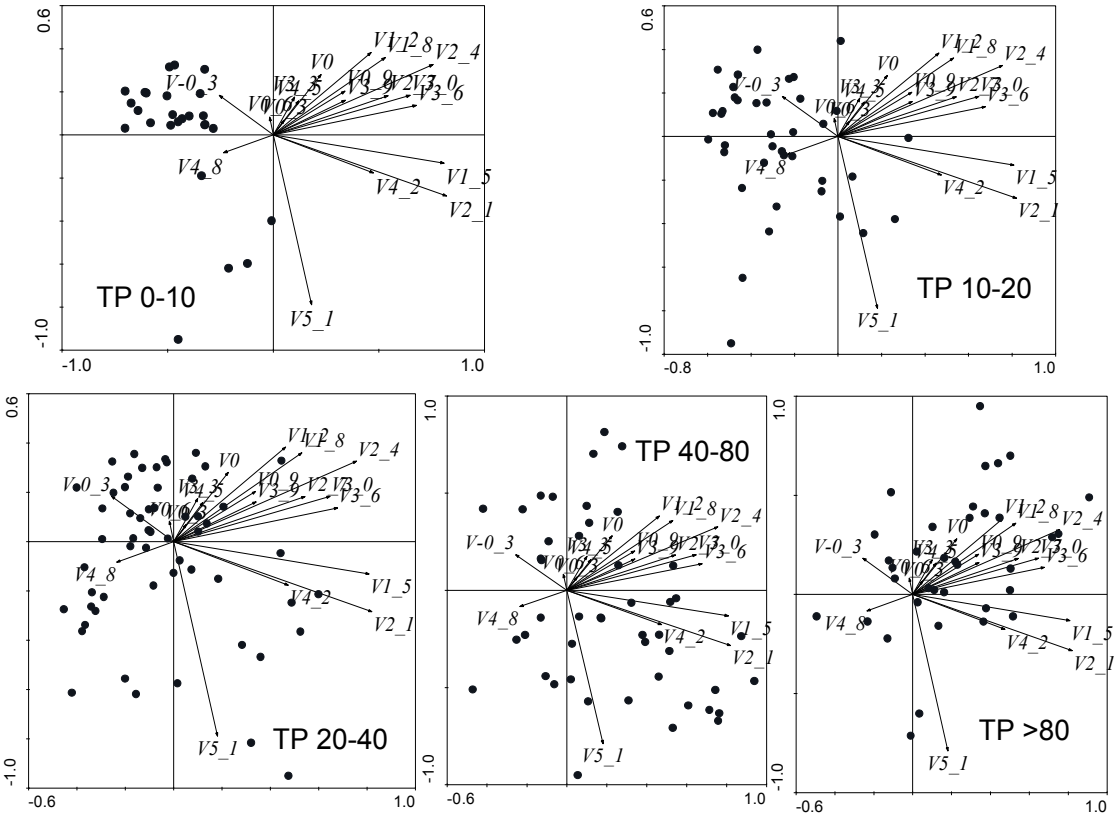

Fig.1. PCA ordination scatterplot of samples (dots) and size classes (arrows) in the five trophic classes.

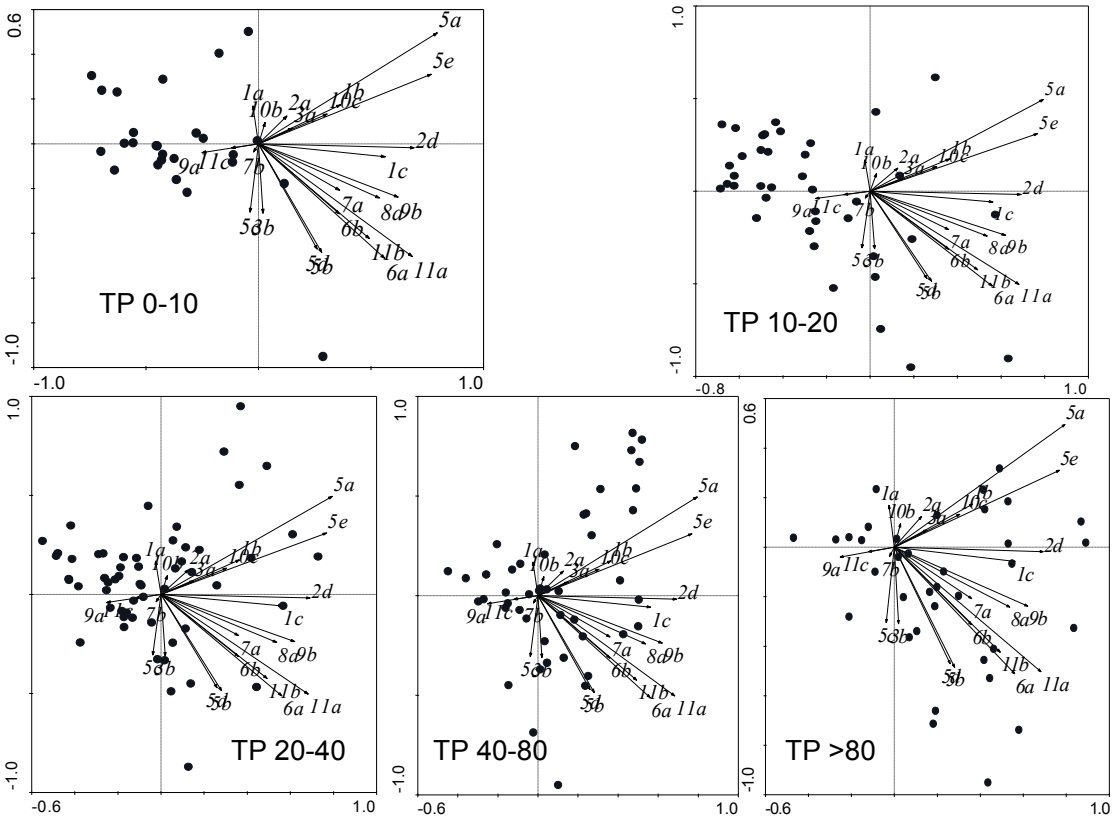

Fig.2. PCA ordination scatterplot of samples (dots) and MF Groups (arrows) in the five trophic classes.

The PCA ordination explained about 44% and 43% for the first two axes for size classes and MF Groups respectively. About the effect of TP changes on size classes, moving from oligotrophic to eutrophic environments, there is a clear shift from smaller to larger algae: in particular, the assemblages are dominated by the size class V-0.3 in the TP range 0-10 µg l^{-1}. In the TP range 40-80 there is the most important decrease of the small organism and the increase of the larger ones.

Concerning the MF Groups, the shift towards eutrophic lakes is coupled with the increased importance of groups 5 (colonial cyanobacteria), 6 (large diatoms), 8a (large coniugatophytes/chlorophytes), 9b (small unicellular chlorococcales) and 11 (naked and gelatinuos colonies of chlorococcales). On the other side, oligotrophic conditions do not seem to select a specific morpho-functional group.

The response of the two indices, Size Phytoplankton Index (SPI) and Morpho-Functional Group Index, against the eutrophication pressure has been evaluated by regression analysis. Both of them show a significant relationship with the TP gradient, with p <0.0001, using a simple linear model (Figs. 3 and 4).

Regression equations are the following:

$$; R^2 = 0.356; n=228; p<0.0001$$

$$; R^2 = 0.351; n=228; p<0.0001$$

Fig.3 Values of the Size Phytoplankton Index: distribution per Country across LogTP values.

Fig.4 Values of the Morpho-Functional Group Index: distribution per Country across LogTP values.

Some differences exist, however, at GIG level.

In the CB-GIG, the two metrics behave in the same general way with respect to the TP gradient, although with a slightly better fit of MFGI:

$SPI = 0.023 LogTP + 1.569$; $R^2 = 0.2294$; $p<0.0001$; $n=122$

$MFGI = 0.033 LogTP + 1.581$; $R^2 = 0.3345$; $p<0.0001$; $n=122$

In the N-GIG the relationships are different, with the SPI metric giving a better response:

$SPI = 0.0239 LogTP + 1.560$; $R^2 = 0.3422$; $p<0.0001$; $n=77$

$MFGI = 0.0221 LogTP + 1.578$; $R^2 = 0.0496$; $p<0.05$; $n=77$

In the M-GIG the response is better for MFGI index:

$SPI = 0.0188 LogTP + 1.559$; $R^2 = 0.1853$; $p<0.02$; $n = 29$

$MFGI = 0.0443 LogTP + 1.548$; $R^2 = 0.3837$; $p<0.001$; $n = 29$

The regression analysis showed that the use of one of the two functional metrics for lakes' classification could give contrasting results, depending on geographic region. The response is dependent on the phytoplankton assemblage composition and on the shift in its functional traits across the TP gradient: for instance, the change in the proportion of the various size classes is not clear in the range 40-80 µg TP (see Fig. 1), as well as the strong response of MF Groups

composition taking place in the upper part of TP gradient (Fig. 2). A metric combining the variable response of the different functional traits (size and morphology) could overcome these problems: the simplest combined metric is the arithmetic mean of SPI and MFGI. After calculating the arithmetic mean, a new multimetric index was obtained, called Functional Traits Index (FTI). The relationship between FTI and LogTP is shown in Fig. 5.

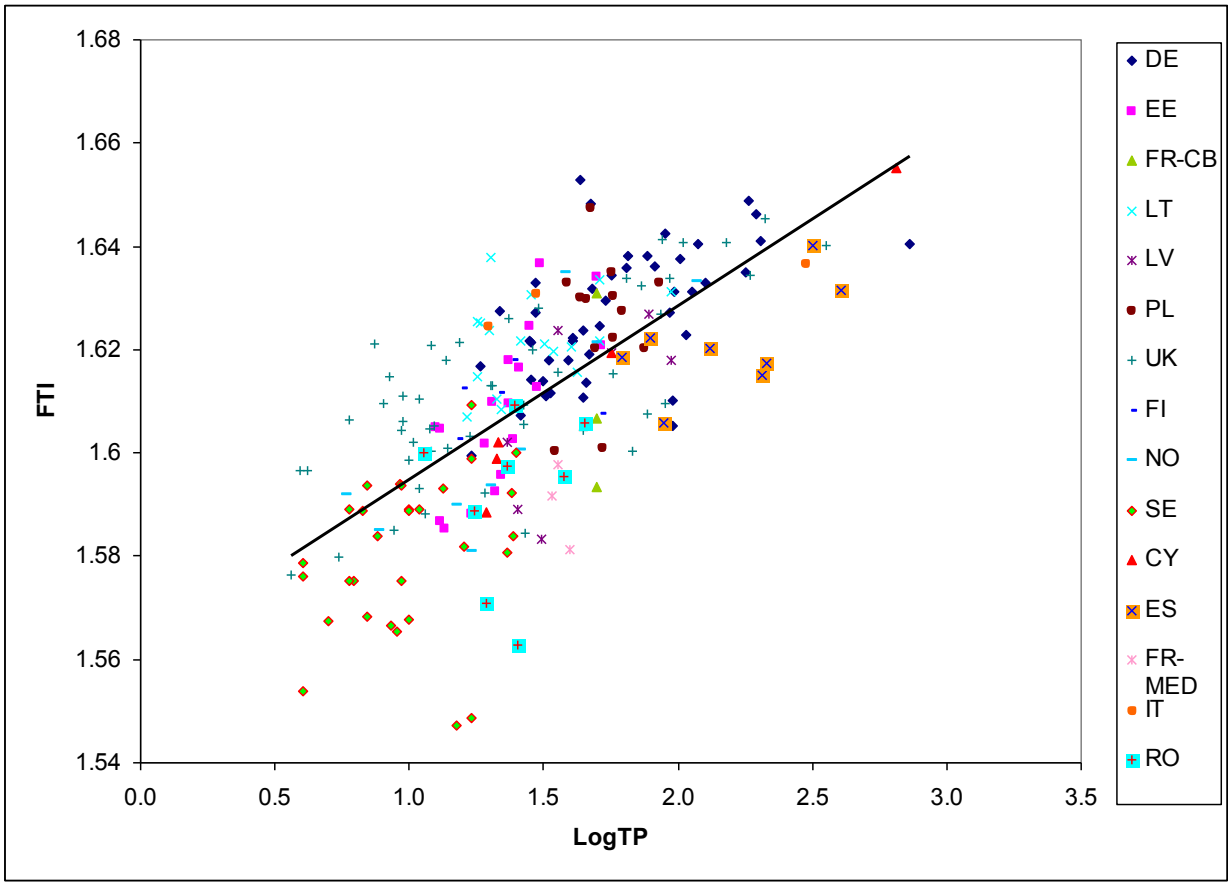

Fig. 5 Values of the Functional Traits Index: distribution per Country across LogTP values.

The linear model parameters for the whole dataset indicate that the fitting improved when using the combined index:

$FTI = 0.0335LogTP + 1.561;\ R^2 = 0.4916;\ p<0.0001;\ n = 228$

The response at GIG level is also better than using the two separate metrics SPI and MFGI for CB and M GIGs:.

$FTI = 0.0282LogTP + 1.575;\ R^2 = 0.3886;\ p<0.0001;\ n = 122$ (CB-GIG)

$FTI = 0.0314LogTP + 1.554;\ R^2 = 0.4956;\ p<0.0001;\ n = 29$ (M-GIG)

For N-GIG the relationship is slightly less powerful than fitting SPI vs LogTP, but the great improvement obtained with the combined index respect to the use of MFGI would justify using the FTI metric insted of the two single metric:

$FTI = 0.0255LogTP + 1.566;\ R^2 = 0.2248;\ p<0.0001;\ n = 77$ (N-GIG)

Effect of colony size

The response of the size classes metric was tested against the variability in the colony dimension of the colony forming taxa. The selection of taxa and their respective size were done as explained in the "Approach" section: the value of the trophic scores as well as the SPI index have been re-calculated each time after assigning the selected species to a new size class. As previously explained, the differences were statistically evaluated by means of the Friedman rank sum test.

As shown in Fig.6, the highest deviation in the trophic scores is found in the size classes V0 and V0.3, namely those including much of the colonial taxa considered in this analysis. However, the Friedman test reports a non significant difference among the series, giving a $p = 0.85$.

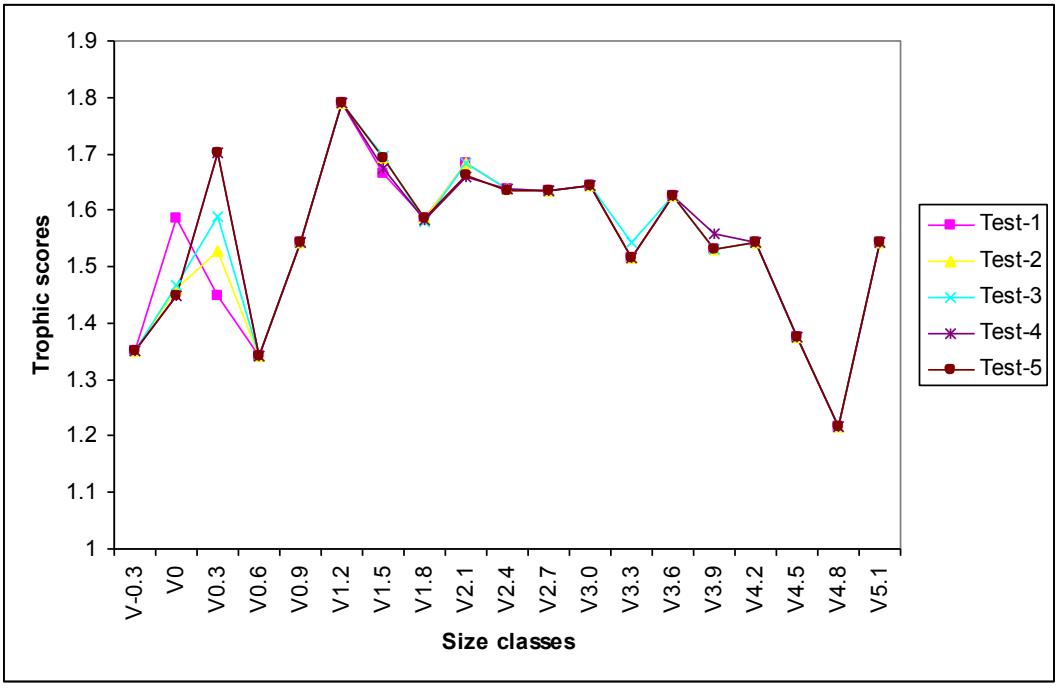

Fig. 6. Trophic scores of each size class after assigning a different size to colonial taxa. From test-1 to test-5 there is an increasing size of the colonies.

The different trophic scores of the size classes were used to calculate five series of SPI values in the lakes calibration dataset. The comparison by Friedman rank test indicates a significant difference among series ($p < 0.0001$): in fact, this difference is sometimes clear, although the general pattern does not change (Fig. 7).

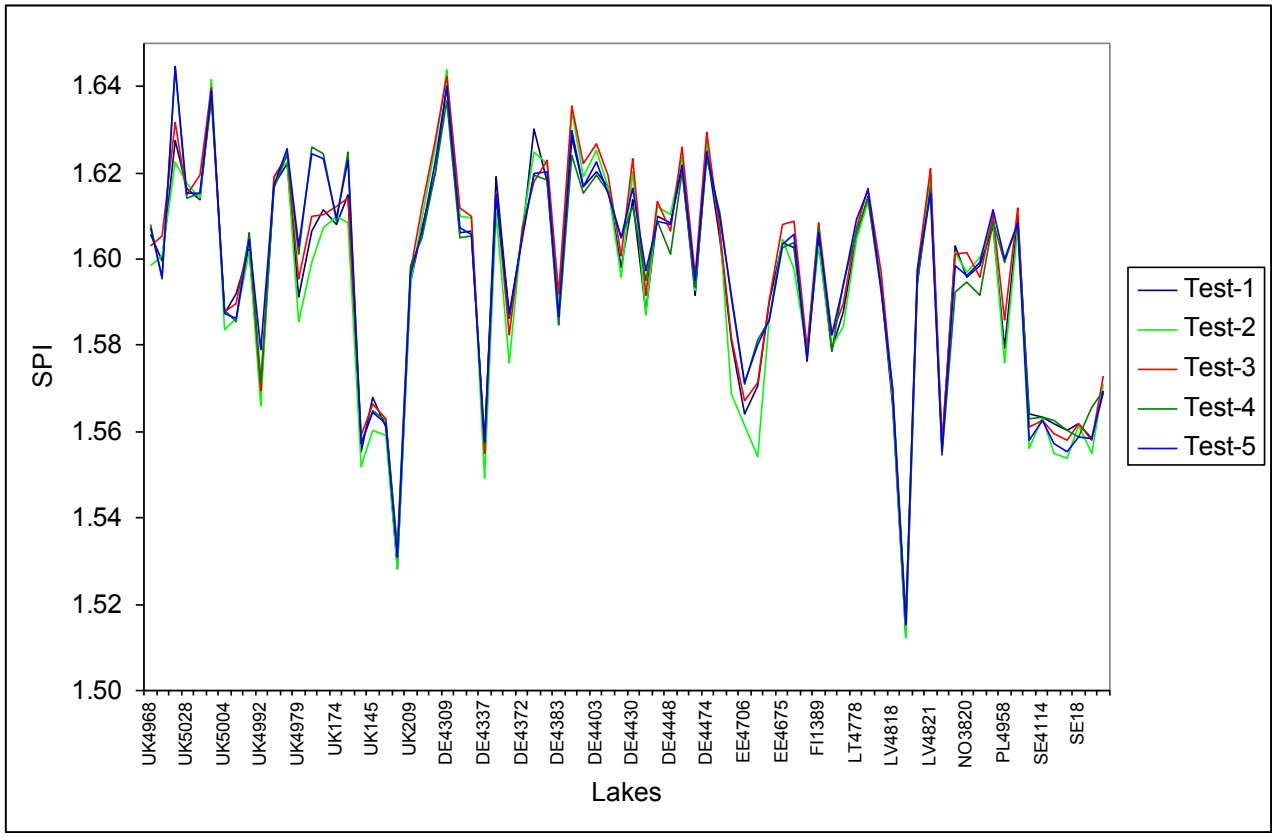

Fig.7 SPI values of the lakes calculated from different values of the trophic scores. Each line was obtained after assigning the selected colony forming species to different size classes.

However, although this difference in SPI values, the response of the SPI metric to phosphorus is always significant, as shown in Table 4.

Table 4. Parameters of the linear models describing the relationship between SPI metric and total P, using a different size for the colony forming taxa.

	Intercept	Slope	R-squared	p value
Test-1 linear model	1.522	0.0448	0.5799	<<0.0001
Test-2 linear model	1.516	0.0478	0.5738	<<0.0001
Test-3 linear model	1.523	0.046	0.5789	<<0.0001
Test-4 linear model	1.524	0.0447	0.5998	<<0.0001
Test-5 linear model	1.521	0.0466	0.6039	<<0.0001

Robustness of metric

According to Hering et al. (2010), the robustness of a metric reflect the effects of the stressor to be assessed (i.e. signal) while other sources of variability (i.e. noise) should have a relatively minor impact. This could be a critical point when using phytoplankton as biological quality indicator, due to the high natural variability of the phytoplanktonic assemblages, both in space and time.

The response to eutrophication gradient of the two traits-based metrics tested in this analysis has been described in the previous paragraphs: respect to the robustness, it is interesting to point out

that the parameters of the three linear models used to evaluate the relationship with total phosphorus in Northern, Central and Mediterranean GIGs have similar values. This would indicate that the gradients of biological quality are equally well reproduced along the whole region where the metric is applied and the response of the functional categories used as indicators is similar across space.

An index must also be robust in time, meaning that the variance due to the natural variability of the population (such as that imposed by the seasonal changes of environmental parameters) must be lower than the variability explained by the response of the metric to the variable degree of the pressure investigated. In this analysis the two sources of variance are given by the repeated sampling at the same site and by the sampling among sites across the trophic spectrum.

In our case, the ANOVA has been used to estimate the amount of variance due to the differences among sites and the amount of variance due to the repeated sampling at the same site. The ratio of respective Sum of Squares has been used as S/N ratio. The results of ANOVA for the whole lakes set, Med, N and CB GIGs in the three metrics are reported in Tables 4, 5 and 6.

Table 4. SPI metric: results of ANOVA for the whole lakes set, Med, N and CB GIGs.

SPI		SS	Df	F	p	Signific.	S/N ratio
MED	Lakes	0.07048	29	6.61	6.24E-12	***	31.65
	Samples	0.00223	3	2.02	0.1177	n.s.	
	Residuals	0.03053	83				
N	Lakes	0.05797	79	3.87	1.3E-15	***	37.23
	Samples	0.00156	3	2.74	0.04	*	
	Residuals	0.0425	224				
CB	Lakes	0,0850	109	4.07	<2E-16	***	115.62
	Samples	0.0007	3	1.28	0.2814	n.s.	
	Residuals	0.0622	325				
All	Lakes	0.3236	228	6.78	<2E-16	***	443.34
	Samples	0.0007	3	1.11	0.34	n.s.	
	Residuals	0.1391	638				

Table 5. MFGI metric: results of ANOVA for the whole lakes set, Med, N and CB GIGs.

MFGI		SS	Df	F	p	Signific.	S/N ratio
MED	Lakes	0.1566	29	15.42	<2E-16	***	86.84
	Samples	0.0018	3	1.72	0.17	n.s.	
	Residuals	0.0287	83				
N	Lakes	0.1869	79	2.74	2.92E-09	***	18.48
	Samples	0.0101	3	3.90	0.01	**	
	Residuals	0.1935	224				
CB	Lakes	0.3764	109	3.79	<2.2E-16	***	14.06
	Samples	0.0268	3	9.79	3.37E-06	***	
	Residuals	0.2964	325				
All	Lakes	0.9119	228	4.83	<2.2E-16	***	111.34
	Samples	0.0082	3	3.17	0,02	*	
	Residuals	0.5492	637				

Table 6. FTI metric: results of ANOVA for the whole lakes set, Med, N and CB GIGs.

FTI		SS	Df	F	p	Signific.	S/N ratio
MED	Lakes	0.0666	29	10.93	<2E-16	***	1416.4
	Samples	0.0001	3	0.07	0.97	n.s.	
	Residuals	0.0172	82				
N	Lakes	0.0881	79	4.95	<2E-16	***	72.6
	Samples	0.0012	3	1.79	0.15	n.s.	
	Residuals	0.0505	224				
CB	Lakes	0.1378	109	4.24	<2e-16	***	26.48
	Samples	0.0052	3	5.82	0.00069	***	
	Residuals	0.0968	325				
All	Lakes	0.4368	228	7.53	<2e-16	***	188.29
	Samples	0.0023	3	2.93	0.03	*	
	Residuals	0.1687	637				

According to Stoddard et al. (2008), the first source can be seen as noise (N), whereas the second one is the signal (S). The S/N ratio quantify the reproducibility of metrics: the authors used a ratio of s/n ≥ 2 to accept the metric.

The variance among lakes is always highly significant, whereas the variance among samples in the same lake is not significant in most cases. Exceptions are given by the SPI metric for NGIG lakes, by the MFGI for N- and CB-GIGs and by FTI in CB-GIG.

Quality classes boundaries and calculation of EQR values

Because of the better response of the multimetric index FTI to the trophic gradient, this metric has been chosen to test the difference between reference and not reference lakes and to calculate the boundaries among the five quality classes and the respective EQR values. The classification of the 228 lakes in reference and not reference sites was based on the information available in the WISER lake database: the final comparison between the two groups has been carried out after the exclusion of the Lithuanian reference lakes, because of their TP concentration, around 20 μl^{-1}, was considered too high. The difference of the two groups of lakes was tested by the *t-test* and resulted highly significant at a $p<0.00001$.

Figure 8 shows the median value and the range of variability of the FTI metric in reference vs. not reference lakes.

The median of the reference lakes group was established as the reference value of the metric, corresponding to the FTI value 1.591. The value corresponding to the 90% quantile of the reference lakes (1.602) has been chosen as H/G threshold.

The G/M, M/P, P/B thresholds were calculated through the relationship between TP and FTI: the dataset has been divided in 10 TP classes, from 4-13 $\mu g\ l^{-1}$ to 404-727 $\mu g\ l^{-1}$, in order to evaluate the degree of change of FTI across increasing TP steps.

Fig. 9 shows median and ranges of variability of FTI in the different TP concentration classes: there is a clear increase of FTI until the class 36-53 $\mu g\ l^{-1}$ and then the metric is more stable.

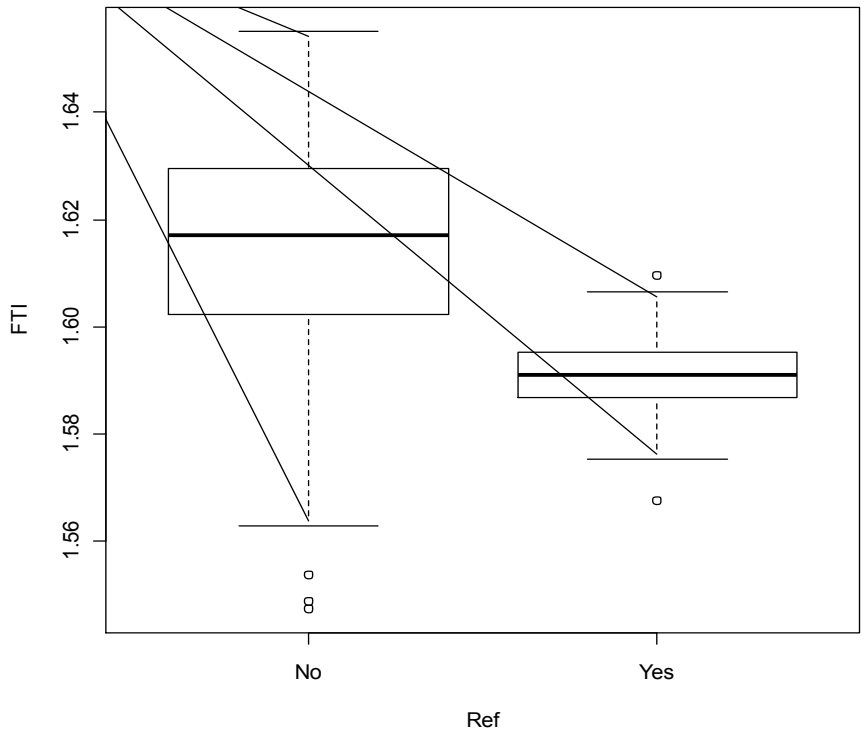

Fig.8 Median and range of variability of FTI Index in reference and not reference lakes.

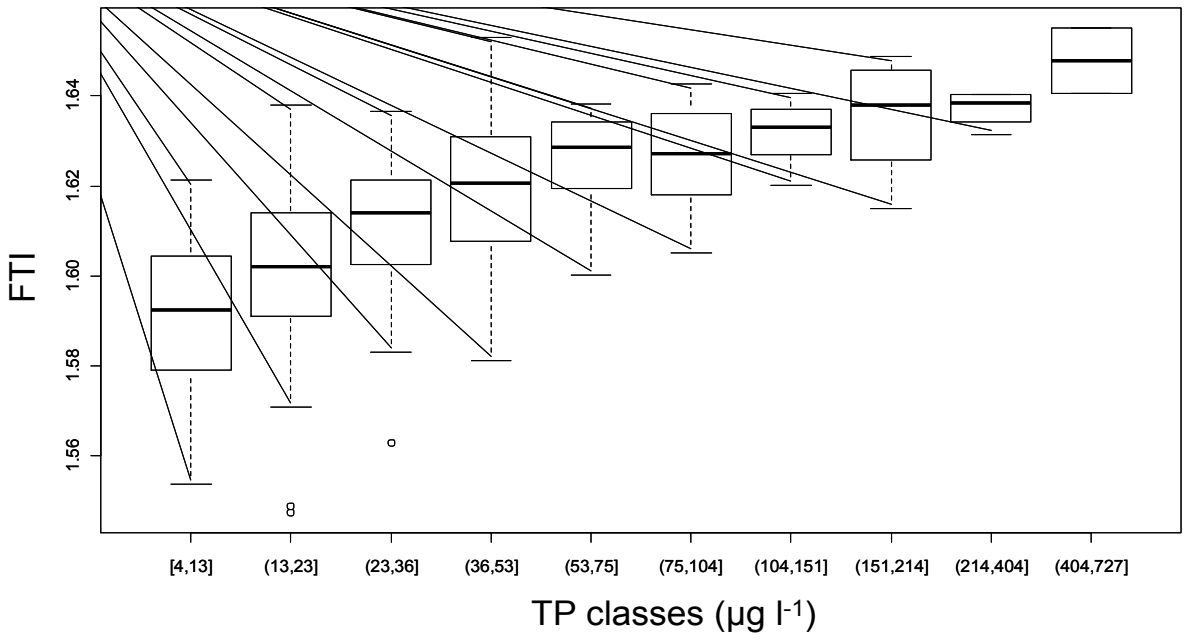

Fig.9 Median and range of variability of FTI Index across increasing TP concentration.

The first class (4-13 µg l^{-1}) includes the reference sites and its mean is significantly different from the second class (13-23 µg l^{-1}) mean (*t-test*; $p<0.001$): this significant change in the metric

value can be seen as a shift between high and good quality status, therefore, the G/M threshold has been set inside this TP class, as the 90% quantile. At the extreme of the linear FTI increase there is the TP class 53-75 µg l^{-1}: only non significant changes of FTI were observed with increasing TP concentrations, so the lakes included in the above TP classes were considered to be in bad quality status. The P/B threshold was set as the 90% quantile of this class. The M/P threshold cannot be immediately derived from the box and whisker plots of FTI vs. TP classes and has been obtained by plotting FTI thresholds against the corresponding normalised EQR, assuming that EQR=1 at the reference FTI value and the H/G, G/M and P/B thresholds correspond to EQR values 0.8, 0.6, 0.2 respectively. EQR values have been calculated using the following equation:

$$EQR_{FTI} = \left(\frac{FTI_{obs} - FTI_{\max}}{FTI_{ref} - FTI_{\max}} \right)$$

FTI thresholds and corresponding EQR values are reported in Table 7.

Table 7. FTI values and corresponding EQR at quality classes thresholds.

Thresholds	FTI	EQR
Reference	1.591	-
H/G	1.602	0.822
G/M	1.614	0.641
M/P	1.625	0.470
P/B	1.636	0.299

Discussion

The use of metrics based on functional traits for describing the relationship between water quality and phosphorus abundance in a lake is not a completely new approach: the classification of phytoplankton assemblages by functional groups (Reynolds et al., 2002) takes into account some parameters commonly affected by trophic change. The analysis presented in this chapter is aimed at the elaboration of water quality indices based on phytoplankton functional traits and represent the first proposal for a quantification of the relationship among functional traits and trophic status. Two types of functional traits were considered: the size spectrum of taxonomic units, following the approach described in Kamenir & Morabito (2009) and the classification of *taxa* in Morpho-Functional Groups, as described in Salmaso and Padisak (2007). Trophic scores and indicator values, derived for each size class and M-F Group, were used to calculate a couple of quality indices, called Size Phytoplankton Index (SPI) and Morpho-Functional Groups Index (MFGI).

As a general pattern, the size-based phytoplankton classification seems to represent the relationship with the trophic gradient better than the MFG classification, as suggested by the results of the PCA analysis. This finding is in agreement with the pattern of nutrient exploitation

in phytoplankton: many observations (see review by Litchman et al., 2010), point out that smaller cells are advantageous in nutrient competition under nutrient limiting condition, due to both their high surface-to-volume ratio and to their higher uptake rate. On the other side, the MFG classification, considering a single size threshold for separating small and large taxa (30-40 µm; Salmaso and Padisak, 2007), is perhaps less sensitive in pointing out these physiological properties.

The general relationship of the two metrics with TP has shown a significant response to the phosphorus gradient, although the analysis of this relationship at a GIG scale deserves further attention. Both indices were significantly correlated with TP concentration in the three GIG considered (N, CB and M): however, in two cases, namely the relationship TP vs. MFGI in N-GIG and SPI vs. TP in M-GIG the correlation was less powerful. In the N-GIG this is due to an increase of scattering of the MFGI value in the TP range 6-15 µg l^{-1}. This phosphorus range mostly includes the Swedish lakes and the Northern UK lakes. The first ones are dominated by MF Groups 1 and 2, including chrysophytes and cryptophytes respectively: the low values of the metric are in agreement with the low trophic status of these lakes, but the high scattering of the points indicates a weak response to the TP gradient. Considering that both chrysophytes and cryptophytes include species with myxotrophic metabolism, this could explain their lower dependence on P availability. On the other side, the Northern UK lakes are scattered towards high values of the metric: these lakes are mostly dominated by MF Groups 6a and 6b, including large diatoms, both centric and pennate. These diatoms, due to their large biomass, could determine an upward shift of the metric even in oligo-mesotrophic lakes. Probably due to the dominance of the large diatoms, in the Northern UK lakes there is a non-significant relationship between MFGI and TP. The relationship is, on the other side, good, considering size classes, because in the SPI metric the scores of the higher classes are quite low.

Concerning the relationship SPI vs. TP in the M-GIG, the low *p* value can be explained by the position of the Italian lakes: two of three are characterised by high SPI value (see Fig. 3), but low TP concentration and the third one by a low SPI and high phosphorus. The classification with SPI is in agreement with the lack of small size classes in the first two lakes: the uncoupling between the metric and the TP value could be due to the low summer TP concentration, very common in the Mediterranean lakes, where, even in the eutrophic lakes, the nutrients supply during summer is mostly connected with the recycling processes rather than with the in-lake storage. Probably, in these types of lakes, the use of TP at mixing, instead of TP during the summer season would give better results. Concerning the third Italian lake (Lake Arancio), this is dominated by small Chroococcales, assigned to a small size class on the basis of cell volume. As a consequence, the SPI value is low, in spite of the high TP concentration: this could be a condition where the use of SPI metric could bring to a wrong quality classification. In fact, a critical point in using the size based classification could be the correct attribution of the colony forming species to a proper size class: our results indicate that, for some selected colony forming species, the use of the cell volume or of different volumes for colonies do not modify,

in most cases, the response of the SPI metric to trophic changes, except, perhaps, for those lakes strongly dominated by small colony-forming species.

The existence of some confounding response of the functional traits to the changes in trophic status and the finding that the relationships are dependent on some peculiarities of the lakes analysed, possibly related with their geographic position, seems to affect, at GIG level, the use of the two functional metrics considered. Moreover, the variable response of SPI and MFGI in the same group of lakes, necessarily means that both metrics should be always used for classification. The possibility to use a combined index would result in a simplification of the final calculation for lake classification: the arithmetic mean of the two functional metrics resulted in a significant improvement of the relationships with LogTP in both NGIG and MGIG lakes, overcoming the problems above mentioned. As a consequence, the global fit (all lakes dataset) also improved. The new combined metric, called Functional Traits Index (FTI), is, therefore, the one suggested for classification: for this metric only quality classes threshold values and EQR are provided.

In order to test the robustness of the combined metric, both SPI and MFGI have been evaluated in this respect. The analysis of the robustness on the whole dataset shown that, for both metrics, the variability due to among lakes differences (Signal) is much more significant than the variability due to different samplings in the same lake (Noise). The MFGI metric gave the worst results: in CB-GIG and N-GIG the among-site variability is almost high as the variability among samples. Concerning CB-GIG, this result is probably due to the high seasonal variability recorded in the Lithuanian lakes, whereas in N-GIG the changes in the contribution of the MF Groups is very pronounced during the growing season in Swedish and Finnish lakes.

A final test was carried out on the robustness of the combined FTI metric: the high seasonal variability of MFGI in CB lakes affected the results of this analysis, although the difference in the significance between lakes variability and season variability indicate that the metric is robust enough.

For most of the lakes included in the dataset only four sampling per year were available, usually taken between June and September: perhaps, considering only samples collected during the same seasonal window could affect the results of the robustness analysis, because the seasonal variability of the phytoplankton assemblage is greatly reduced. However, for a smaller part of the lake dataset, it has been possible to evaluate the robustness over a longer growing season (April-October): in the UK and German lakes, where 6 sampling dates were considered, the S/N ratios for FTI amounted to 19 and 105 respectively.

Conclusions and recommendations

The response of the functional traits to trophic gradient appears to be promising towards the use of these ataxonomic composition metrics for lake quality evaluation. The repeatability of the results using data from different sampling (high S/N ratio) confirms this finding.

As a general pattern, size spectra seem much more sensitive to trophic changes, with a relationship strongly driven by the increase or decrease of the smaller size classes. However, the lacking or the low importance of these small size classes in the upper part of the trophic spectrum, could give a weaker response to TP.

On the other side, the Morpho Functional Groups, shown, in some cases, confounding relationships with the TP gradient and a higher variability respect to the seasonal changes of assemblage composition.

The use of a multimetric index, called FTI, that is a simple arithmetic mean of size classes and MF Groups indices, allowed solving the problems due to the contrasting response of size classes and MF Groups in some lakes, thus improving the relationships with LogTP as well as the discrimination between reference and not reference sites.

Concerning the correct attribution of the colony forming species to the proper size class, the test carried out on some selected colony forming species indicate that, in general, the use of the cell volume or of different volumes for colonies do not modify the response of the SPI metric to trophic changes. However, care must be taken under the strong dominance of colony forming *taxa* (i.e. small celled Chroococcales), where the attribution of these organisms to the proper size class could be critical for the correct classification of the lake.

Therefore, the use of FTI is recommended, instead of each of the other two metrics (SPI and MFGI) separately: because of this reason, for FTI only, quality classes thresholds and EQR values are reported.

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Appendix 1 – Trophic scores and indicator values of size classes and MFGroups

Size classes			Morpho Functional Groups		
Class	*TS*	*VI*	*MFGroup*	*TS*	*VI*
V-0.3	1.35	2	1a	1.49	3
V0	1.61	6	1b	1.54	3
V0.3	1.45	5	1c	1.72	5
V0.6	1.35	1	2a	1.52	1
V0.9	1.54	3	2d	1.63	2
V1.2	1.78	4	3a	1.59	3
V1.5	1.66	3	3b	1.69	6
V1.8	1.59	4	5a	1.68	4
V2.1	1.68	3	5b	1.73	6
V2.4	1.64	3	5c	1.39	1
V2.7	1.63	3	5d	1.58	3
V3.0	1.64	3	5e	1.72	5
V3.3	1.52	3	6a	1.69	3
V3.6	1.62	3	6b	1.58	2
V3.9	1.54	3	7a	1.58	4
V4.2	1.54	2	7b	1.59	5
V4.5	1.38	3	8a	1.60	2
V4.8	1.22	1	9a	1.34	1
V5.1	1.55	3	9b	1.62	2
			10b	1.49	2
			10c	1.59	6
			11a	1.73	3
			11b	1.64	2
			11c	1.15	6