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Chapter 1 Introduction

The enrichment of ecosystems with plant nutrients, or eutrophication, is one of the most widespread pressures affecting European freshwaters. There are numerous socio-economic problems associated with eutrophication, particularly with increasing frequency and intensity of harmful algal blooms (HABs). These include detrimental effects on drinking water quality, filtration costs for water supply, access for water-based activities, and conservation status (particularly sensitive fish species, such as salmonids and coregonids). Phytoplankton blooms, are a widespread feature of freshwater lakes across lowland Europe. There is strong evidence that the development of phytoplankton blooms has been increasing in both frequency and intensity in recent decades (Smith 2003). This is widely believed to be due to nutrient enrichment, but also in response to warmer and drier summer conditions (Paerl & Husiman 2009, Weyhenmeyer et al. 2002). In this report we examine two candidate metrics for phytoplankton blooms for use as common metrics in Europe in the WFD Intercalibration (IC) process.

Common Metrics: the IC Process

Introduction to WFD and IC process and need for common metrics – **to be added.**

Steps in development of a common metric (Hering et al., 2010):

Step 1: Setting the background

Step 2: Identification of candidate metrics

Step 3: Testing the relationship of candidate metrics and national assessment methods

Step 4: Testing the relationship of candidate metrics and stress gradients

Step 5: Testing the robustness of candidate metrics

Step 6: Metric selection

Step 7: Normalisation of metrics

Step 8: Combination to a multimetric index

Phytoplankton Bloom metrics: the background

The EC Water Framework Directive (WFD) requires the ecological status of water bodies to be assessed on the condition of their biological quality elements (Article 8, annex V). For lakes this includes phytoplankton. Annex V of the WFD outlines three features of the phytoplankton quality element that need to be considered in the assessment of the ecological status of lakes:

1. Phytoplankton composition
2. Phytoplankton abundance and its effect on transparency conditions

3. Planktonic bloom frequency and intensity

Classification schemes for phytoplankton abundance, and its effect on transparency conditions, have been established based on phytoplankton chlorophyll *a* (Carvalho et al., 2006; Sondergaard et al. 2005) and chlorophyll schemes have been successfully Intercalibrated to ensure standardised quality classes exist across regions of Europe (Poikane et al., 2010). Several schemes also exist for phytoplankton composition (Mischke et al., 2008; Pasztaleniec & Poniewozik 2010, Wolfram et al., 2009; others). There has, however, been least progress in developing classification schemes for phytoplankton bloom frequency and intensity. No consistent definition for an algal bloom even exists across Europe. The metric should, however, incorporate some measure of both bloom intensity (spot measures of magnitude/abundance) and how frequently they occur (or potentially could occur) over a particular specified time period (e.g. within a 3 monthly summer sample or over 6 year WFD reporting period).

Further relevant information in the WFD includes the normative definitions for phytoplankton in lakes associated with five ecological status classes. These definitions indicate that declining ecological quality is associated with more frequent and intense phytoplankton blooms; blooms becoming persistent during summer being a characteristic for defining moderate and poor status (Table 1.1).

Table 1.1 Qualitative criteria for assessing Ecological Status in terms of eutrophication impacts on phytoplankton (modified from ECOSTAT Eutrophication Guidance, 2005)

Ecological Status	WFD normative definition	Primary impacts on phytoplankton	Secondary impacts on phytoplankton
High	Undisturbed conditions or minor changes	None	None
Good	Slight change	Slight changes in composition, abundance or frequency and intensity of blooms	None
Moderate	Moderate change	Moderate change in composition and abundance begins to have significant undesirable disturbance. Persistent blooms may occur in summer. Pollution tolerant species more common	Occasional impacts on other biological elements, transparency and oxygen
Poor	Major change	Pollution sensitive species no longer common. Persistent blooms of pollution tolerant species	Secondary impacts common & occasionally severe.
Bad	Severe change	Totally dominated by pollution tolerant species	Severe impacts common

Definitions of phytoplankton blooms

A bloom is generally perceived as a significant increase in biomass, meaning there is an unbalance between phytoplankton growth and loss processes. The spring diatom bloom is a regular phenomena in most temperate lakes (i.e. most lakes in Europe) and is a response to increasing daylength and temperature and a replenished supply of nutrients (particularly silica) over winter. The spring diatom bloom is terminated by a number of factors, including stratification/sedimentation, silica depletion and early summer increases in zooplankton grazers. The spring bloom initiation, intensity, and duration is, therefore, driven largely by factors other than trophic status (nitrogen and phosphorus) of the water, which would make it difficult to establish relations between spring bloom intensity and nutrient pressures.

The normative definition has a more specific focus on persistent algal blooms in summer. For this reason algal blooms will only be considered for the summer growth season. There are a number of characteristics of a phytoplankton bloom:

- High phytoplankton abundance relative to typical levels of abundance for that time of year.
- Uneven community – dominance by one or two species
- Abundance of nuisance species e.g. potentially toxic cyanobacteria, phytoplankton associated with strong odour problems

A number of potential (candidate) metrics could, therefore, be considered to represent algal blooms and these two will be examined in the following two chapters:

- Evenness of phytoplankton community (incorporating chlorophyll maxima)
- Cyanobacteria: actual biovolume or relative % biovolume

Chapter 2: Evenness metric

Introduction

The aim of this study is to develop a robust measure that relates phytoplankton diversity to eutrophication pressure. Phosphorus concentrations are used as a proxy for pressure. The rationale background to analyse diversity of phytoplankton is that

- Diversity, and especially evenness, is expected to decrease during blooms
- Evenness is an established metric in the Estonian phytoplankton method (Ott 2006 modified in Birk 2010)

Evenness is not as greatly influenced by the number of taxa detected in a sample as other diversity indices, like species richness or the Shannon index of diversity (Spatharis et al. 2011). Species richness is influenced strongly by the skill and effort of the phytoplankton analyst. Furthermore, species richness is related to productivity of lakes in a hump-shaped distribution (Mittelbach et al. 2001): Few species are generally found under oligotrophic conditions, followed by an increase in taxa number in eutrophic waters and a decline under hypertrophic conditions. In contrast, evenness measures the balance in contribution of taxa to total abundance or biomass, and is less confounded by issues of species identity (Wilsey & Potvin 2000). Concerning the focus of this study, evenness is expected to be a sensitive measure of algal blooms even in situations with a species-rich community, but a strong dominance of few species above and beyond the sampling effect. Eutrophication as the main stressor for phytoplankton is expected to affect evenness by increasing the probability that a few tolerant species with higher growth rates can dominate. Longhi and Beisner (2010) found evenness to decrease with total phosphorus concentrations in Canadian lake phytoplankton.

A phytoplankton community with a high evenness index (J') is thought to be less sensitive to stress and more stable (Steiner et al. 2005). Ptacnik et al. (2008b) found a positive effect of high evenness on the resource use efficiency. Both findings underlie that a high evenness in the phytoplankton community is a character which is expected to be found in undisturbed lakes. Such lakes are marked as reference lakes in the European intercalibration process, and are detected by a low level of land use in their lake catchment areas (Poikane et al. 2010). This group of reference lakes can be used as the reference also for the diversity characteristics of different lake types.

The summer period was chosen for the analysis of evenness as summer algal blooms are more routinely monitored across Europe. The summer period is also more in accordance with the normative description in Annex V of WFD. According to Hutchinson's diversity-disturbance relationship in phytoplankton (Sommer et al. 1993), the phytoplankton diversity is expected to be lower in steady state conditions, as is characteristic of summer, than in transition periods (spring, autumn, mixing or flushing events in shallow lakes). So it may be problematic to detect nutrient pressure effects on phytoplankton evenness in summer and in very shallow lakes. It has, however, been demonstrated by several authors that steady-state equilibria can be established

in summer by cyanobacteria even in very shallow lakes (Mischke & Nixdorf 2003; Nixdorf et al. 2003, Stoyneva 2003) or in Mediterranean reservoirs (Nasseli-Flores & Barone 2003).

Methods

Data

Phytoplankton data were taken from the WISER data base WP31_data_2010_08_04.mdb provided by the WISER and GIG partners (see fig. 1.1). It contains taxonomically harmonized phytoplankton data from 1590 lakes spanning 4 GIGs; the bulk of the data were from the Northern (890 lakes) and Central-Baltic GIGs (602 lakes; Table 3.1). A detailed description of the data base is given in the WISER deliverable 3.1-1 (Phillips et al., 2010: chapter 2). TP data were widely available and used to represent nutrient pressure. The environmental data were screened and water bodies with total phosphorus concentrations outside of the range of 1-1000µg l⁻¹ were omitted. Both evenness index values, chlorophyll a and nutrient data were summarised as a summer mean abundance using data spanning the months July, August and September. For each lake, only the last year of available data was used in the analysis, except of 120 N-GIG lakes, which had been introduced to the data base with a second, more recent investigation series under new lake codes. Data were initially extracted using the data extraction tool (Dudley 2010), this ensured a consistent approach to averaging and matching environmental and biological data. Following this screening, data from 1704 lake years were available for analysis.

The evenness index was calculated for each single sample according to Pielou's evenness index:

$$J' = \frac{H'}{H'_{\max}}$$

H' max is the maximum value of H' (Shannon index), if all taxa are even distributed. As in the national Estonian method the taxa contribution to total biovolume (=biomass) is investigated.

Since up to 100 species were detected in some samples whilst the examination of others revealed only 3 – 10 species, the taxonomic level of determination was strongly uneven in the whole data set. The influence of the different skill and effort among individual phytoplankton analysts to affect the evenness index calculation was of concern. Thus, a sub-data set was constructed by restricting the number of taxa to 20 per sample by selecting only the most 20 abundant taxa.

Statistical analysis

Statistical analysis was carried out using SPSS 9.0. Scatter plots and simple linear regression of evenness (response) to total phosphorus concentrations (TP) (pressure) were applied to check pressure sensitivity in the whole datasets and in sub-datasets for each lake type.

To visualize the response and response range, box plot graphs were explored by dividing the TP scale into TP classes with ecological meaningful boundaries.

Box plots and t-tests were carried out on the reference lake data set in comparison to the impacted lake group, which were identified by the chlorophyll a mean concentration from July to September with the agreed lake type specific threshold at good / moderate boundary (G/M).

Linear regression models for N and CB GIG lake data sets were developed for evenness to TP.

Critical bloom densities (CBD) were defined as when chlorophyll a was more than 150% G/M concentrations. These CBDs were used to check the influence of bloom intensity (chlorophyll maximum) on evenness in comparison to situations of low evenness and low chlorophyll a concentrations.

For index development, CBD and linear regression models with evenness for N or CB GIG were tested combined or alone.

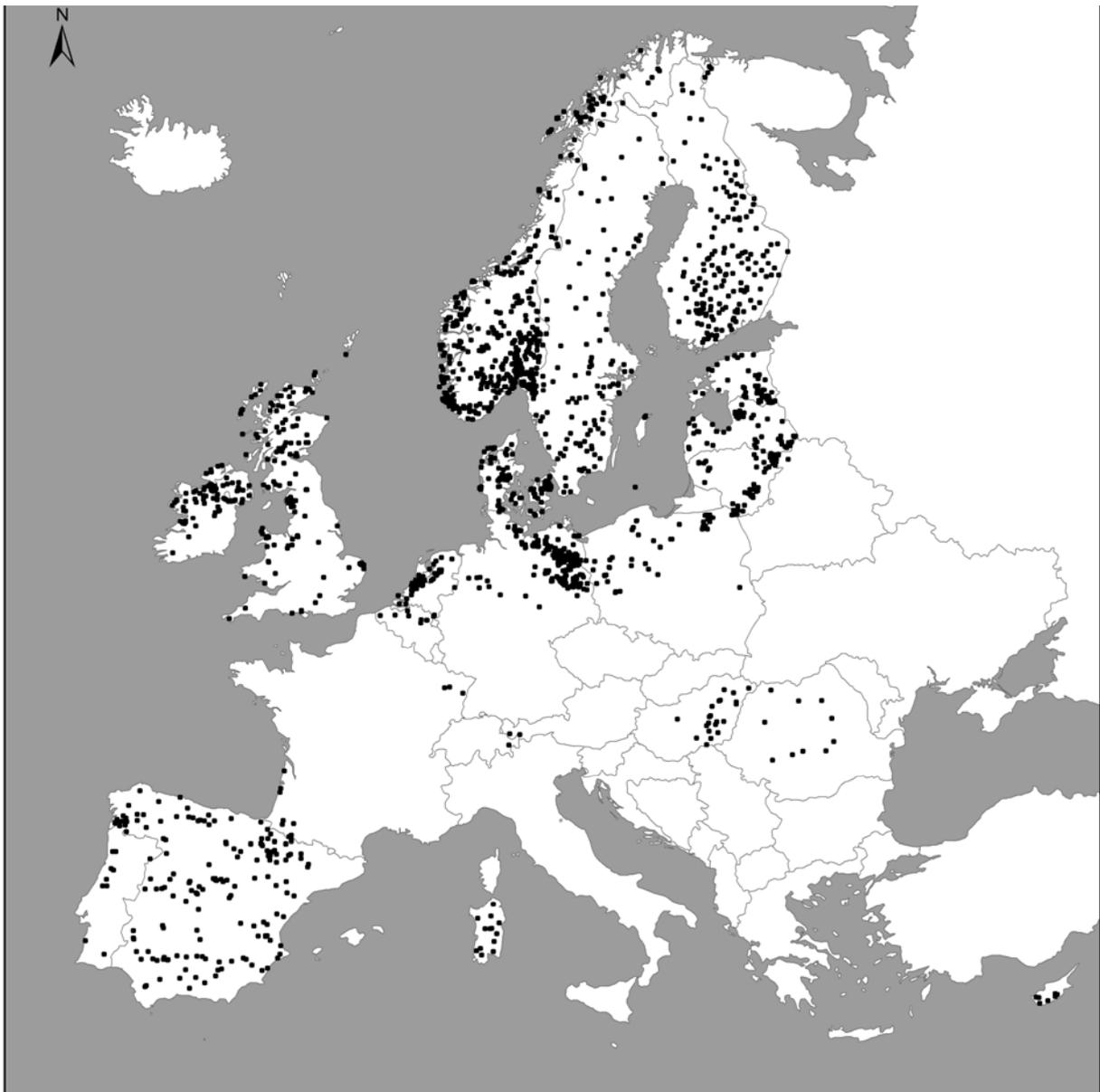


Fig 1.1: Distribution of the 1590 lakes from all types and regions in WISER data base.

Results

Pooling all lake data together, there is a slight sensitivity of the evenness response to nutrient pressures (represented by total phosphorus concentrations) ($r^2 = 0.1395$ linear regression). Pooling the data into ecological meaningful TP classes, an overall decrease of evenness with increasing TP class can be observed (Fig 2.1). Still, the ranges of evenness in each TP class overlap strongly. Thus, the differences between the subsequent TP classes are not significant.

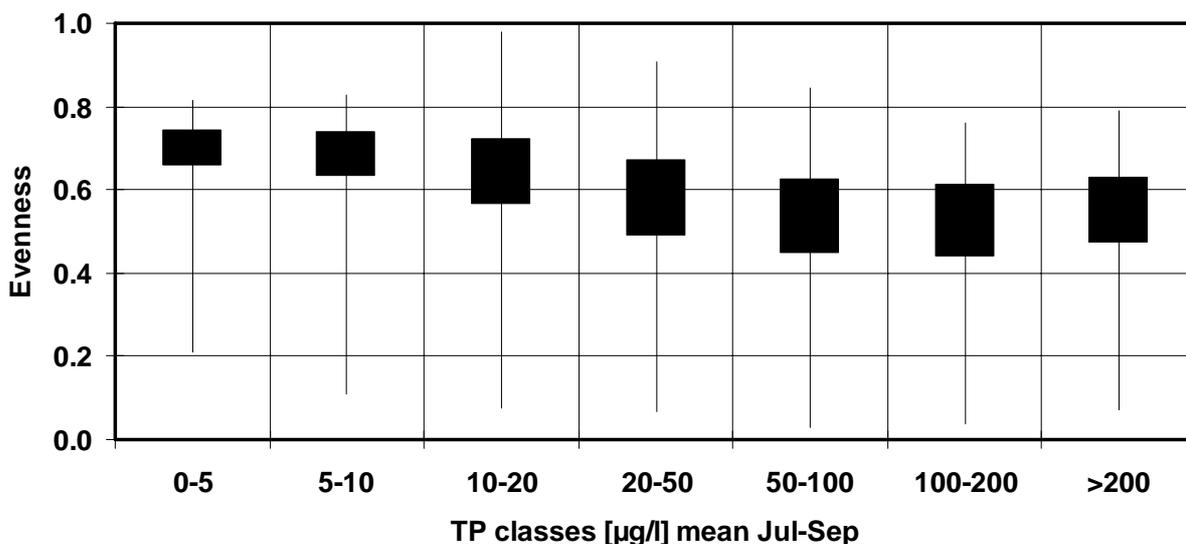


Fig 2.1: Box plots of observed mean evenness in seven TP classes in 1649 lakes from all types and regions in WISER db pooled.

Separating the data set into lake types, evenness was significantly correlated to TP in 2 lake types of the Central Baltic (CB) and in 7 types of the Northern (N) GIG (see table 2.1).

There were too few lakes or no significant relationship between evenness and TP in the following lake types: L-EC2, L-CBU, L-CBX, L-MU, L-N10, L-N11, L-N3, L-N5, L-N6a, L-N6b, L-N7, L-N8 and L-N8b.

In general the relationship of evenness to TP shows a negative trend. The observed decline was much steeper in the sensitive lakes of the N-GIG than in the CB-GIG (grouped in last rows in table 2.1). There was one exception with a positive trend of evenness to TP: the *Lobelia*-lake type L-CB3, in which reference lakes also have a low evenness in their phytoplankton community (mean $J' = 0.54$).

In order to find a critical TP value when evenness significantly decreases, the N- and the CB-GIG datasets were subdivided along a subsequently lowered test TP threshold: The first significant differences in evenness (0.001 Spearman correlation and T-Test) between the subgroups were found at TP level above and below $25\mu\text{g/l}$ in both GIG's (see also fig. 2.2). In the

group with TP concentrations below 25µg/l, the evenness was on average 1.0 unit lower than in the group with TP >25µg/l.

Tab. 2.1: Correlation factor of linear regression of TP log to evenness index (J') in lake types (N = cases per type), number of reference lakes, J' mean of reference lakes with standard deviation (SD) and test of difference of reference lakes to other lakes of this type.

Lake type	Lake type characters	N	Spearman corr	N reference lakes	mean and SD of J' in reference lakes	p = Levene T-test ref to others
L-EC1	Eastern Lowland; shallow?; high alkalinity	16		0		
L-CB1	Lowland; shallow; high alkalinity; retention time 1 - 10y	360	-0.300***	31	0.64 ±0.119	0.141
L-CB2	Lowland; very shallow; high alkalinity; retention time 0.1 - 1y	199	-0.247***	4	0.69 ±0.140	0.993
L-CB3	lowland; silicious; shallow; small; Lobelia type)	24	0.090	9	0.54 ±0.106	0.311
L-M7 ^{a)}	Med. Reservoirs, deep, large, siliceous, 'wet areas'	8	-0.143	0		
L-M8 ^{a)}	Med. Reservoirs, deep, large, calcareous	29	-0.006	0		
L-N1	Lowland; shallow; moderate alkalinity; clear	66	-0.494***	18	0.70 ±0.071	0.070**
L-N2a	Northern Lowland, shallow, low alkalinity, clear)	101	-0.237**	58	0.71 ±0.058	0.000***
L-N2b	Northern Lowland, deep, low alkalinity, clear	98	-0.136	71	0.68 ±0.105	0.475
L-N3a	Northern Lowland, shallow, low alkalinity, meso-humic	132	-0.477***	58	0.65 ±0.149	0.680
L-N3b	Northern Lowland,*XXXX	28	-0.367	8	0.61	0.153
L-N8a	Northern Lowland, shallow, moderate alkalinity, meso-humic)	58	-0.566***	13	0.70 ±0.082	0.216
L-N9	Northern Lowland,*XXXX	33	-0.232**	22	0.69 ±0.055	0.000***
L-NU	Northern Lowland, missing typology data	88	-0.483***	11	0.68 ±0.085	0.059
L-NX	Northern Lowland, lakes not covered by typology	238	-0.437***	111	0.66 ±0.113	0.000***
N-GIG sensitives	Group: N1, N2a,N3a, N8a,N9, NU,NX	848	-0.436***			
CB-GIG sensitives	Group: CB-1, CB-2	559	-0.261***			

Footnote -legend to Tab.2.1: *** $p = 0.01$; ** $p = 0.05$

^{a)} = the WISER data set of the Mediterranean lakes and reservoirs was not completed for lake types and TP concentrations for the analysis. For a more detailed test of evenness to pressure data, see the discussion section.

The linear regression model between J' and TP was significant (Anova $p = 0.00$) but with high residuals for

CB-GIG sensitive lakes types:

$$J' = 0.704 + -0.078 * \log TP \quad (r^2 = 0.057)$$

N-GIG sensitive lakes types:

$$J' = 0.777 + -0.140 * \log TP \quad (r^2 = 0.152)$$

Tab. 2.2: Predicted values of evenness (J') by $\log TP$ in sensitive lake types of CB GIG and N GIG based on linear regression models. TP ranges not covered by data are left empty. Mean J' in group of reference lakes is given.

TP $\mu\text{g/l}$ example	$\log TP$	J' CB GIG	J' N GIG	J' in ref lakes CB	J' in ref lakes N	EQR normalized	
1	0.000		0.777				
2	0.301		0.726				
				0.648 \pm 0.1143	0.673 \pm 0.1114	1	REF
5	0.699	0.649	0.658				
10	1.000	0.626	0.607				
25	1.398	0.595	0.539	0.595	0.539	0.6	G/M
50	1.699	0.571	0.488				
100	2.000	0.548	0.437				
200	2.301	0.525	0.386	0.525	0.386	0.8	P/B
1000	3.000	0.470					

The two linear regression models are applied to the data as a metric and tested in combination with a critical bloom density value. The G/M boundary is set at $25\mu\text{g/l}$ TP as a (weak) breaking point indicated as the point of first significant change of evenness (see text above).

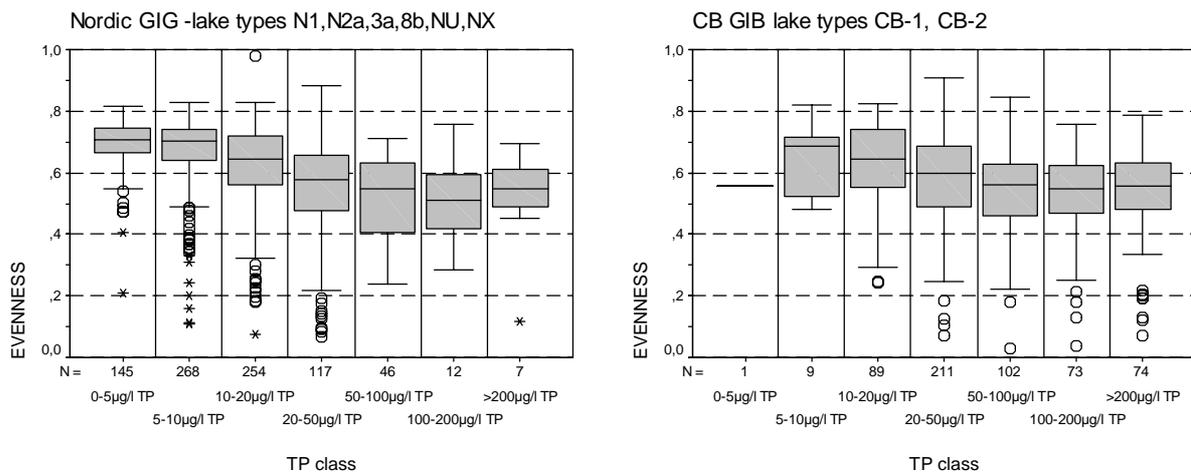


Fig 2.2: Box plots of observed evenness in seven TP classes in lake types with detected significant sensitivity of evenness to TP (see Tab. 2.1).

Test of sensitivity of evenness in selected seasonal period

The summer period was chosen for evenness analysis of summer algal blooms as this is in accordance with the normative description in Annex V of the WFD and, for practical purposes, most samples were available for this period. According to figure 2.3, the period from July to September is suitable as a sensitive period to observe a decline of evenness with increasing pressure.

Phytoplankton diversity was expected to be higher in transition periods (spring, autumn) than in steady-state conditions, such as during summer stratification. A decline of evenness from spring to summer can be observed in our data set, although this pattern is slight; the most obvious gradient in evenness in relation to TP occurred in March (Figure 2.3). This month is, however, not sampled in all regions of Europe, mainly in lakes of the CB GIG.

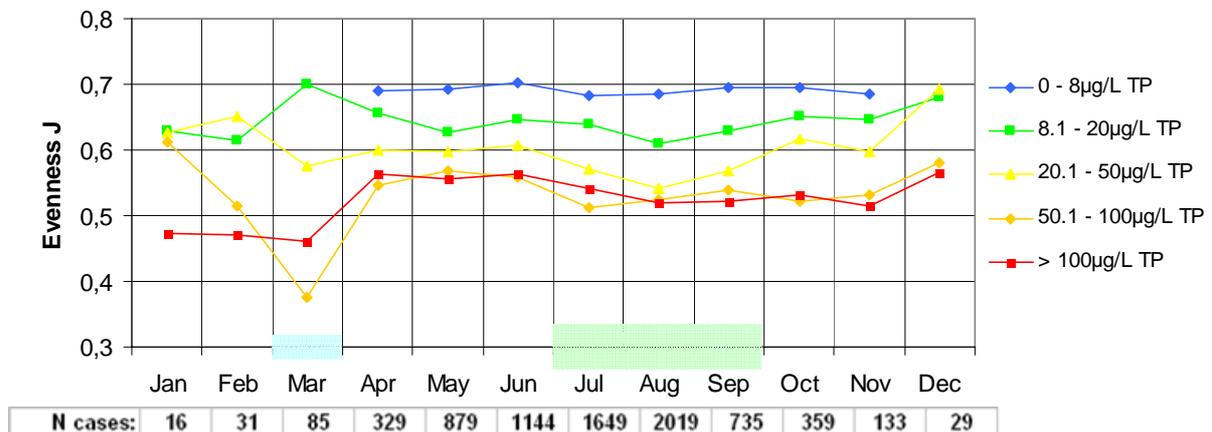


Fig 2.3: Seasonality of evenness (monthly means) in 6 TP classes (all lake types and regions in WISER db pooled). At the bottom the numbers of samples per TP class are given.

Examination of the variability of evenness in reference lakes

The reference lakes were examined in order to describe the natural range of evenness in unimpacted lakes and to use the mean of evenness in reference lakes as the anchor point of the index scale. Reference lakes were marked in the data base by the data providers but were not available for all lake types in the data set (see table 2.1). The mean of evenness within the available reference lakes ranged between 0.64 and 0.71 per applicable lake type except for L-CB3 reference lakes which had a very low J' index of 0.54 (see also fig 2.4). This lake type was not included further in the evenness analysis as they appear to be less sensitive to TP.

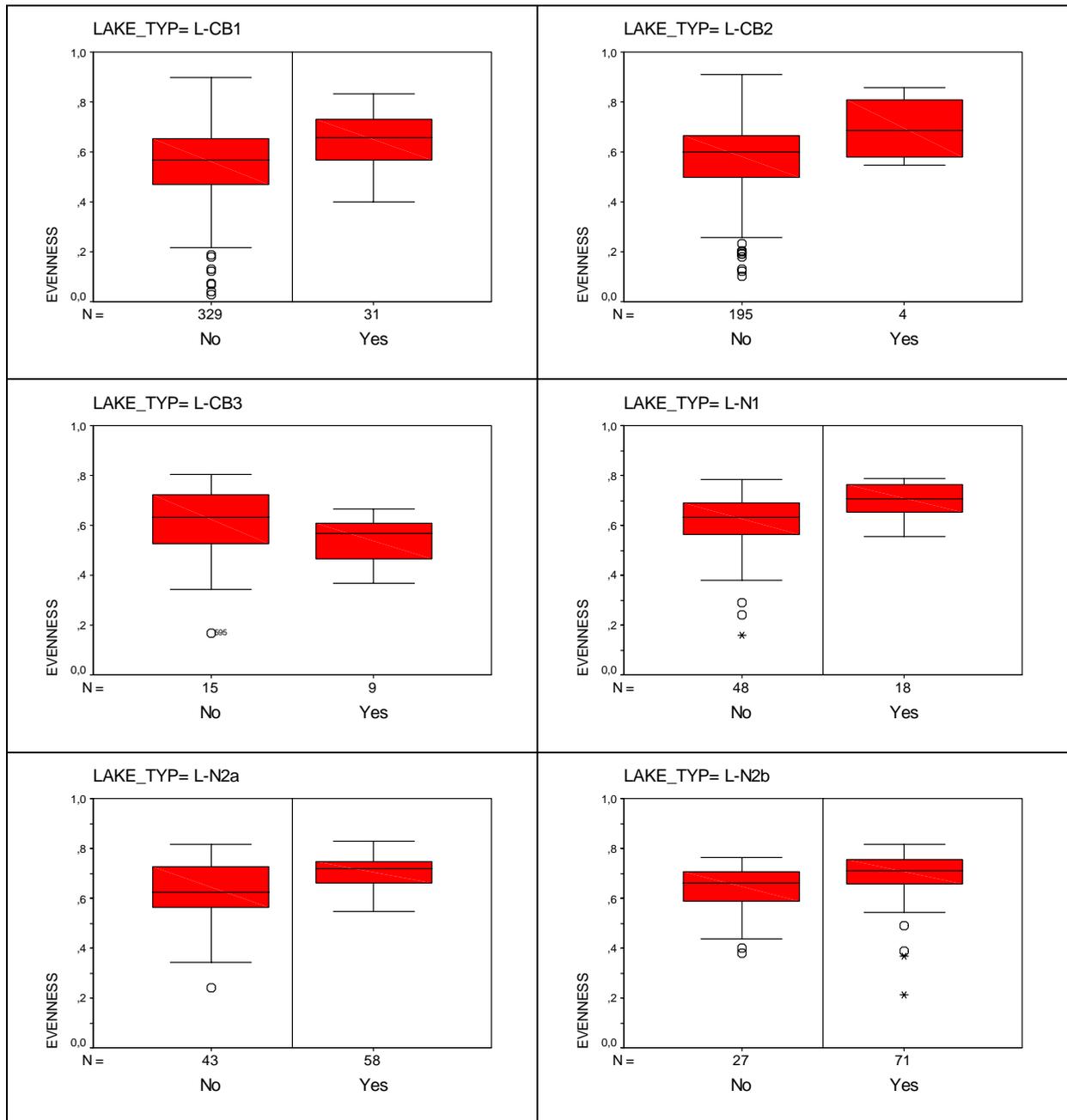


Fig 2.4: Distribution of evenness (means Jul-Sep) in reference lakes (YES; right box plot) and in other lakes (Lake group “No”) shown for selected lake types (see also table 2.1). At the bottom the numbers of cases are given.

When t-tests were carried out on the reference lake data set in comparison to non reference lakes, the difference between these groups were significant only in lake types L-N2a, L-N9 and L-NX (see table 2.1 in last column).

Chlorophyll a as a co-dominant factor in an evenness metric

In this section, two characteristics of blooms are integrated: evenness in combination with thresholds for maximum chlorophyll a concentrations. What is the reason to combine both

aspects? During the seasonal succession of the phytoplankton, diversity can be low not only because of high nutrient pressure, but also in other ‘stress’ situations such as high grazing pressure by zooplankton, co-limitation by other key elements (e.g. Fe or Mn) or possibly other reasons such as shading by suspended sediment. In these cases, the phytoplankton may be dominated by a few taxa but their density, or abundance, may still be low. These situations should not, therefore, be classified as a bloom. For this reason, the density of a bloom should be checked before any evenness measure is applied; the phytoplankton density should be clearly above the seasonal mean chlorophyll a concentration expected for that lake type.

Assumption: Blooms are only relevant, when the chlorophyll a concentration is clearly above the lake-type specific G/M boundary. In this analysis, a critical bloom density (CBD) is defined when chlorophyll a concentrations are a factor of x1.5 the G/M boundary value taken from the IC decision table (European Union C (2008) 6016).

Table 2.3: Definition of critical bloom densities per lake type by chlorophyll a (chl_a µg/l) to combine with an evenness metric.

IC lake type ID	critical bloom density (1.5 * G/M)	G/M chl_a	% bloom density found in Jul-Sep mean of chl_a
EC-1	75.00	50*	18.8
L-CB1	15.00	10	40.4
L-CB2	34.50	23	44.2
L-CB3	15.00	10	25.0
L-M7	12.15	8.1	42.9
L-M8	7.65	5.1	19.4
L-N1	13.50	9	15.2
L-N2a	10.13	6.75	5.0
L-N2b	9.00	6	1.0
L-N3a	15.00	10	6.1
L-N5	6.75	4.5	1.8
L-N6a	11.25	7.5	5.7
L-N8a	19.13	12.75	12.1

* value supposed; all others from EU intercalibration decision table EU C(2008) 6016

71% of all lakes in the data set could be classified using these bloom density thresholds; for the remaining lakes, not falling within an IC type, G/M boundaries were not available (see table 2.3). Whereas quite a high proportion of CB GIG lakes exhibit bloom situations (25-44%), lakes in the Northern GIG seldom surpass the critical bloom density (1-15%) (Table 2.3).

It is assumed that lakes which surpass the CBD exhibit lower evenness index values than those are below the CBD. In general this assumption holds true (Figure 2.5).

Lakes surpassing the CBD generally have a low evenness and show no sensitivity for TP ($r^2 = 0.0009$ linear regression). These lakes lie well below the evenness observed in reference lakes.

In lakes below CBD (without a bloom), in general, an increase of nutrient pressure leads to decreasing evenness from 0.69 in TP class 0-5µg/l to 5.0 in TP >100µg/l (figure 2.5). Despite

this, the linear regression between evenness and TP shows a low correlation ($r^2 = 0.1072$ linear regression).

Bloom cases are seldom in TP concentration below $20\mu\text{g/l}$ and, as expected, the risk of blooms increases with TP (see increasing N with CBD in bottom table in figure 2.5). Still, at very high TP values ($>100\mu\text{g/l}$ TP) about one third of the lakes do not reach the CBD but do indicate a pressure situation by their low evenness index.

Here, we conclude that the parameter evenness is not independent from chlorophyll concentration and not dependant on TP, when a bloom density has been reached. The evenness is especially sensitive to TP when no bloom density exists, and evenness strongly decreases with increasing TP (Fig 2.5).

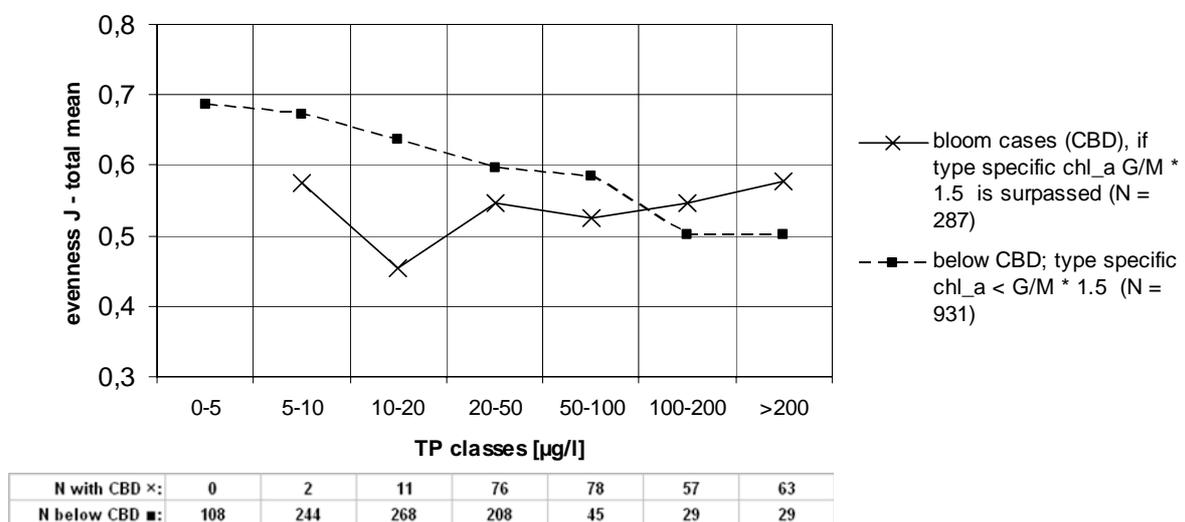


Figure 2.5: Mean value of evenness index with increasing TP classes split into cases, when summer mean of chlorophyll a concentration surpass or remain below the lake type specific critical bloom density (CBD). The number of cases available for each TP class is given at the figure base. TP values larger $1000\mu\text{g/l}$ are excluded.

Effect of exclusion of rare species > 20 taxa on evenness (E)

In general there is only a slight tendency that evenness increases with total number of taxa per sample (Figure 2.6a). In more than 80% of all samples ($N = 5896$) more than 19 taxa were detected and quantified. To detect the effect of rare species, the evenness of samples was calculated using all taxa in the count and compared with the evenness calculated when only the top 20 most abundant taxa were included in the evenness calculation (Figure 2.6b). This showed that the evenness index was influenced by rarer taxa mainly at higher evenness values. The correlation between the two data sets (with all taxa or with 20 most dominants) was, however, very high ($r^2 = 0.9461$) and the difference was not significant. Therefore it is concluded, that it is not necessary to reduce taxa lists to the 20 most dominant taxa.

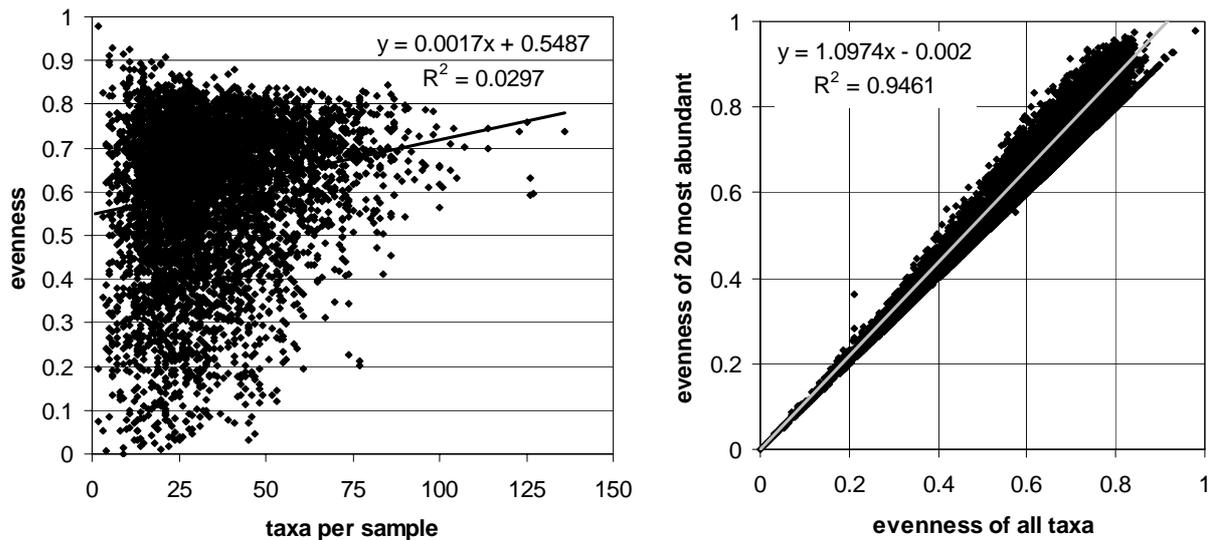


Figure 2.6: Left plot: Effect of taxa number per sample on evenness result. Right plot: Comparison of evenness results when all or only the 20 most abundant species are used (5897 sample from Jul - Sep). Grey line indicates the linear regression line.

When using only the most dominant 20 taxa, evenness values increase in the 25% percentile with 0.09 units and deviate mainly at values larger than 0.7 (see fig 2.6). The greatest effects (0.09 - 0.22 evenness unit) were observed well above the upper angle point of the metric scale (EQR 1 with evenness 0.65 - 0.67), which is set by the distribution of evenness in the reference lake population (see table 2.2 and fig 2.7).

Defining and test of a diversity bloom metric

In this study a bloom metric is tested which integrates the aspect of bloom intensity (thresholds for maximum chlorophyll a concentrations) and an aspect of diversity (evenness). Combining both aspects, blooms can be defined as situations when “phytoplankton biomass is remarkably higher than the seasonal mean and the bloom is dominated by few taxa”.

The metric is suggested to be applied only at those lake types, which exhibit significant correlation between pressure and parameter (see table 2.1). These lake types are referred to as “TP sensitive lakes” of N and CB GIG in the following text.

In the section “Chlorophyll a as a co-dominant factor in an evenness metric” it is explained how the CBD (Critical Bloom density) is derived from the good-moderate boundary of chlorophyll a.

To predict TP and EQR by evenness, the linear regression models for N and CB GIG were converted to regression functions given in table 2.4.

The EQR value 1 was set at the mean evenness within the reference lake group of N-GIG and CB-GIG respectively. The EQR value 0.6 (G/M boundary) was set as the evenness value detected at 25 $\mu\text{g/l}$ TP by linear regression for each eco-region group. The EQR value 0.2 (P/B) was set as evenness value at 200 $\mu\text{g/l}$ TP. In the next step, the H/G and the M/P boundaries of

evenness were determined by simple interpolation between the set points high-lighted in figure 2.7.

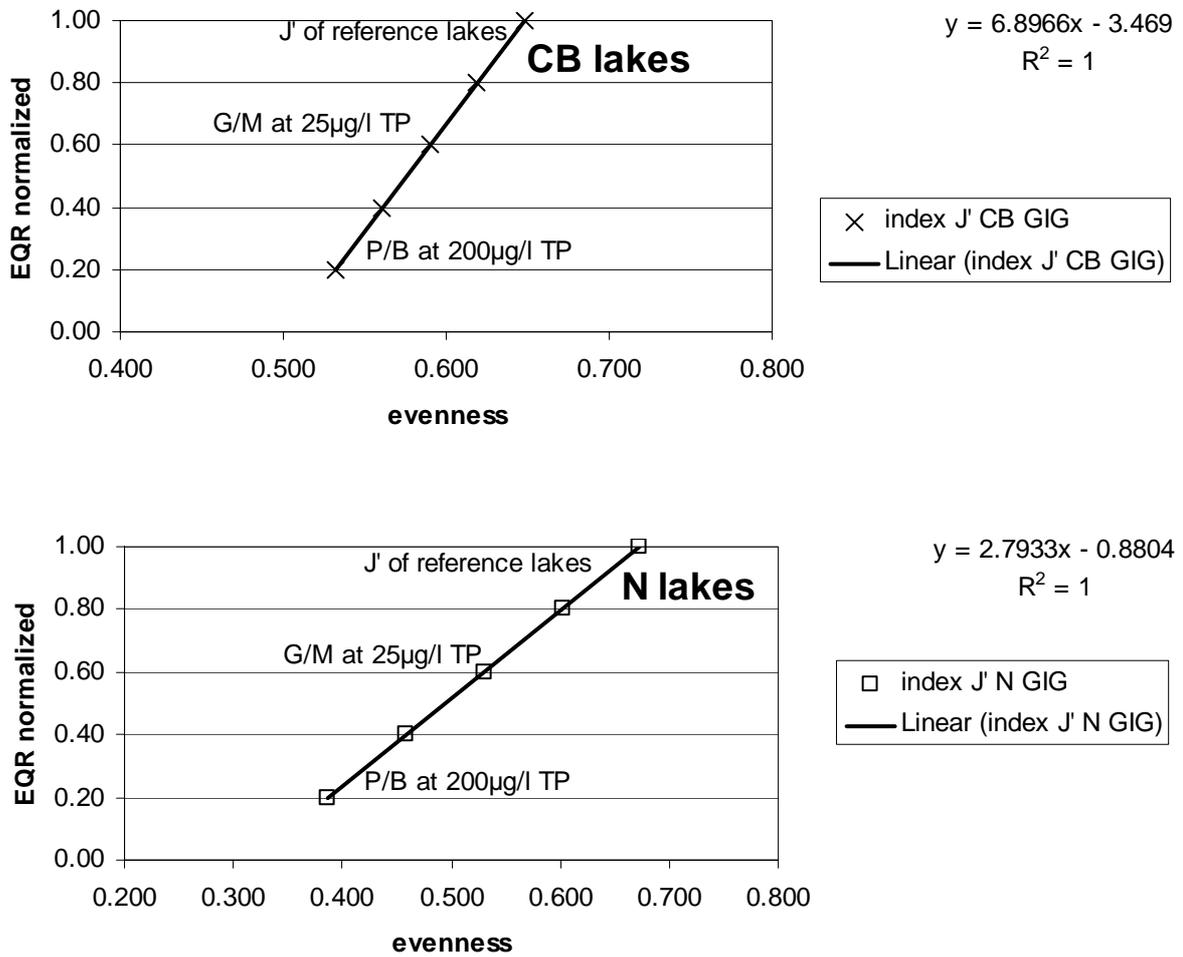


Figure 2.7: Linear relationship of evenness to EQR for Northern and Central Baltic lake group. EQR: 0.8 – 1 = “high status”; 0.6 – 0.8 = “good status” so on. Set points are explained in the text.

Tab. 2.4: Diversity models to predict TP(log) and ecological quality ratio (EQR; 0.8 – 1 = high; 0.6 – 0.8 = good; etc.) in CB GIG and N GIG lakes according to the linear regression.

	TP predicted by J'	EQR predicted by J' a)
J' CB-GIG model	$TP_{predictCB} = -12.821 * J' + 9.0256$	$EQR_{CB} = 6.8966 * J' - 3.469$
J' N-GIG model	$TP_{predictN} = -5.8824 * J' + 4.5706$	$EQR_N = 2.7933 * J' - 0.8804$

a) = resulting EQR values >1 are set as 1 and EQR values <0 are set as 0.

Access queries have been written which allow each diversity model to be applied to all samples collected from July to September and from the appropriate GIG in the WISER database. The resulting EQR values were plotted against the TP-classes (see fig. 2.8 and 2.9) and were analysed for significant correlation to pressure.

For N- GIG lakes, the predicted EQR values are significantly different in the TP classes ($<20\mu\text{g/l}$ TP; see fig 2.8) and they decrease significantly (Spearman 0.001) when correlated to mean TP concentrations in XY- plots (not shown here).

Lakes with TP mean concentrations below 20 are assessed by evenness mainly as high or good status (EQR >0.6) except of the few cases, in which the critical bloom density (CBD; $N = 7$) was surpassed. In CBD situations the EQR (predicted by evenness (J')) classify these lakes mainly as moderate to poor status (fig. 2.8 green box plots).

Thus, in N-GIG lakes the combination of a diversity metric with a threshold for chlorophyll a (see CBD) increases the prediction value of the metric by excluding all low level bloom densities.

The distribution of predicted EQR's are significantly different in the N-lakes below CBD ($N = 424$) from lakes surpassing CBD ($N = 31$), which underlies the co-correlation of evenness with chlorophyll a (see section).

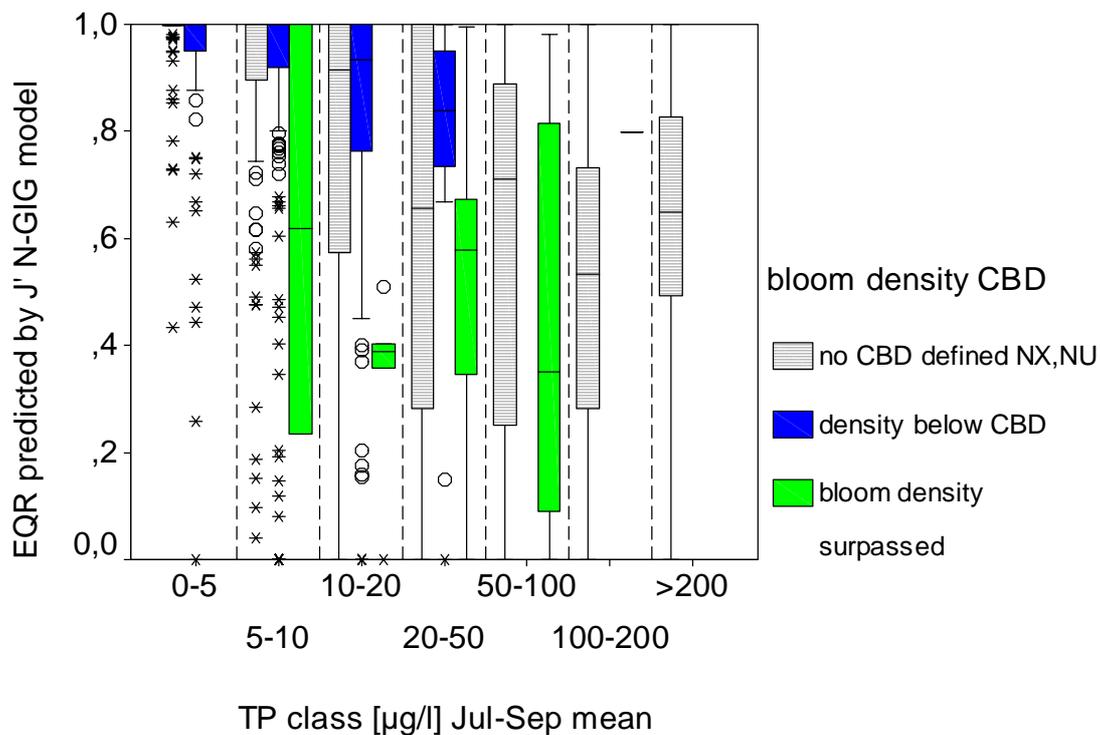


Fig 2.8: Box plots of predicted EQR by J' N-GIG model (see table 2.4) in seven TP classes in TP-sensitive lakes of the N-GIG (see table 2.1). The results are divided in cases above and below critical bloom density (CBD).

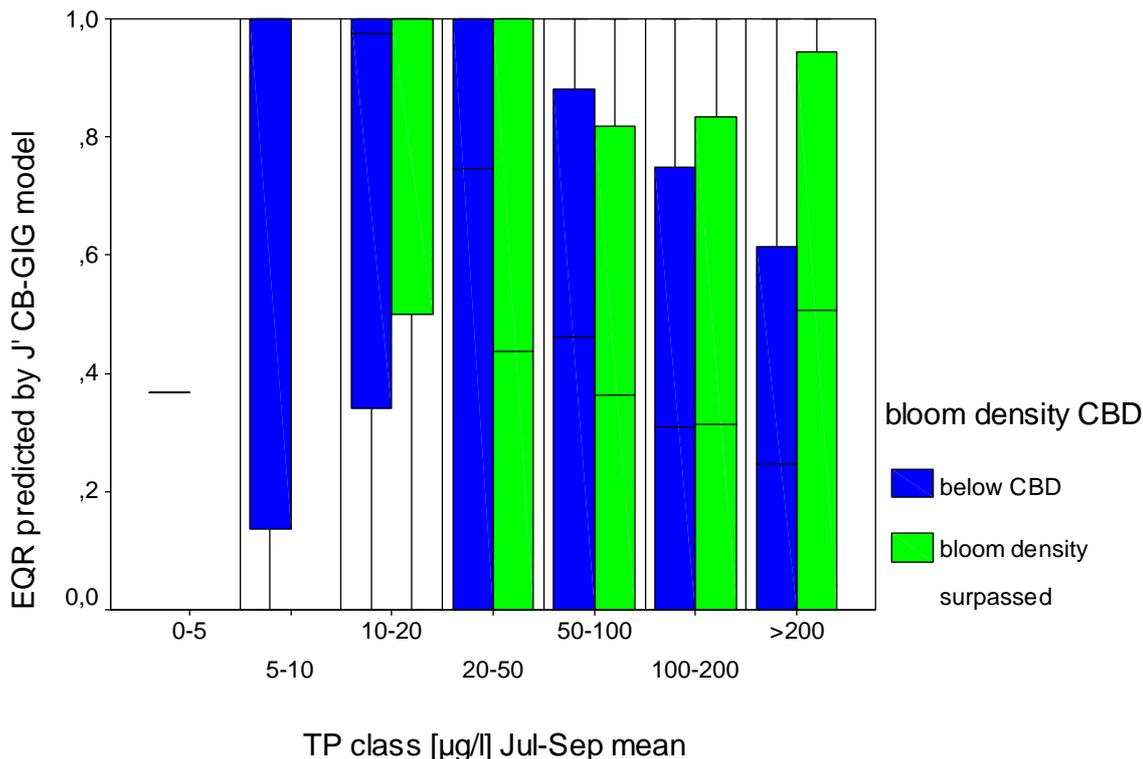


Fig 2.9: Box plots of predicted EQR by J' CB-GIG model (see table 2.4) in seven TP classes in TP-sensitive lakes of the CB-GIG (see table 2.1). The results are divided in cases above and below critical bloom density (CBD). EQR: 0.8 – 1 = high; 0.6 – 0.8 = good; etc.)

For TP-sensitive CB-GIG lakes the diversity model predicts the EQR classes less sensitive for TP classes (see figure 2.9) than for N-GIG lakes. The difference of predicted EQR in cases \leq TP 20 or $>$ 30 $\mu\text{g/l}$ are not significant (T-test 0.077 level). The means are well distinguished (see figure 2.9) but the overlapping of the distribution ranges of predicted EQR's between TP classes is too strong.

The significant level of regression correlation between EQR and TPmean is 0.01, when all cases are used (corr = -0.291**). In the case group “CBD is surpassed”, the correlation to TP is not significant (corr = -0.137).

If a CBD threshold would be applied to CB GIG lakes, then about the half of all cases (42%) would be selected. 63% of these cases with bloom density would be assessed as less than good by low evenness index.

Discussion and Recommendations for IC

The diversity index evenness (J') was calculated and analysed for the summer phytoplankton communities of 1590 European lakes. Evenness distribution exhibits a significant correlation to pressure (here total phosphorus (TP) concentration) in several of the most common lake types (see table 2.1) covering about 80% of all investigated lakes.

Since the evenness distribution is very broad in each pressure class and it is not very distinct different between reference and impacted lakes, the final application should implement the results of the WISER uncertainty analysis, which will also assess the risk that a site is classified wrong by this potential metric.

According to the tests presented here and tests carried out thereafter by the GIG leaders for phytoplankton element, the following general recommendations are given to which lake groups the evenness metric could be applied as bloom metric:

Alpine GIG lakes: Alpine lakes were not available in the WISER data base. Blooms are rare at TP concentrations of less than 20–25 $\mu\text{g L}^{-1}$, and most Alpine lakes are below this pressure concentration. Even under moderate status, many lakes do not have persistent blooms during summer months. Therefore, it can be expected that blooms, with decreasing effect on evenness, are rare.

Eastern Continental GIG lakes: All lakes (EC-1) of this GIG have very high level of pressure, and benchmark lakes (N = 18) were identified by criteria others than TP pressure ranges as for example with “low/moderate fishing”, “no artificial modifications of the shore line” so on. When investigating the distribution of evenness within the benchmark lake group and comparing this with those in the impacted lakes (N = 37), the median evenness not differ and evenness values range mainly between 0.6 and 0.8 in both lake groups (see presentation of Gábor Borics at EC GIG meeting in Ispra at 2-5. November 2010). In conclusion, the evenness metric is not recommended for lakes in the EC- GIG.

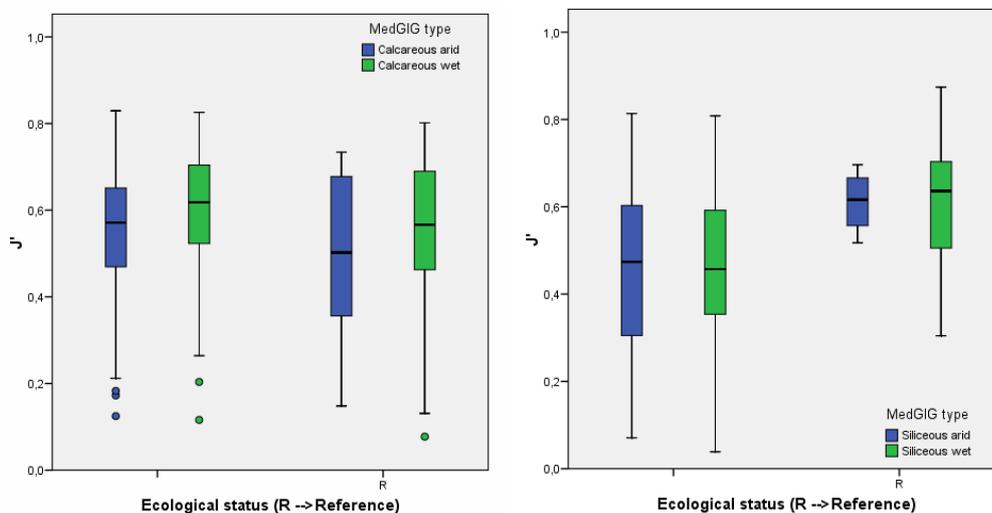


Fig 2.10: Box-plot distribution of evenness (J') in reference or MEP lakes (R) and in non-reference lakes (non-MEP) shown for arid and wet calcareous lakes (left graph) and for siliceous lake types (right graph) in Med GIG (source of graph: de Hoyos et al.2011, Milestone Report 4).

Mediterranean GIG lakes: Almost all calcareous reservoirs have TP concentrations below 10 $\mu\text{g L}^{-1}$ and no blooms can be expected. Both wet and arid calcareous reservoirs have a similar distribution of evenness in the reference or MEP lake group (Maximum Ecological Potential) when compare to the impacted lake group, while evenness is about 0.1 higher in siliceous MEP lakes compared to non-MEP lakes (see fig. 2.10). Furthermore, the median values of evenness

not differ significantly in lake groups with increasing TP concentrations and the decrease of the evenness value is low (see Caridad de Hoyos & José Pahissa in MedGIG presentation on phytoplankton 2010). In conclusion of the Milestone Report 4, the evenness metric is not recommended for reservoirs in the Med GIG.

Northern GIG lakes: The analysis presented here demonstrates that evenness significantly decreases in almost all N-GIG lakes types (L-N1; L-N2a; L-L3a; L-N8a; L-NU; L-NX) with increasing pressure. A combination with a threshold for a critical bloom density is recommended in order to exclude cases, in which evenness is low, but no bloom exists according the intensity. For more details see the following text.

Central Baltic GIG lakes: The analysis presented here demonstrates that evenness significantly decreases in almost all CB-GIG lakes types (L-CB1 and L-CB2) with increasing pressure. Because of an overall higher range of eutrophication in this GIG, a combination with a threshold for a critical bloom density is not necessary. For more details see the following text.

Combining the diversity metric with a threshold for chlorophyll a (see CBD) in N-GIG lakes by excluding all low level bloom densities increases the prediction value and pressure sensitivity of the metric. Thus, it is necessary to combine evenness index with a threshold for CBD. The decline of diversity is not redundant to an increase of biomass, but an additional functional response of the phytoplankton community to an increase of nutrient pressure.

N-GIG lakes, which have high chlorophyll a concentrations (>CBD), are rare in the data set and generally have evenness indices well below those observed in the reference lakes.

Diversity bloom metric for N-GIG lakes is suggested as:

If chlorophyll a concentration surpass 150% of the G/M boundary (CBD) for chlorophyll (see Poikane et al. 2010), then the ecological status can be assessed by calculating the normalized EQR derived from the evenness index (J'):

$$EQR = 2.7933 * J' - 0.8804$$

Applying this metric on the data, only 9% of all cases are selected by the CBD threshold. 58% of these cases (N = 18) are identified as less than good by low evenness index.

So, the additional assessment value of the diversity bloom metric for N-GIG lakes is the dissimulation of unbalanced phytoplankton communities (low evenness) from those blooms with high diversity. The member states of the N-GIG should consider evenness as a possible bloom metric.

Diversity bloom metric for CB-GIG lakes is suggested as:

The ecological status can be assessed by calculating the normalized EQR derived from the evenness index (J'):

$$EQR = 6.8966 * J' - 3.469$$

Applying this metric on the whole CB GIG data, 52% of all case (N = 560) are identified as less than good (EQR <0.6) by low evenness index.

The additional assessment value of the diversity bloom metric for CB-GIG lakes is the detection and gradual assessment of unbalanced phytoplankton communities (low evenness) at all levels of biomass density.

If it is accepted that an unbalanced phytoplankton communities can be used as a response to high pressure, the suggested evenness metric is able to detect and assess such degradations even in cases when the total biomass is not able to detect the pressure status by remaining below the G/M boundaries. In sensitive N -GIG lakes this is a very seldom case, since in this lake group high biomasses (chlorophyll a) are well correlated to low evenness values, but frequently in impacted lakes of CB-GIG (TP > 25µg/l) the critical bloom density nor the G/M boundaries is surpassed and here, the evenness give additional information.

The fact that evenness also can be very low at low pressure (TP < 25µg/L) in some very shallow CB-L2 lakes (Lake Maardu and Järise (2008, EE); Lake Keenaghan (2007, UK); Loch Hempriggs and Loch Watten (2008; UK)) must be further investigated.

The suggested diversity metric is little influenced by the number of detected taxa per sample, but it is strongly recommended that at least 20 taxa should be detected in each phytoplankton sample to ensure reliable evenness estimation as for indicator taxa index. This is not an exceptional strong and unrealistic claim for the quality assurance of the phytoplankton data, since for more than 80% of all samples more than 19 taxa and in mean 35 taxa were already quantified.

Chapter 3: Cyanobacteria abundance

Introduction

Cyanobacteria present hazards to the health of humans and other animals when large populations flourish to produce blooms and particularly when these accumulate on lake surfaces or along shorelines as scums. Cyanobacterial blooms constitute a major health hazard as they frequently produce numerous potent toxins (cyanotoxins) that can result in a range of adverse health effects from mild, e.g. skin irritations and gastrointestinal upsets, to fatal (Codd et al., 1999; Codd, et al., 2005). Concern over cyanobacterial blooms has led to the development of World Health Organisation (WHO) and national guideline levels for usage of recreational and drinking waters (Chorus & Bartram, 1999; WHO, 2003; 2004). Blooms of cyanobacteria can, therefore, restrict fishing and recreational usage and also increase costs of water treatment for drinking water and industrial uses. Incorporating a metric for cyanobacterial abundance in the assessment of ecological status is, therefore, of great relevance to the ultimate goal of the WFD, the sustainable use of our freshwaters. Developing a bloom metric based solely on the abundance of cyanobacteria should also complement other metrics being developed for the abundance (chlorophyll a) and composition of the phytoplankton community in general.

It is a widely held view that the increasing magnitude and frequency of cyanobacterial blooms is primarily related to the widespread nutrient enrichment, of freshwaters. There have been several studies showing empirically that bloom frequency is related to the general nutrient status of a lake (Gorham et al., 1974; Dokulil & Teubner 2000; Downing et al., 2001; Reynolds & Petersen, 2000; Schindler, 2008). Supporting evidence of a relationship between nutrient enrichment and cyanobacterial abundance is largely derived from long-term studies of enrichment at a few selected individual sites, usually lowland, alkaline, eutrophic lakes, and often examining individual cyanobacterial species. There have been a couple of published studies examining the relative % abundance of cyanobacteria across eutrophication gradients in large datasets (Downing et al., 2001; Ptacnik et al., 2008), but a more comprehensive quantitative analysis of the relationship between nutrients and actual cyanobacterial abundance across a wide range of lake types at a regional or European scale has not been carried out.

This study, therefore, aims to explore the relationship between cyanobacterial abundance and nutrient pressures in a range of lake types across Europe and if sufficiently strong, recommend a common metric for phytoplankton blooms that can be adopted for Intercalibration. The chapter describes how the metric could be used to assess ecological status, how status class boundaries can be set and their fit with the normative definitions, and how reference conditions and EQR can be estimated.

Methods

Data

Phytoplankton data were available from 1710 lakes spanning 4 GIGs; the bulk of the data were from the Northern (1010 lakes) and Central-Baltic GIGs (602 lakes) (Table 3.1). TP data were widely available and used to represent nutrient pressure. Both phytoplankton and nutrient data were summarised as a summer mean abundance using data spanning the months July, August and September. For each lake, only the last year of available data was used in the analysis.

Table 3.1. No. of lakes with cyanobacteria and nutrient data, by GIG and lake type.

GIG	Lake Type	No. of Lakes
Central-Baltic	L-CB1	367
	L-CB2	204
	L-CB3	24
	L-CBU	4
	L-CBX	3
Easter Continental	EC-1	15
	EC-2	3
Mediterranean	L-M8	37
	L-MU	36
	L-M7	7
Northern	L-NX	238
	L-N3a	133
	L-N2a	101
	L-N2b	94
	L-NU	93
	L-N6a	73
	L-N1	65
	L-N5	60
	L-N8a	54
	L-N9	31
	L-N3b	28
	L-N6b	12
	L-N11	9
	L-N7	6
	L-N8	5
	L-N8b	5
	L-N3	2
L-N10	1	

Table 3.2. No. of reference and non-reference lakes, by GIG.

GIG	Not reference	Reference	% reference
C	558	44	7%
E	19	0	0%
M	68	11	14%
N	548	462	46%
Grand Total	1193	517	30%

Statistical Analysis

In the context of harmful cyanobacterial blooms, for a precautionary approach it would be better to consider the potential maximum cyanobacterial abundance that the current environment could bring about. For modelling the relationship between cyanobacterial abundance (biovolume) and pressure (TP), it is, therefore, more relevant to model maximum abundance instead of mean abundance. The majority of biological response modelling approaches in current use [e.g. simple linear least squares regression, general linear models (GLM) or generalized additive modelling (GAM)] are, however, based on the estimation of mean or median responses to environmental factors. Modelling the upper bounds of species–environment relationships should relate much more to the most limiting resource (Hiddink & Kaiser 2005), while the variation or scatter below the upper boundary reflects the limiting effect on the abundance of environmental attributes other than the limiting factor of interest (Cade et al. 1999).

One method which models the relationship of variables at different levels of a distribution is quantile regression (Koenker & Bassett 1978). Quantile regression extends the idea of linear regression by estimating all parts of the distribution of the response variable (cyanobacteria abundance) conditional on the predictor variable (nutrient pressure), rather than only the mean distribution (Rocchini & Cade 2008). For each quantile in the range 0 to 1, different weights are given to the proportion of data that are above that quantile to those that are below the quantile. For example, the 0.8th quantile will have 20% of the dataset above this quantile weighted differently to the 80% of the dataset that is below. Linear quantile regression does not require an assumption about the error-term of the distribution (i.e. a normal distribution) of the dataset to be made compared to other parametric tests and it is robust to outliers within the data (Cade *et al.* 2009, Cade & Noon 2003). Each quantile can give different estimates of the slope of the regression line, therefore, identifying relationships between variables that least squares regression may miss in a dataset. So for This is especially so when the dataset has a heterogeneous distribution (Fleeger *et al.* 2010).

Extensions to linear quantile regression have been applied to model bivariate or count data in the form of nonparametric quantile regression. There are different ways of applying nonparametric quantile regression, for example, using polynomial regression splines, quantile smoothing

splines (penalty methods) or triogram methods (Koekner 2005). For example in one method of applying polynomial regression splines, the data is split into subsets according to values of the predictor variable, for each quantile. Each subset is then fitted locally with a linear quantile regression. Non-parametric quantile regression can be applied to datasets with highly skewed distributions that cannot be transformed to normal. The quantile regression approach can also be used to model relationships between variables which show a non-linear trend. This can be carried out by either transforming the response and/or predictor variables to give a linearized form (logarithmic), and then applying linear quantile regression (Cade & Guo 2000), or a non-linear model such as a 2-parameter logarithmic model can be defined directly.

Quantile regression was, therefore, used to model responses of cyanobacterial abundance (actual biovolume) against TP concentrations. In this analysis, a number of percentile cyanobacterial responses were modelled, the median (50%), 75%, 90% and 95%. For example, the 90% model would give an estimate of the cyanobacterial abundance along the TP gradient where 90% of data points were below that abundance level. Linear, non-linear and non-parametric quantile regression were all applied to the data in R (R Development Core Team, 2010), using routines available in the *quantreg* library (Koenker, 2009). Non-parametric quantile regression was applied using the function *rqss* in the *quantreg* package which fits a smoothing spline using a roughness penalty term. The non-linear quantile regression models are described below.

Akaike's information criterion (AIC) values for the linear and the non-parametric quantile regression models were used to compare model fit to the data. AIC values are a measure of the goodness of fit of a statistical model. The AIC is not a test of the significance of a model; it is a test between models. Given a data set, several competing models may be ranked according to their AIC, with the one having the lowest AIC being the best. For, non-linear quantile regression, AIC values cannot be calculated for each quantile, therefore, deviance is reported as a measure of goodness. Deviance is the measure of discrepancy between the fitted values produced by the regression model and the values of the data. Deviance is defined as -2 times the difference in the log-likelihood between the fitted regression model and a saturated model that fits the data perfectly (Crawley, 2009). For a continuous variable, deviance is calculated as:

$$D = \sum_{i=1}^n (y_i - \mu)^2$$

where n is the sample size, y_i is the observed data point and μ is the mean of the y variable. The lower the deviance value then the better the fit of the model to the dataset.

Non-parametric quantile regression models do not enable predictions or simple interpretations of model equations. Therefore, non-linear exponential quantile regression was applied to the datasets to enable this. For the Central Baltic GIG dataset the following 3-parameter asymptotic equation was used:

$$\text{Log}_{10}(\text{Cyanobacteria volume} + 1) = a / (1 + b * \exp(-c * \text{Log}_{10}(\text{total phosphorus})))$$

where , a =cyanobacteria value where the fitted curve begins to reach a maximum

b =a - position on the y-axis where the convex curve starts

c =position of x-axis where the initial change in slope occurs i.e where the concave curve starts.

For the Northern and Mediterranean lake datasets, a 2-parameter logistic model was fitted to the data:

$$\log(\text{cyano biovolume} + 1) \sim (a * \exp(b * \log(\text{TP})))$$

where a is the intercept of the line and b is the slope of the line.

In this study, this quantile modelling approach is combined with WHO thresholds for cyanobacterial abundance to identify ‘probabilities’ of exceeding health risk thresholds. WHO guidelines for safe recreational waters (WHO, 2003) outline three health risk categories: low, medium and high. High risk is assigned when surface scums are present, where cell densities and toxin concentrations tend to be very high. Medium and low risk waters are those where cyanobacteria cells are at or above 100,000 and 20,000 cells ml⁻¹ respectively. These can be converted to a biovolume (mm³ L⁻¹) by multiplying by typical cyanobacterial cell volume. For this study these were converted based on a typical *Microcystis aeruginosa* cell diameter of 4.5 μm, equivalent to approximately 2 mm³ ml⁻¹ as a low risk threshold and 10 mm³ ml⁻¹ for a medium risk threshold.

Results

General test for sensitivity to eutrophication pressure – all lakes

Considering the whole lake dataset together it can be seen that there is a weak, positive relationship between \log_{10} cyanobacteria and \log_{10} TP ($r^2 = 0.1978$, Fig. 3.1). A polynomial regression gives an improved fit ($r^2 = 0.2621$), but there is still a large amount of scatter in the data. Clearly there are a number of factors, other than TP, that limit cyanobacterial abundance, but the relationship does suggest that there is a take-off or threshold response above approximately $10 \mu\text{g L}^{-1}$ TP. In the context of harmful cyanobacterial blooms, a precautionary approach would also better consider the potential maximum abundance that eutrophication can bring about. Regression modelling of the maximum cyanobacterial abundance may, therefore, be more appropriate than modelling mean abundance.

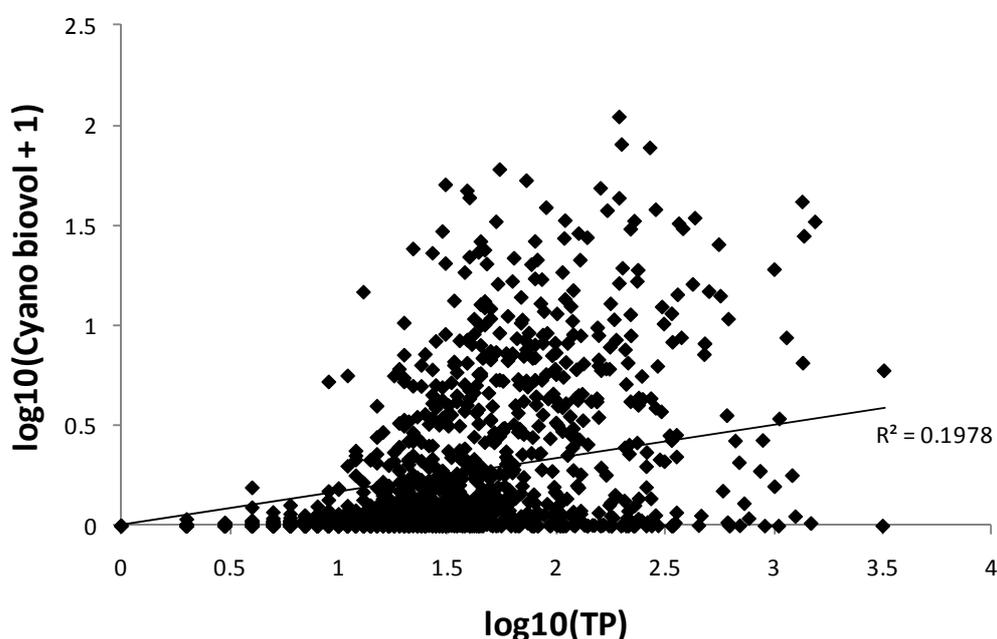


Fig. 3.1 Scatter plot for \log_{10} cyanobacteria and \log_{10} total phosphorus ($\mu\text{g L}^{-1}$) for lakes across all European regions with fitted polynomial regression line

For this reason, quantile regression was carried out on all 1710 lakes in the WISER database. A linear quantile model was initially fitted to the data, however, comparison of AIC values for linear and non-parametric quantile regression models highlight the much poorer fit of linear models (Table 3.4). Models for 0.05, 0.10 and 0.25 quantiles had the lowest AIC values, due to the large proportion of low or zero values for cyanobacteria biovolume (Fig. 3.2), but the relationships between cyanobacteria biovolume and TP were more or less flat and not significant for these quantiles: 0.05 ($p=0.98$), 0.10 ($p=0.80$) and 0.25 ($p=0.20$). The non-parametric quantile regression models indicate a highly significant relationship between cyanobacteria biovolume and TP for quantiles 0.5, 0.75, 0.90 and 0.95 ($p<0.001$).

Table 3.4 AIC values for both linear and non-parametric quantile regression models relating cyanobacterial biovolume to TP concentrations in European lakes.

Model type	Quantile						
	0.05	0.10	0.25	0.50	0.75	0.90	0.95
Linear quantile	4675	4302	3723	3201	283	2634	2575
Non- parametric quantile	183	139	283	490	691	918	1068

Non-parametric regression models are based on rank differences and, therefore, cannot, be used to describe or visualise the relationship or make simple interpretations/predictions from the model. Therefore, (parametric) non-linear quantile regression was applied to the dataset to enable this. The resulting non-linear regression models for quantiles 0.05-0.95 are shown in Figure 3.2.

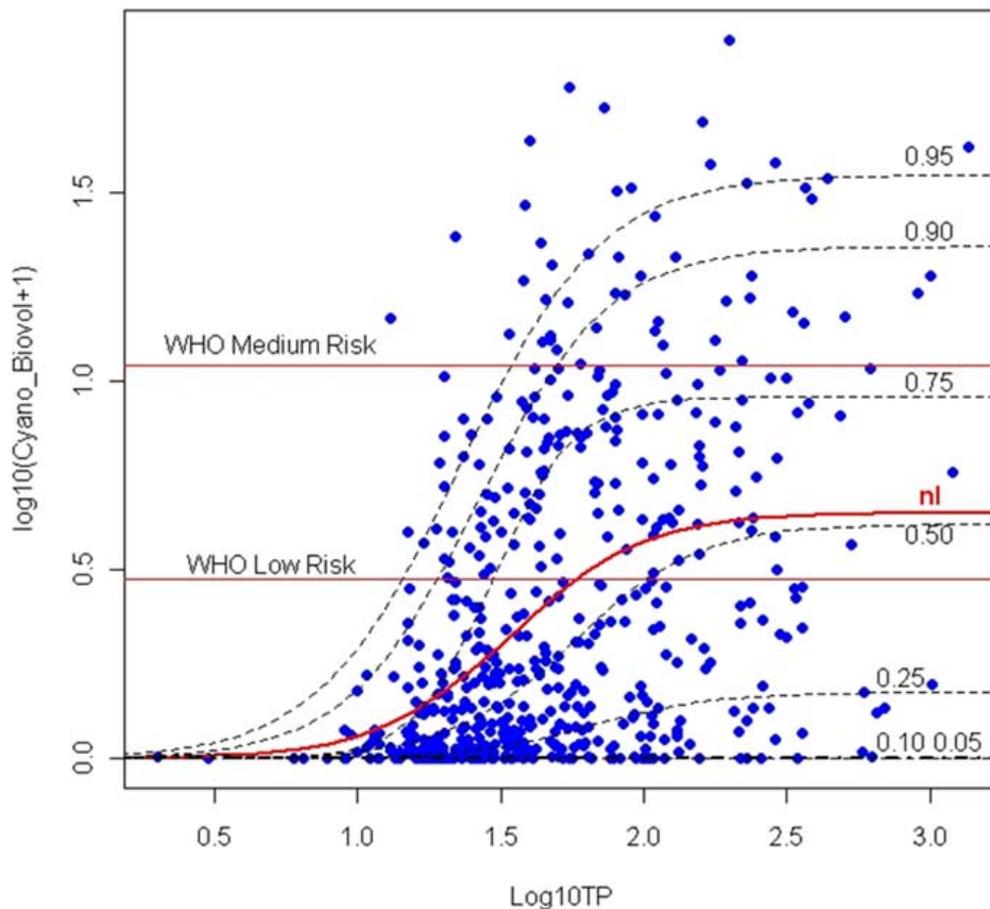


Figure 3.2: Scatter plot for \log_{10} cyanobacteria and \log_{10} total phosphorus for lakes across all European regions. Quantile regression curves (0.05 – 0.95) using a fitted sigmoid non-linear model are displayed. Thresholds relating to approximate WHO (2003) low and medium risk categories are also indicated. Nl = Non-linear regression fit to mean of data

Central-Baltic Lakes

Cyanobacterial biovolumes were plotted against TP concentrations for all 602 lakes in the Central-Baltic GIG dataset (Fig. 3.3). There was a highly significant positive correlation between cyanobacterial biovolume and TP concentrations (Spearman’s Rank correlation, $r_s = 0.44$, $p < 0.001$). For non-parametric models there was a highly significant relationship between cyanobacteria biovolume and TP for quantiles 0.50, 0.75, 0.90 and 0.95 ($p < 0.001$), whereas there was no significant relationship for quantiles 0.25 ($p = 0.05$), 0.10 ($p = 0.99$) and 0.05 ($p = 0.98$).

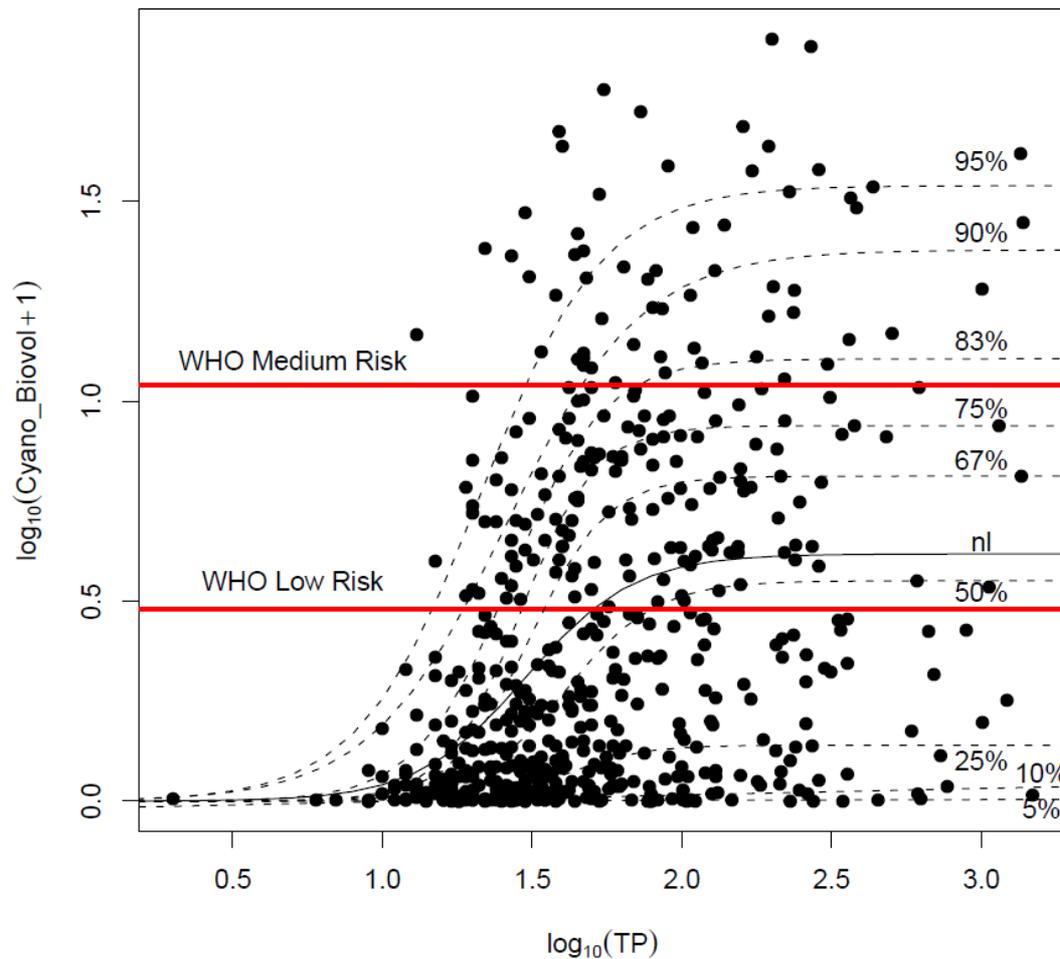


Figure 3.3 Scatter plot of cyanobacterial abundance against TP in CB GIG lakes. Fitted non-parametric quantile regression curves are shown for a number of quantiles between 5% and 95%. The cyanobacteria biovolumes approximating to WHO risk levels are also shown. nl = non-linear regression fit to mean of data.

Non-linear quantile regression allows parameter estimates to be derived to describe the shape of the relationship. The CBGIG lake dataset is best described by a 3-parameter asymptotic relationship (Table 3.5). No p-values or AIC values can be calculated with non-linear quantile regression, instead, deviance values are reported to examine the fit of the non-linear models to the data (Table 3.6). The model is a better fit when the deviance is minimized, indicating that the most robust models are for the 0.95 and 0.90 quantiles.

Table 3.5 Parameter estimates derived using non-linear quantile regression for CB GIG lakes

Quantile	Parameter a ±s.e	Parameter b ±s.e	Parameter c ±s.e
0.25	0.14 ± 0.04*	480462.41 ± 0.001	8.07 ± 0.38*
0.50	0.55 ± 0.06*	48464.06 ± 0.001	6.70 ± 0.23*
0.67	0.81 ± 0.07	121806.74 ± 0.001	7.85 ± 0.21*
0.75	0.94 ± 0.04*	141601.08 ± 0.001	8.12 ± 0.16*
0.83	1.11 ± 0.07*	11279.57 ± 0.001	6.54 ± 0.15*
0.90	1.38 ± 0.12**	542.51 ± 611.43	4.46 ± 0.86*
0.95	1.54 ± 0.07*	679.27 ± 762.33	4.91 ± 0.85*

Table 3.6 Deviance values for the CB GIG lake non-linear quantile regression models

Quantile	0.50	0.67	0.75	0.87	0.90	0.95
Deviance	88.54	89.24	80.46	66.43	47.92	28.94

The equations in Table 3.5 can be used to determine the likelihood (certainty) that the low and medium risk WHO threshold levels for Cyanobacteria abundance are exceeded for a given TP concentration (Table 3.7). Only quantile curves which pass through these risk threshold levels could be tested. For example, at a TP concentration of 19 $\mu\text{g L}^{-1}$ there is a 90% likelihood of being below the WHO low risk threshold, at 30 $\mu\text{g L}^{-1}$ this has reduced to a 75% likelihood, and at 78 $\mu\text{g L}^{-1}$ there is only a 50% likelihood of being below the WHO low risk threshold.

Table 3.7 TP concentrations for a given likelihood (quantile) of being below low and medium risk WHO threshold levels for Cyanobacteria volume.

WHO Threshold	Quantile	TP
Low	0.50	78
	0.67	35
	0.75	30
	0.83	25
	0.90	19
	0.95	15
Medium	0.83	71
	0.90	46
	0.95	30

Reference conditions

Reference lakes had generally lower cyanobacterial biovolumes than non-reference lakes (Figure 3.4) with a highly significant difference between the population means of reference and non reference lakes (2 sample t-test, $p < 0.001$).

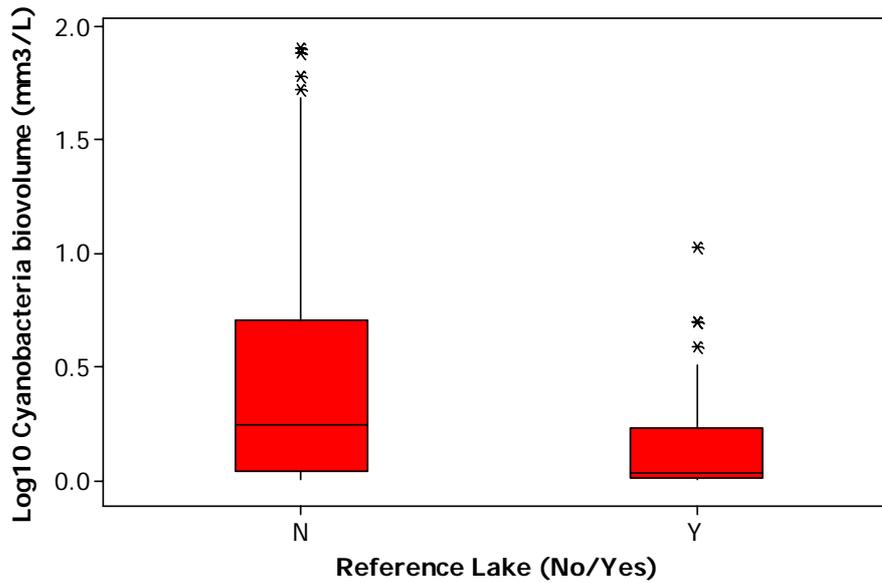


Figure 3.4. Boxplots of cyanobacteria biovolume in non-reference (N) and reference (Y) lakes in the CB GIG.

Figure 3.5 shows the relationship between cyanobacterial biovolume and TP concentrations for Central-Baltic GIG reference lakes. There is difficulty fitting the non-linear quantile regression models that were used for all lakes in the Central-Baltic GIG to the reference CB lakes only. The range of TP concentrations for the reference lakes is more constrained than the range for non-reference lakes – with reference lakes typically less than $50 \mu\text{g L}^{-1}$. The cyanobacteria levels for reference lakes do not, however, go above the WHO medium risk threshold. Only 90% and 75% quantile curves pass through the WHO low risk threshold. Figure 3.5 shows that 75% of reference lake samples were below the low risk threshold, but 5 out of 44 reference lakes (11%) were still above it. To derive type-specific reference conditions, descriptive statistics (mean, median, 75% and 90%) were produced for cyanobacterial biovolumes by each lake type (Table 3.8). One L-CB1 lake had a much higher TP ($70 \mu\text{g L}^{-1}$) and cyanobacterial biovolume ($9.68 \text{ mm}^3 \text{ L}^{-1}$) than all other reference lakes and was excluded from this analysis of reference conditions. The median value is recommended for defining reference condition as it is less affected by outliers than the mean. One of only four L-CB2 reference lakes exceeded the WHO low risk threshold. For this reason, the 75th percentile statistic was chosen as a more suitable as a potential measure for defining the high/good status class boundary. This does, however, mean that 1 in 4 reference lakes may not be classified as high status and it may be, therefore, be more appropriate to simply base the high/good boundary at just below the low risk threshold.

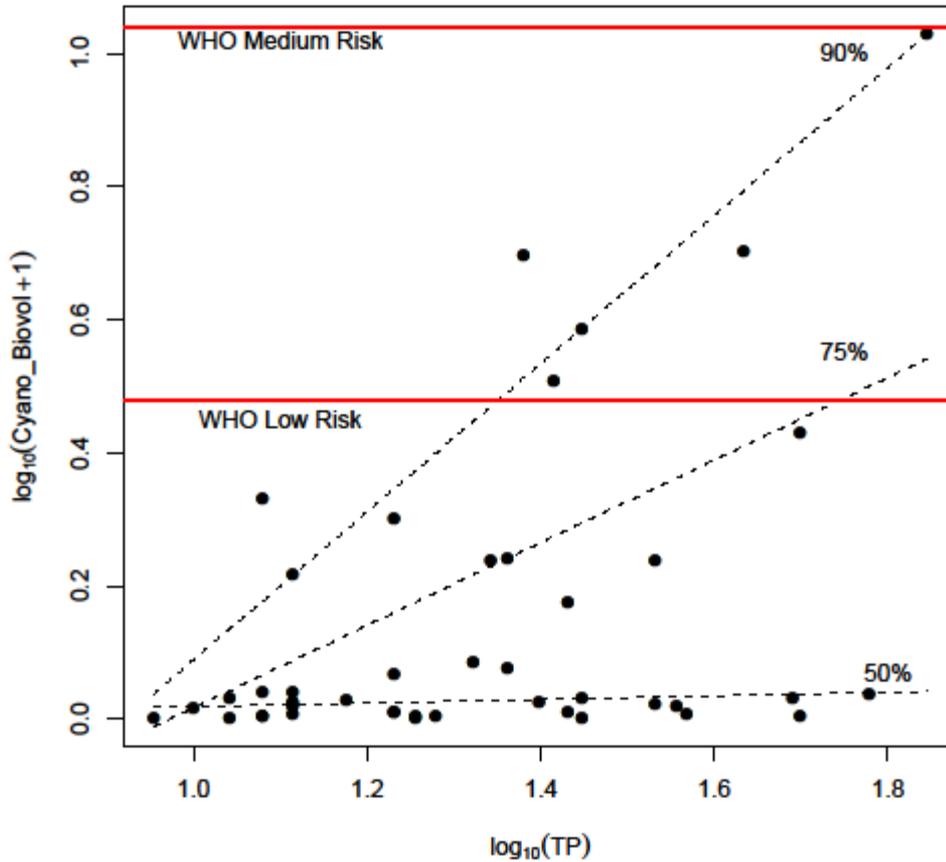


Figure 3.5. Cyanobacteria biovolume vs TP in CB GIG reference lakes. Fitted non-parametric quantile regression curves are shown for 90%, 75% and 50% quantiles. The cyanobacteria biovolumes approximating to WHO risk levels are also shown.

Table 3.8. Number of reference lakes (n) by GIG type and corresponding median, 75th and 90th percentile values for cyanobacterial biovolume.

	L-CB1	L-CB2	L-CB3
N	30	4	9
median	0.07	0.34	0.01
75%	0.71	1.38	0.05
90%	1.24	2.96	0.40

Mediterranean Lakes

Data from 79 lakes in the Mediterranean GIG dataset were used to examine cyanobacterial biovolumes in response to TP concentrations (Fig. 3.6). Cyanobacterial biovolume showed a significant positive correlation with TP (Spearman’s correlation $r_s = 0.30$, $n = 79$, $p\text{-value} = 0.007$). Non-linear quantile regression was applied to the data using the following 2-parameter logistic model:

$$\log(\text{cyano biovolume}+1) \sim (a * \exp(b * \log(\text{TP})))$$

where a is the intercept of the line and b is the slope of the line.

The model can be used to determine the likelihood (certainty) that the low and medium risk WHO threshold levels for cyanobacteria abundance are exceeded for a given TP concentration. Only quantile curves which pass through these risk threshold levels could be tested. (Figure 3.6). The change in gradient of the curves for the Mediterranean lakes is less defined than those for the CB GIG region. This suggests that for the cyanobacteria biovolume levels to increase above the WHO threshold levels a larger change in TP is needed, For example, at a TP concentration of $41 \mu\text{g L}^{-1}$ there is a 90% likelihood of being below the WHO low risk threshold, at $120 \mu\text{g L}^{-1}$ this has reduced to a 83% likelihood (1 year in 6), and even at $324 \mu\text{g L}^{-1}$ there is still a 50% likelihood of being below the WHO low risk threshold (Table 3.9).

Table 3.9 TP concentrations for a given likelihood (quantile) of being below low and medium risk WHO threshold levels for Cyanobacteria volume (MedGIG lakes data only).

WHO Threshold	Quantile	TP
Low	0.50	324
	0.67	269
	0.75	234
	0.83	120
	0.90	41
	0.95	24
Medium	0.50	501
	0.67	525
	0.75	513
	0.83	347
	0.90	224
	0.95	110

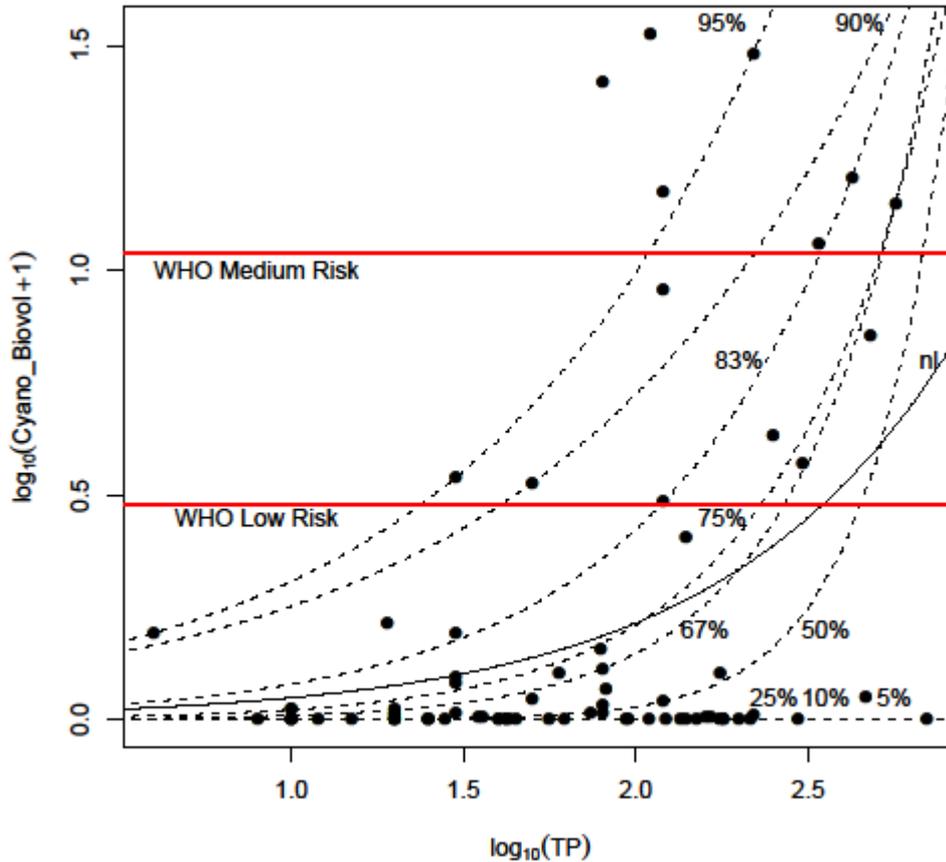


Figure 3.6 Scatterplot of cyanobacterial abundance against TP in Med GIG lakes. Fitted non-parametric quantile regression curves are shown for a number of quantiles between 5% and 95%. The cyanobacteria biovolumes approximating to WHO risk levels are also shown. *nl* = non-linear regression fit to mean of data.

Reference conditions

There are only 11 reference lakes within the Mediterranean dataset. Reference lakes had generally lower cyanobacterial biovolumes than non-reference lakes (mean ref = 0.013; mean non-ref = 0.228) (Fig. 3.7) with a highly significant difference between the population means of reference and non reference lakes (2 sample t-test, $p < 0.001$). The cyanobacteria biovolume values for all the 11 reference lakes are all substantially lower than the WHO low risk threshold ($2 \text{ mm}^3 \text{ L}^{-1}$) despite some very high TP concentrations (Fig. 3.7).

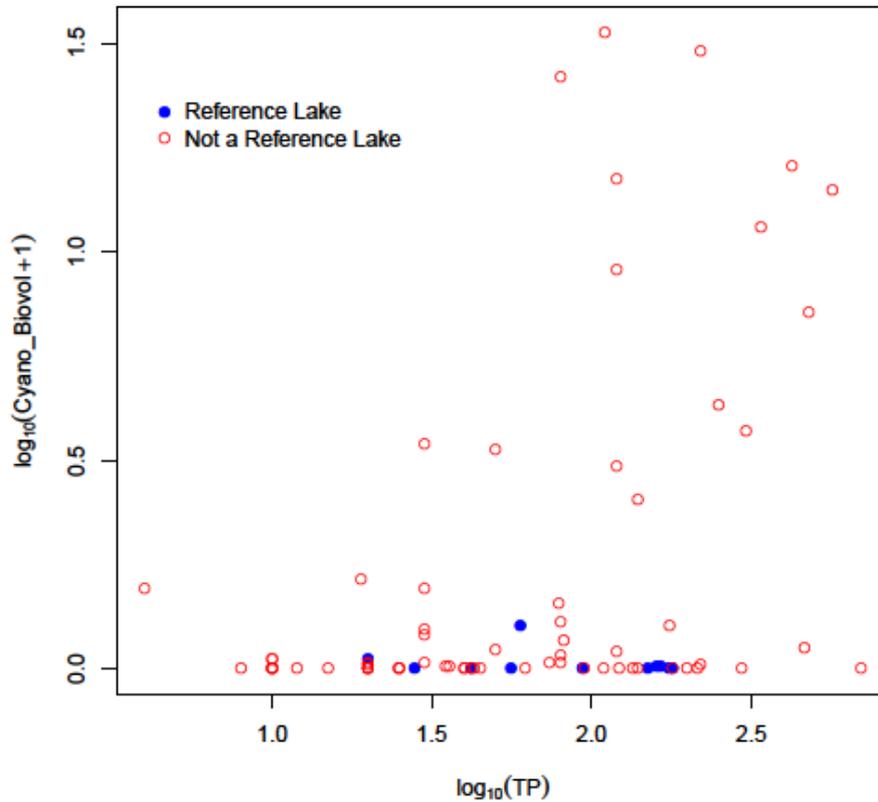


Figure 3.7. Cyanobacteria biovolume vs TP in Med GIG reference and non-reference lakes.

To derive type-specific reference conditions, descriptive statistics (mean, median, 75% and maximum) were produced for cyanobacterial biovolumes by each lake type (Table 3.10). The median value is recommended for defining reference condition as it is less affected by outliers than the mean. Reference conditions for L-M7 are based on only 1 reference lake and should be considered very uncertain. Median and 75% values were generally very low, well below the WHO low risk threshold of approximately $2 \text{ mm}^3 \text{ L}^{-1}$, the highest reference value was $0.277 \text{ mm}^3 \text{ L}^{-1}$ for a L-M8 lake (Table 3.10). Although all reference lakes in the Med GIG had maximum cyanobacterial densities below the WHO low risk threshold, for consistency with the CB GIG (and chlorophyll classifications), the 75th percentile statistic could potentially be used as a potential measure for defining the high/good status class boundary.

Table 3.10. Number of reference lakes (n) by Med GIG type and corresponding mean, median, 75th percentile and maximum values for cyanobacterial biovolume ($\text{mm}^3 \text{ L}^{-1}$).

ICType	N	Mean	Median	75%	Max.
L-M7	1	0.060	0.060		0.060
L-M8	10	0.032	0.001	0.012	0.277

Northern Lakes

Data from 1010 lakes in the Northern GIG dataset were used to examine cyanobacterial biovolumes in response to TP concentrations (Fig. 3.8). Cyanobacterial biovolume showed a significant positive correlation with TP (Spearman’s Rank Correlation, $r_s = 0.46$, $n = 1010$, p -value < 0.001). Very few N GIG sites (10 out of 1000) have high TP concentrations and so, as for the MedGIG, a 2-parameter logistic quantile model was the best fit for the data:

$$\log(\text{cyano biovolume}+1) \sim (a * \exp(b * \log(\text{TP})))$$

where a is the intercept of the line and b is the slope of the line.

Deviance values are reported to examine the fit of the non-linear models to the data (Table 3.11). The model is a better fit when the deviance is minimized, indicating that the most robust models are for the 0.95 and 0.90 quantiles.

Table 3.11 Deviance values for the N GIG lake non-linear quantile regression models

Quantile	0.50	0.67	0.75	0.87	0.90	0.95
Deviance	88.54	89.24	80.46	66.43	47.92	28.94

The models can be used to determine the likelihood (certainty) that the low and medium risk WHO threshold levels for cyanobacteria abundance were exceeded for a given TP concentration (Figure 3.8). The slopes of the quantile curves are much steeper than for the MedGIG, indicating that to increase cyanobacterial biovolume levels above the WHO thresholds a smaller change in TP is needed. For example, at a TP concentration of $35 \mu\text{g L}^{-1}$ there is a 95% likelihood of being below the WHO low risk threshold, at $65 \mu\text{g L}^{-1}$ this has reduced to a 90% likelihood and at $138 \mu\text{g L}^{-1}$ there is a 75% likelihood of being below the WHO low risk threshold (Table 3.12).

Table 3.12 TP concentrations for a given likelihood (quantile) of being below low and medium risk WHO threshold levels for Cyanobacteria volume.

WHO Threshold	Quantile	TP
Low	0.67	234
	0.75	138
	0.83	74
	0.90	65
	0.95	35
Medium	0.67	490
	0.75	282
	0.83	151
	0.90	123
	0.95	85

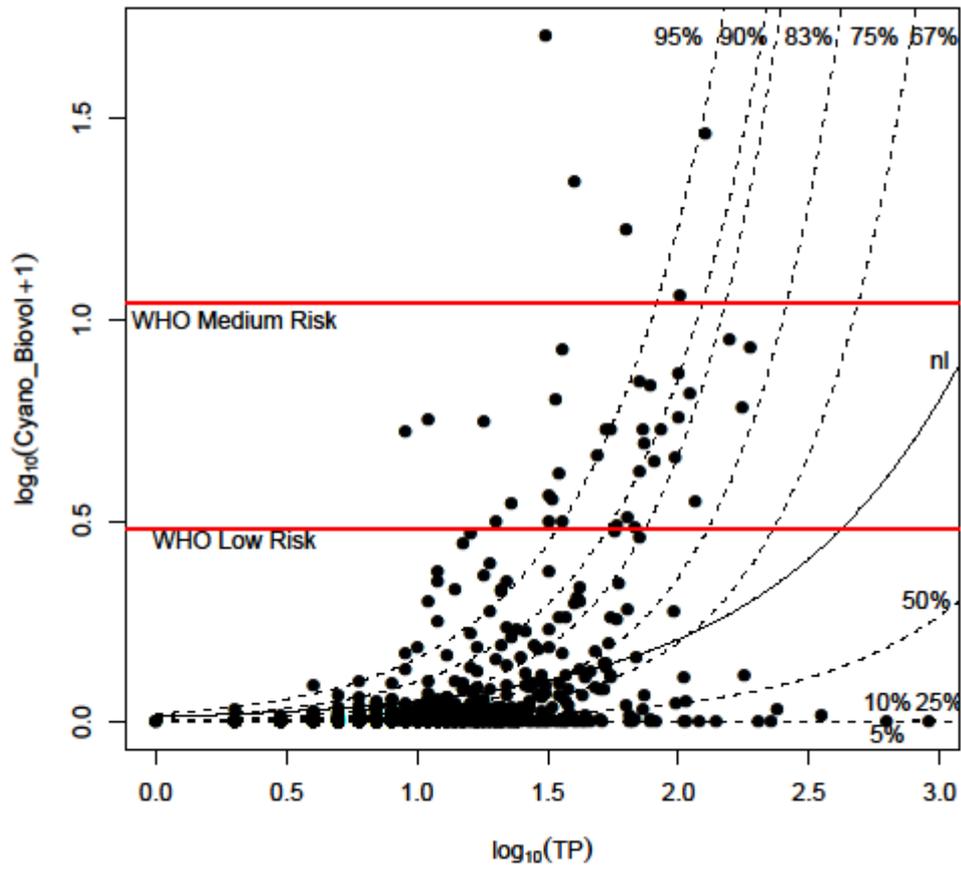


Figure 3.8 Scatterplot of cyanobacterial abundance against TP in Northern GIG lakes.

Reference conditions

There were 463 reference lakes in the N GIG. Reference lakes had generally lower cyanobacterial biovolumes than non-reference lakes (Fig. 3.9) with a highly significant difference between the population means of reference and non reference lakes (2 sample t-test, $p < 0.001$).

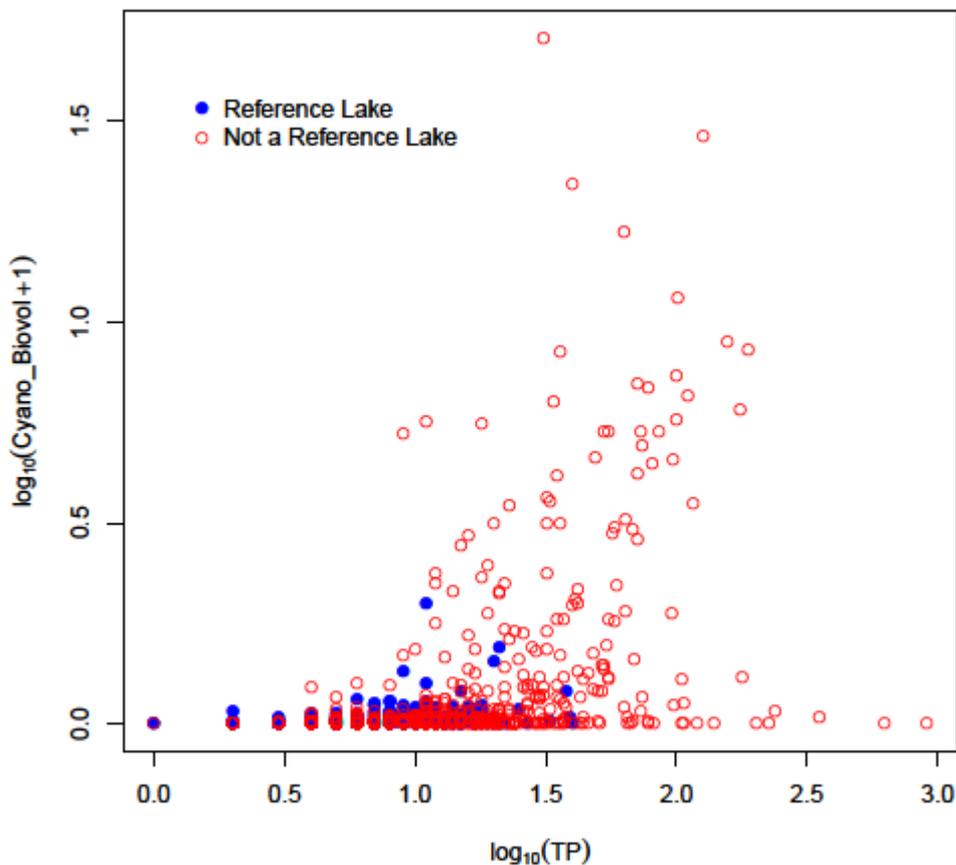


Figure 3.9. Cyanobacteria biovolume vs TP in Northern GIG reference and non-reference lakes.

To derive type-specific reference conditions, descriptive statistics (mean, median, 75% and maximum) were produced for cyanobacterial biovolumes by each lake type (Table 3.13). The median value is recommended for defining reference condition as it is less affected by outliers than the mean. Reference conditions for lake types with less than 5 reference lakes should be considered uncertain. Median and 75% values were generally very low, well below the WHO low risk threshold of approximately $2 \text{ mm}^3 \text{ L}^{-1}$, the highest reference value was $0.987 \text{ mm}^3 \text{ L}^{-1}$ for a L-N8a lake (Figure 3.10; Table 3.13). Although all reference lakes in the N GIG had maximum cyanobacterial densities below the WHO low risk threshold, for consistency with the CB GIG (and chlorophyll classifications), the 75th percentile statistic is recommended as a potential measure for defining the high/good status class boundary. This is felt to be particularly more appropriate in the NGIG where risk levels are generally much lower.

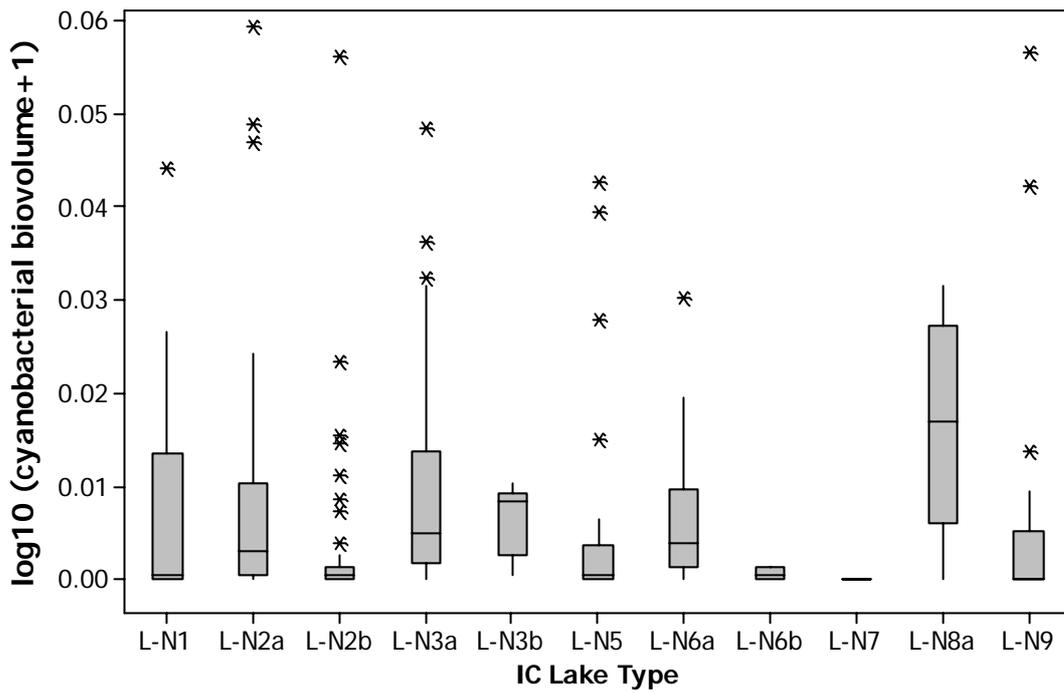


Figure 3.10. Boxplots of cyanobacteria biovolume in N GIG reference lakes by IC type.

Table 3.13. Number of reference lakes (n) by GIG type and corresponding mean, median, 75th percentile and maximum values for cyanobacterial biovolume ($mm^3 L^{-1}$).

ICType	N	Mean	Median	75%	Max.
L-N7	5	0.000	0.000	0.000	0.000
L-N9	22	0.015	0.000	0.012	0.139
L-N1	18	0.019	0.001	0.032	0.107
L-N2b	70	0.009	0.001	0.003	0.207
L-N3	1	0.001	0.001	N/A	0.001
L-N5	41	0.020	0.001	0.009	0.426
L-N6b	3	0.001	0.001	0.003	0.003
L-N2a	59	0.018	0.007	0.024	0.146
L-N6a	41	0.035	0.009	0.023	0.553
L-N3a	58	0.021	0.012	0.032	0.118
L-N8b	1	0.018	0.018	N/A	0.018
L-N3b	8	0.015	0.020	0.022	0.024
L-N11	2	0.032	0.032	N/A	0.036
L-N8a	12	0.115	0.040	0.065	0.987

Application

Deriving EQRs for a cyanobacterial blooms metric

An EQR score of between 0 and 1 was calculated using the following formula:

$EQR = (O1 - E0) / (E1 - E0)$ where

O1 - Observed cyanobacterial biovolume

E0 – Maximum cyanobacterial biovolume (most degraded)

E1 - Expected cyanobacterial biovolume (median of type specific reference site score)

The median of type-specific reference scores are shown in Tables 3.8, 3.10 and 3.13. EQRs >1 are, therefore possible and should be set at 1 for use in the combined BQE assessment.

Using absolute maximum cyanobacterial biovolume observed results in a very limited EQR range, therefore, the upper anchor was based on at least 85% of non-reference lakes in the GIG.

For CB GIG this was the 85% = $7.39 \text{ mm}^3 \text{ L}^{-1}$

For Med GIG this was the 85% = $2.70 \text{ mm}^3 \text{ L}^{-1}$

For N GIG this was the 95% = $3.01 \text{ mm}^3 \text{ L}^{-1}$

As cyanobacterial biovolumes above this do occur, EQRs <0 are possible and should be set at 0 for the combined BQE assessment.

Example L-CB1

Ref (Median L-CB1 reference lakes) = $0.07 \text{ mm}^3 \text{ L}^{-1}$

Maximum (85% of non-reference lakes) = $7.39 \text{ mm}^3 \text{ L}^{-1}$

Good quality lake - observed = $1.5 \text{ mm}^3 \text{ L}^{-1}$,

$EQR = (1.5 - 7.39)/(0.07 - 7.39) = 0.8$

Poor quality lake - observed = $5.9 \text{ mm}^3 \text{ L}^{-1}$,

$EQR = (5.9 - 7.39)/(0.07 - 7.39) = 0.2$

Status class boundaries

Cyanobacterial biovolumes representing status class boundaries were derived in relation to the frequency a site could be above or below the WHO low and medium risk thresholds i.e. based on the frequency and intensity of cyanobacterial blooms over a 6-year monitoring period. Quantitative values for these are outlined in Table 3.14. Qualitatively these describe the following ecological situations:

H/G: All years are well below the low risk threshold ($2 \text{ mm}^3 \text{ l}^{-1}$) and at least 75% of reference lakes.

Good status: All years being below the low risk threshold of $2 \text{ mm}^3 \text{ l}^{-1}$

G/M: Five out of six years being below the low risk threshold ($2 \text{ mm}^3 \text{ l}^{-1}$). One year exceeds.

Moderate status: At least 3 of 6 years being below the low risk threshold, 3 being above.

M/P: 4 of 6 years are above the low risk threshold one of which is above the medium risk threshold ($10 \text{ mm}^3 \text{ l}^{-1}$)

Poor status: 5 or more of 6 years being above the low risk threshold, one of which is above the medium risk threshold ($10 \text{ mm}^3 \text{ l}^{-1}$)

P/B: At least 3 of 6 years being above the medium risk threshold and the other 3 years being above the low risk threshold.

Bad status: worse than the P/B EQR

Table 3.14. Biovolumes used to derive status class boundaries based on the frequency and intensity of cyanobacterial blooms over a 6-year monitoring period

	H/G	G/M	M/P	P/B
Cyano biovol yr 1	1	2	2	2
Cyano biovol yr 2	1	2	2	2
Cyano biovol yr 3	1	2	3	2
Cyano biovol yr 4	1	2	3	10
Cyano biovol yr 5	1	2	3	10
Cyano biovol yr 6	1	3	10	10
Mean cyano biovol	1.00	2.17	3.83	6.00

Discussion and Recommendations for IC

Cyanobacteria Metric

All GIGs have pointed out methodological difficulties regarding the use of blooms in lake phytoplankton assessment systems, e.g. lack of a common definition of a bloom, inadequate sampling frequency for detecting a bloom etc;

- Alpine GIG has agreed not to include blooms in their classification systems because blooms do not occur in Alpine lakes under high and good status, even under moderate status in many lakes, therefore, bloom metrics have high uncertainty and little relevance;
- Central Baltic and Eastern Continental GIG have suggested that their assessment systems do represent the quality element as a whole and that there is no need for a more specific bloom metric (assessment systems include bloom metrics indirectly). Some countries have a cyanobacteria bloom metric (NL and BE) and others may consider adopting a bloom metric based on either % or absolute abundance of cyanobacteria.
- Mediterranean GIG has recognized the value of incorporating bloom metrics in their assessment systems: They consider the WISER candidate metric of cyanobacteria absolute as a promising metric. Following the WISER uncertainty analysis, they will consider replacing % cyanobacteria metrics with the metric based on absolute abundance
- Northern GIG. Following the WISER uncertainty analysis, some MS will consider adopting a metric based on absolute abundance of cyanobacteria

WISER recommendations

- Cyanobacterial blooms are probably the most widely recognized ecological response to eutrophication, of interest to the public, water managers and policy makers. They have explicit consequence for ecosystem services (access to safe, clean water for drinking and recreation). Therefore, a cyanobacteria bloom metric is a great opportunity for making ecological science relevant with the general public and policy makers;
- As remote-sensing and in-situ fluoroprobes become more widespread, the frequency and spatial variability issues become less relevant and a cyanobacteria metric potentially becomes a low-cost metric
- Cyanobacterial bloom metrics based on actual abundance, rather than % abundance, are relevant to water users and have been shown in this report to have a robust relationship with pressure;
- A bloom metric should not be discarded by MS unless the WISER uncertainty analyses shows that they are too variable within a lake or in relation to eutrophication pressure that its inclusion greatly weakens the overall phytoplankton BQE assessment (which incorporates metrics for chlorophyll and composition).

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