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Strength and uncertainty of phytoplankton metrics for assessing eutrophication impacts in lakes

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Abstract

Phytoplankton constitute a diverse array of short-lived organisms which derive their nutrients from the water column of lakes. These features make this community the most direct and earliest indicator of the impacts of changing nutrient conditions on lake ecosystems. It also makes them particularly suitable for measuring the success of restoration measures following reductions in nutrient loads. This paper integrates a large volume of work on a number of measures, or metrics, developed for using phytoplankton to assess the ecological status of European lakes, as required for the Water Framework Directive (WFD). It assesses the indicator strength of these metrics, specifically in relation to representing the impacts of eutrophication. It also examines how these measures vary naturally at different locations within a lake, as well as between lakes, and how much variability is associated with different replicate samples, different months within a year and between years. On the basis of this analysis, three of the strongest metrics (chlorophyll-a, Phytoplankton Trophic Index (PTI) & cyanobacterial biovolume) are recommended for use as robust measures for assessing the ecological quality of lakes in relation to nutrient enrichment pressures and a minimum recommended sampling frequency is provided for these three metrics.

Keywords: ecological indicator; eutrophication; Water Framework Directive; WFD; chlorophyll; cyanobacteria; trophic index

Introduction

The phytoplankton community forms a key component of primary production in lakes. The fact that phytoplankton have short generation times and derive their nutrients from the water column makes this biological quality element the most direct and earliest indicator of the impacts of changing nutrient conditions on lake ecosystems (Lyche-Solheim et al., 2012). There are numerous socio-economic problems associated with eutrophication-related increases in phytoplankton abundance, particularly with increasing frequency and intensity of toxic cyanobacteria blooms. These include detrimental effects on drinking water quality, filtration costs for water supply, recreational activities, and conservation status. The phytoplankton community is, therefore, a key indicator of the health and functioning of freshwaters in relation to eutrophication pressure, and for measuring the success of restoration measures following reductions in nutrient loads. The European Water Framework Directive (WFD) requires the ecological status of surface waters to be assessed on the condition of their biological quality elements (BQEs) (EC, 2000). As part of this, Annex V of the WFD specifically outlines three features of the phytoplankton BQE that need to be considered in this assessment for lakes:

1. Phytoplankton biomass or abundance and its effect on transparency conditions
2. Phytoplankton composition
3. Planktonic bloom frequency and intensity

Here we briefly review national metrics for lake phytoplankton that have been developed for the WFD. We then compare six metrics assessed in the EC WISER project, focusing particularly on metrics for phytoplankton composition and blooms. Metrics for phytoplankton biomass are relatively standardised using chlorophyll a or total biovolume (Poikane et al., 2011) and reference conditions and status class boundaries had already been widely agreed for chlorophyll for European lakes (e.g. Carvalho et al.,2008; Poikane et al., 2010; Wolfram et al., 2009). The other two features, outlined in the next sections, required further specific developments for the WFD, as highlighted in Birk et al. (2012) & Poikane et al. (2011). Here we summarise the strength of all these metrics in relation to eutrophication pressure and sources of uncertainty based on analysis of temporal and spatial variability in metric scores. We recommend which of the studied metrics are most suitable for assessing ecological status in the WFD (compliant with Annex V, EC 2000) and the minimum sampling requirements for robust assessment. Finally we discuss the gaps in current assessment schemes, particularly in relation to lake functioning and more integrated measures of eutrophication pressure that incorporate information across a number of biological elements.

Biomass, abundance and transparency

In general, as nutrient concentrations increase, phytoplankton biomass or abundance shows more frequent and sustained peaks throughout summer and transparency declines (Reynolds, 1984). There are exceptions to this, such as shallow macrophyte-dominated lakes, where top-down control by zooplankton grazers can limit phytoplankton biomass (Jeppesen et al., 1997), highlighting a need for a holistic approach to ecological assessment. Phytoplankton biomass or abundance is generally measured as “biovolume”. Alternatively, concentrations of the photosynthetic pigment chlorophyll *a* (chl-a) are used as an approximate measure, widely adopted in national (e.g. Carlsson, 1977; Wolfram et al., 2009), European (EC, 2008) and international (OECD, 1982) lake monitoring and classification schemes. Measurements of chlorophyll *a* can be problematic in that concentrations vary depending on algal composition and their physiological state (Reynolds, 1984). For example, cyanobacteria have less chl-a per unit biomass than green algae (Chlorophyta). Direct counts and measurements of algal biovolume are potentially, therefore, a more accurate measure of phytoplankton biomass or abundance. Biovolume measurements are, however, much more time-consuming to make and often more prone to errors between different analysts, so can be more affected by issues of cost-saving, accuracy and precision.

One of the first classification schemes developed for phytoplankton abundance was that of Carlson (1977) who used chl-a (and Secchi disc depth) as a measure of “trophic status”. The most widely recognised classification in terms of chl-a, is that developed during the OECD programme on eutrophication (OECD, 1982). This programme developed quantitative regression models relating chl-a concentrations to total phosphorus concentrations and outlined chlorophyll standards for different trophic classes (oligotrophic, mesotrophic and eutrophic) based on expert opinion. Since then, these regression equations have been explicitly refined for European lake types (Phillips et al., 2008). More recently, reference-based classification schemes for chl-a have been developed in individual Member States specifically for the WFD (e.g. Carvalho et al., 2008; Søndergaard et al. 2005; Wolfram et al., 2009) and chl-a standards have been successfully compared between European member states in an “Intercalibration” (IC) process to ensure that standardised quality classes exist in specific lake types across several geographical regions of Europe (EC, 2008; Poikane et al., 2010). For this reason the WISER project did not re-visit assessment schemes for phytoplankton biovolume or chl-a. It has, however, examined sources of uncertainty in the measurement of chl-a and on the basis of this provided recommendations for WFD sampling programmes, and these are summarised and discussed in this paper.

Composition

In general, most algal classes are found in lakes spanning the entire nutrient gradient. The only exceptions to this are chrysophycean algae that are characteristic of nutrient poor (and low alkalinity) waters (Järvinen et al., 2012; Maileht et al., 2012). Compositional changes due to nutrient enrichment usually become apparent at the generic and species level. For example, of the diatoms, *Cyclotella* Kützing species are frequently associated with nutrient poor lakes and *Stephanodiscus* Ehrenberg species tend to dominate following enrichment (Bennion, 1994; Wunsam & Schmidt, 1995). Cyanobacteria, such as the large colonial and filamentous genera *Microcystis* Kützing, *Aphanizomenon* Morren and *Anabaena* Bory also tend to increase in abundance in response to increasing nutrient concentrations (Reynolds, 1984). Phytoplankton compositional responses to eutrophication can also be considered in terms of functional groups (Reynolds et al., 2002) and this may be important for encapsulating the philosophy of ecological status in the WFD, which should be “an expression of the quality of the structure and functioning of the system”. Trait-based, functional classifications are increasingly being used in ecology because of their connection with ecosystem functioning. Among phytoplankton functional traits, cell size is a key feature, being related to the efficiency of many eco-physiological processes (nutrient assimilation, photosynthetic efficiency, respiration, buoyancy), most of which are affected in some way by nutrient changes (Capblanq & Catalan, 1994). Phytoplankton body size is also related to ecosystem functioning as it affects the transfer of energy through the food web as zooplankton grazers specialise on different algal sizes (Jansson et al., 2007). Following a more functional approach, a phytoplankton assemblage can be described in terms of size spectra (Kamenir & Morabito, 2009) or Morpho-Functional Groups (Reynolds et al., 2002; Salmaso & Padisák, 2007).

In recent years, a large number of national assessment systems for phytoplankton composition have been under development for the WFD, including taxonomic and functional approaches (Poikane, 2009). One of the key actions identified by the WFD is to carry out a European benchmarking or "Intercalibration" (IC) exercise to ensure that these assessment systems are comparable and, in particular, that good ecological status represents the same level of ecological quality everywhere in Europe (EC, 2000, Annex V). In this paper, we very briefly review the national metrics submitted by the end of the 2nd phase of the Intercalibration process (November 2011) and review three compositional metrics, developed in WISER, for potential use as a "common metric", a common measurement scale for comparison of national metrics in the Intercalibration process. These three composition metrics are:

1. Phytoplankton Trophic Index (PTI) – a taxonomic-based sensitivity index
2. Size Phytoplankton Index (SPI), an index based on size classes
3. Morpho-Functional Group Index (MFGI) – a combination of size and functional group

Bloom frequency and intensity

There is no consistent agreement on a definition of a phytoplankton bloom, although it is always used in relation to an abundant crop of a particular class of algae. Annex V of the WFD indicates that a bloom metric should incorporate some measure of both bloom intensity (measures of magnitude/abundance) and how frequently they occur over a particular specified time period (e.g. frequency within a summer period or frequency over the 6 year WFD reporting period). The term "bloom" has been associated with surface scums of cyanobacteria for hundreds of years (McGowan et al., 1999). Cyanobacteria are widely recognised to increase in dominance and abundance in response to increasing nutrient concentrations, often resulting in dense, mono-specific blooms during summer in eutrophic waters (Carvalho et al., 2011; Watson et al., 1997). Lake ecologists also use the term "bloom" to refer to spring and autumn increases in diatoms (Reynolds, 1984) and marine biologists refer to blooms of diatoms or dinoflagellates (Carstensen et al., 2007). Annex V of the WFD characterises moderate status lakes as those in which "persistent phytoplankton blooms" may occur during summer months and, for this reason, almost certainly had in mind summer blooms of cyanobacteria. Mischke et al. (2011) proposed three characteristics of a summer phytoplankton bloom in lakes:

- High phytoplankton abundance
- Uneven community - dominance by one type of algae, usually one or two species
- Abundance of nuisance species e.g. potentially toxic cyanobacteria

With these characteristics in mind, we review the strength and uncertainty of two potential bloom metrics examined in the WISER Project (see Mischke et al., 2011 for full details):

1. Pielou's Evenness Index (J) (incorporating a critical abundance threshold)
2. Cyanobacterial abundance (actual biovolume – not relative % abundance)

Methods

Review of national assessment methods

National assessment methods have been collated into an online database (Birk et al., 2010; 2012) and reviewed for WFD-compliance as part of the Intercalibration process (Poikane, 2009;

2011). Based on existing metric classifications (Karr and Chu, 1999; Hering et al., 2006), metrics were grouped into the following types: (1) abundance metrics (e.g. chl-a and total biovolume), (2) composition metrics (e.g. percentage cyanobacteria), (3) sensitivity/tolerance metrics (e.g., trophic indices) and (4) richness/diversity metrics (e.g., evenness or diversity indices). Note that sensitivity / tolerance metrics often form the basis of the composition metric in national schemes for the WFD, and the composition metrics specifically related to cyanobacteria have sometimes been adopted as a bloom metric for WFD purposes.

Strength of WISER composition and bloom metrics

The sensitivity of the WISER phytoplankton metrics to eutrophication pressure was assessed from regression analyses of dose-response curves along total phosphorus (TP) gradients using large scale pan-European datasets from >1500 lakes from 21 countries (Moe et al., 2012; Schmidt-Kloiber et al., 2012). Full details of the data and methods are provided in Phillips et al. (2010; 2012) and Mischke et al. (2011).

Uncertainty and Sampling Guidance

Spatial and analytical sources of variability of the six WISER phytoplankton metrics were assessed using data from 32 European lakes, sampled in 2009 as part of a WISER multi-scale field campaign to understand sources of variation in phytoplankton metrics. Spatial variability in metric values between three different open water sampling locations were examined: the deepest point, a location around the mean depth and a depth intermediate between the two, as well as variability between lakes, between samples within a location and analytical variability (see Thackeray et al., (2011; 2012b) for full details of sample design and methods).

The pan-European WISER phytoplankton dataset from >1500 European lakes was also used to carry out analyses to compare temporal and between-lake variation in phytoplankton metrics at the European scale (Thackeray et al., 2012a; 2012b). Three phytoplankton metrics were examined: chl-a concentration, PTI (Phillips et al., 2010; 2012) and total cyanobacterial biovolume (Mischke et al., 2011). Linear mixed effects (LME) models were used to resolve temporal aspects of metric variation, specifically metric variability between months and between years, and to compare this variation to that apparent between lakes that span a wide pressure gradient. LME models were constructed to take into account modifications of the typical pattern of seasonal metric change as a result of lake characteristics (such as latitude, altitude, humic type) and TP (Thackeray et al., 2012a). Using this formulation, within-year metric uncertainty is taken to be the monthly variation in metric scores that occurs around the pattern that is typical for a specific lake type. Separate analyses were carried out on lake data from three geographical regions, known as GIGs (Geographical Intercalibration Group): Central European and Baltic region, Northern region and the Mediterranean region. Using the estimated variance parameters from the LME models, a measure of sampling variance was calculated to describe the degree of uncertainty in the mean observed value of each metric for a waterbody, when based upon collecting samples from different numbers of years, and/or months within years:

Monthly and inter-annual scale temporal sampling variance of water body mean =

$$\frac{\sigma^2_{y} \text{ x } (1- [N_{year}/\max_{year}])}{N_{year}} + \frac{\sigma^2_{m} \text{ x } (1- [N_{month}/\max_{month}])}{(N_{month} \text{ x } N_{year})}$$

Where:

σ^2_y = year-level metric variance from mixed effects model

σ^2_m = month-level metric variance from mixed effects model

N_{year} = number of years sampled

N_{month}= number of months sampled per year

Max_{month} = maximum number of months that can be sampled per year [for total cyanobacteria and PTI, max_{month} =3 (July-September); for Chl-*a*, max_{month} =6 (April-September)]

Max_{year}= maximum number of years that can be sampled per reporting/monitoring period [set at 6 years; a WFD river basin monitoring cycle]

Based on this analysis, we are able to recommend minimum sampling frequencies for these three metrics. Where possible, two alternative sampling frequencies have been recommended for a given metric (each yielding a near-equivalent degree of temporal sampling uncertainty) to enable flexibility in operational monitoring programmes, whilst retaining comparable confidence in classification.

Results

Review of national metrics

24 European countries reported on 26 lake phytoplankton assessment methods comprising 87 metrics. Most of the national methods for the phytoplankton BQE comprise either 2 metrics (one of them related to phytoplankton biomass, another to taxonomic composition) or 4 - 5 metrics (including several parameters both for biomass and species composition). Only one national method contains just one metric (Swedish metric for assessing impacts of acidification).

Table 1. Overview of the phytoplankton metrics used in European Union Member State assessment schemes for the Water Framework Directive (Birk et al., 2010; 2012).

Metric type	Metric	Number
Biomass metrics		**40**
	Chlorophyll-a	23
	Phytoplankton biovolume	13
	Average of chlorophyll-a and biovolume	3
	Secchi depth	1
Sensitivity / tolerance metrics		**23**
	Indices based on indicator species	13
	Indices based on taxonomic groups	8
	Indices based on indicator values of functional groups	2
Composition metrics		**13**
	Relative abundance of Cyanobacteria	9
	Cyanobacteria biovolume	2
	Relative abundance of other algal groups	2
Richness / diversity metrics		**7**
	Evenness index	2
	Taxa richness	2
	Diversity index	3
Bloom metrics		**4**
	Cyanobacteria biovolume	4
Total		**87**

Of the 87 metrics reported, almost half of the metrics characterise phytoplankton abundance (46 %), while composition metrics were largely of two types: indices of sensitivity/tolerant taxa (26%) and abundance of specific taxa (15%) (Table 1). Richness/diversity metrics were rarely used (8%) and only 4 (5%) national metrics were specifically termed "bloom" metrics, although another 11 of the 13 composition metrics were also based on the relative or absolute abundance of cyanobacteria (Table 1) and could potentially be considered as bloom metrics.

The most frequently used biomass metric is chl-a (23 metrics), used alone or together with total biovolume. Almost all European Union Member States (MS) included some version of sensitivity / tolerance metrics where 3 patterns can be distinguished: (1) The most frequent sensitivity indices are based on indicator taxa lists and their trophic scores and weighting factors (e.g., Brettum, 1989; Dokulil &Teubner, 2006; Mischke et al., 2008; Salmaso et al., 2006; Swedish EPA, 2010), (2) other indices were based on biovolume of a given algal group, or on the ratios between the biovolumes of several algal groups (Catalan et al., 2006; Nygaard, 1949, adapted by Ott, 1995); 3) only two MS used indices based on a functional group approach (Reynolds et al., 1989) where indicator values were assigned to each functional group (Padisák et al., 2006).

Strength of WISER composition and bloom metrics

Of the six WISER phytoplankton metrics tested, PTI (r^2 = 0.67), and chl-a (r^2 = 0.63, for lakes with TP<100µg/l) had the strongest relationships with TP (Table 2). The weakest relationships with TP were generally found for the evenness metric, although the SPI and MFGI were also weak in some GIGs (Table 2). Full details of metric strength are provided in Phillips et al. (2010; 2012) & Mischke et al. (2011).

Table 2. Relationship strength between six WISER phytoplankton metrics and total phosphorus as a proxy of eutrophication pressure. GIG = Geographical Intercalibration Group. CB = Central European and Baltic region, N = Northern region, M = Mediterranean region. Data summarised from Phillips et al. (2010) and Mischke et al. (2011). GAM = Generalized Additive Model. All other relationships are based on linear regression models.

Metric	Metric description	Pressure	r^2	GIG	p	N
Chl-a	Chl-a (µg/l)	Eutrophication (Total-P)	0.63	all	<0.001	16949
PTI	Phytoplankton Trophic Index	Eutrophication (Total-P)	0.67 (GAM)	all	<0.001	1500
SPI	Size Phytoplankton Index	Eutrophication (Total-P)	0.23 0.34 0.19	CB N M	<0.0001 <0.0001 <0.05	122 77 29
MFGI	Morpho-Functional Group Index	Eutrophication (Total-P)	0.33 0.05 0.38	CB N M	<0.0001 <0.05 <0.001	122 77 29
J’	Pielou’s Evenness Index	Eutrophication (Total-P)	0.19 0.07	N CB	<0.001 <0.001	716 559
Cyanobacteria bloom intensity	Cyanobacteria biovolume (mg/l)	Eutrophication (Total-P)	0.34 (GAM)	All	<0.001	1710

Uncertainty and Sampling Guidance

For all six WISER metrics, between 65% and 96% of the variance in metric scores was due to variability between lakes (Table 3). Within-lake variability caused by natural spatial variation, as well as variability related to sampling and analyses, was generally low for these six metrics (Table 3). Not considering temporal variability, the most precise metrics with the lowest within-lake variance are chlorophyll, cyanobacteria biovolume and the taxonomic composition index PTI. The most important within-lake variance component for these metrics was sub-sampling. However, as the total within-lake variance is so low for these metrics (ca.5-10%), the error caused by sub-sampling is minor.

Table 3. Metric precision given as the proportion of total metric variance that occurred between and within-lakes. The major within-lake variance component is also highlighted. See Table 2 for description of metrics. Data taken from Thackeray et al. (2012b).

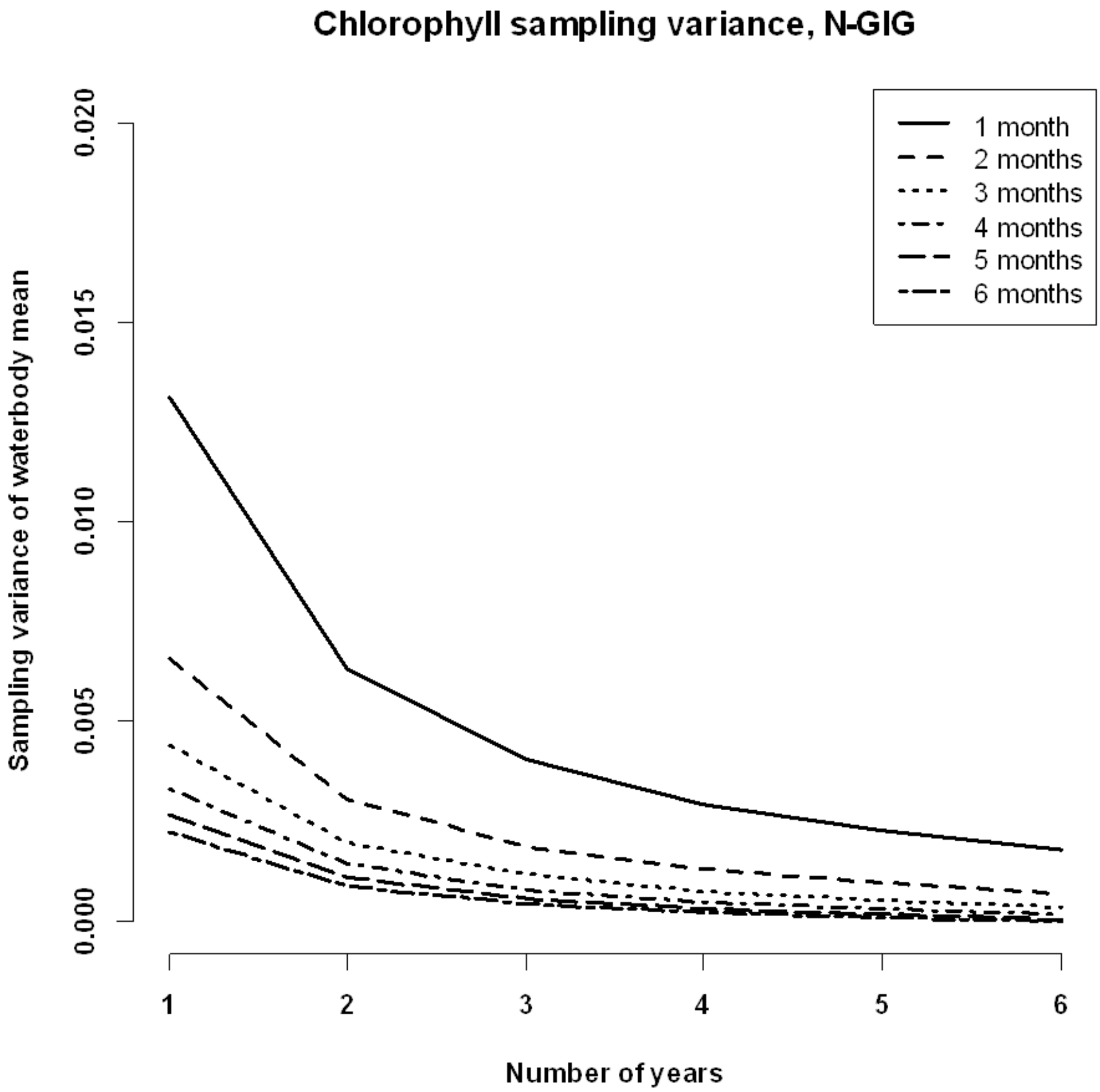
Metric	Between-lake variance	Within-lake variance	Major within-lake variance component (excluding temporal variability)
Chl-a	0.96	0.04	Sub-sampling
PTI	0.88	0.12	Sub-sampling
SPI	0.65	0.35	Analyst
MFGI	0.86	0.14	Sub-sampling
J’	0.69	0.31	Analyst
Cyanobacteria bloom intensity	0.94	0.06	Sub-sampling

The analysis of temporal variability only examined three candidate metrics (chl-a, PTI and cyanobacteria biovolume) but highlighted different levels of variability for these three metrics in different regions of Europe (Thackeray et al., 2012a). Based on the analyses presented in Thackeray et al. (2012a), Table 4 summarises our recommended minimum sampling frequencies for chl-a, PTI and the cyanobacterial bloom metric. It should be noted that, based on typical Member State sampling regimes and analytical practicalities (Birk et al., 2010; 2012), the analysis limited the maximum number of months that can be sampled per year for the cyanobacteria biovolume and PTI metrics to 3 months (July-September), whilst for chl-a this was extended to a possible 6-month sampling frequency (April-September). As an example of this analysis, Fig.1 illustrates the extent to which uncertainty in the chl-a metric in lakes in Northern Europe can be reduced when sampling increasing numbers of years and months within years. From these analyses, it can be seen that the sampling variance (and associated uncertainty) in chl-a reduces markedly when increasing the number of months sampled (between Apr-Sep) and when sampling multiple years. The all-lake (cross-GIG) and Northern Europe (N-GIG) analyses suggest that sampling variance can be reduced dramatically by sampling in 2 months, in each of 3 years, or alternatively, a similar level of uncertainty can be obtained sampling 3 months in each of 2 years. Due to the higher level of temporal variability for chlorophyll a in CB-GIG, a greater degree of replication is needed to achieve this same reduction in sampling variance, therefore, we recommend at least 3 monthly samplings for 4 years to achieve comparable levels of uncertainty in metric scores (Table 4).

Table 4. Minimum recommended sampling frequencies for three phytoplankton metrics in three GIGs based on analysis of variability in the cyanobacteria biovolume and PTI metrics within 3 summer months (July-September), and for chl-a within 6 months (April-September). For example for NGIG, chl-a should be sampled at least once in 2 different months (Apr-Sep) in each of 3 different years, or alternatively, once in 3 different months (Apr-Sep) in each of 2 different years, meaning 6 samples altogether (see Thackeray et al., 2012a for full details). Where alternatives are given, these yield very similar levels of metric uncertainty and the first alternative should not be considered optimal compared to the second.

	CB-GIG	**M-GIG**	**N-GIG**
Chl-a	3 months for 4 years	3 months for 3 years	2 months for 3 years or 3 months for 2 years
PTI	2 months for 4 years or 1 month for 6 years	3 months for 3 years or 1 month for 6 years	3 months for 3 years or 1 month for 6 years
Cyanobacteria biovolume	1 month for 6 years	1 month for 6 years	1 month for 6 years

Figure 1. Changes in temporal sampling variance for chl-a in the N-GIG when sampling in different numbers of years and months (Apr-Sep) within years (see Thackeray et al., 2012a for full details).



Discussion

Recommendations of metrics for Intercalibration and National Schemes

Lake phytoplankton are widely adopted around the world as a highly sensitive, early-warning indicator of water quality. European environmental legislation, the EU Water Framework Directive (WFD), formalises this, requiring the use of phytoplankton for the assessment of the ecological status of lakes. For lakes, the most widespread pressure is nutrient enrichment. There is, therefore, a great need to develop robust metrics that quantify the response of phytoplankton communities to nutrient pressure. Annex V of the WFD specifically outlines three features of the phytoplankton quality element that need to be considered in the assessment for lakes (abundance, composition and blooms). The review of national metrics revealed that many MS used chl-a as a biomass or abundance metric and many used some form of index based on indicator taxa lists and their trophic scores as a composition metric (e.g. Dokulil & Teubner, 2006; Mischke et al., 2008; Salmaso et al., 2006).

Our analysis strongly supports the use of both chl-a and the PTI metric in a common metric for the Intercalibration exercise. These two metrics have both the strongest relationships with TP (Table 2) and also some of the lowest within-lake variance (Table 3). Our analysis shows that non-taxonomic morpho-functional approaches (SPI & MFGI) had weaker relationships with TP and higher within-lake variance (particularly the SPI). The reasons for this are not clear but may simply be due to the smaller number of indicator groups, compared with genera- or species-based indices, and greater weighting given to biovolume estimates in the size-based indices. The uncertainty in the latter could potentially be reduced through improved counter training or more automated methods for assigning size-classes, such as the use of flow cytometry (Garmendia et al., 2012).

Of the two bloom metrics developed and tested in WISER, cyanobacterial biovolume is recommended over evenness as it had a stronger and significant relationship with TP (Table 2) and had very low levels of within-lake variance (Table 3). This metric effectively represents the intensity of summer blooms, but does not represent bloom frequency. The wording of the normative definition in Annex V of the WFD mentions "persistent blooms during summer" which tends to suggest high frequency monitoring is needed. With the currently used labour-intensive in-lake sampling and counting methodologies this is clearly not practical for any European country. New technologies based on fluorometry, citizen monitoring of cyanobacterial blooms (e.g. Finland) or new hyper-spectral European satellite platforms (e.g. MERIS and Sentinel 2; see, for example, Bresciani et al., 2011), could, however, make higher frequency monitoring a real possibility in the near future.

Uncertainty and Sampling Guidance

Differences in sampling locations in a lake, sample replicates or analytical variability accounted for just a small proportion of the variability in metric scores for the strongest metrics representing the three features of abundance (chl-a), composition (PTI) and blooms (cyanobacteria biovolume). The full analysis by Thackeray et al. (2012b) importantly indicates that, for these three metrics at least, the variability between lakes is significantly related to differences in total phosphorus concentrations, i.e. these metrics are sensitive to eutrophication pressure and show little "noise" in relation to within-lake or analytical variability. Although these metrics appear very robust to differences in the location of sampling points within a lake, it has to be stressed that the WISER field campaign only compared three different open water sampling locations. It did not examine sampling from the edge of a lake or the outflow and so cannot be used to approve or disapprove of any method based on these locations. It does, however, highlight that only a single open water location needs to be sampled, as replicate sampling of the open water will have little effect on uncertainty in status assessments.

It has been shown that in some small, well sheltered lakes small-scale horizontal patchiness of the phytoplankton can result in differences in assessment results (Borics et al., 2011). There are also some more predictable exceptions, where spatial heterogeneity can be expected to be greater, and where more than 1 sampling location should be considered. This includes large lakes (e.g. surface area >10 km^2) or lakes with clearly distinct separated bays. In these cases, several integrated samples could be taken and mixed before analysis. If, however, nutrient loading pressures are likely to impact differently in different basins of large, morphologically complex lakes, then these basins should be designated as distinct water bodies and their status assessed separately. With the development of satellite technology in the near future, high resolution, multi-spectra satellite imagery may enable improved spatial representation of the open-water of large lakes for parameters such as chl-a and cyanobacteria biovolume (Hunter et al., 2010).

It should also be pointed out that the within-lake and analytical variability may have been particularly low in the WISER field exercise as sampling methods were standardised and many of the phytoplankton counters attended a training workshop to standardize counting methods and identification prior to sample analysis. The results highlight the value of good training, standard methods and quality control checks for increasing confidence in assessment results.

Frequency of sampling

The phytoplankton community is notoriously dynamic between years, over a year, and even within a season. Developing an ecological assessment scheme using phytoplankton requires minimising the effects of seasonal variability associated with the changing physical and biological structure of the water column and magnifying the signal related to nutrient pressures. The ambition to capture seasonal succession and variability greatly differs between European countries. Sampling frequencies vary from once in the summer period to monthly sampling throughout the year (Poikane, 2009). These variations in sampling can contribute to differences between assessment results and may require different standards between countries (e.g. some countries may set chlorophyll standards based on growing season means whilst other countries standards may be based on annual means). A strict and agreed definition for the growing season is not possible across large geographical regions, such as Europe. The duration and the onset of the ice-free period vary by longitude (Atlantic-continental influences), latitude (Norway to Spain) and altitude. Despite this, the methods review highlighted that the period from July to September is a common period for phytoplankton sampling in European lakes. The WFD has a six-year reporting period. For this, the WISER temporal uncertainty analysis indicates that generally at least 3 samplings of these summer months is necessary for at least 3 years to minimise the effects of seasonal and inter-annual metric variability. Although the temporal analysis revealed that for some metrics in some regions sampling one summer month every year for six years gave a comparable level of uncertainty, it must be stressed that this is based on the study of a large population of lakes. For many individual lakes, summer variability may be much higher and a single monthly sample within a year for six years is likely to lead to high uncertainty in assessment results (e.g. Søndergaard et al., 2011).

The cyanobacteria biovolume metric shows a different uncertainty pattern than the rest. Based on only summer sampling (July to September), inter-annual variability appeared much greater than monthly variability within the summer and, therefore, frequency of sampling for this metric would be better targeting different years. The reasons for this are not clear, but may be related to the fact that this metric is based on only a single algal class and cyanobacteria are known to be sensitive to a number of factors, including temperature and water column stability (Dokulil & Teubner, 2000). It may be that, unlike the other metrics, at a broad lake scale, lakes either have cyanobacteria or do not (e.g. low alkalinity lakes, Carvalho et al., 2011). Lakes that do not have cyanobacteria clearly have little seasonal or inter-annual variability in cyanobacteria. Our analysis suggests that in lakes that are prone to cyanobacteria, variability is between years, rather than between summer months, i.e. weather conditions during one summer season are generally fairly stable, whilst between years can vary greatly. This is a clear hypothesis that could be tested in a future study and in fact further analysis could help strengthen the relationship of this metric with eutrophication pressures. For example, if additional climate-related factors, such as annual flushing rates, are shown to be a major source of variability, then these could be incorporated into the assessment scheme (through typology or shifting climate-related reference conditions).

Wider conclusions on assessment of eutrophication and recovery

Despite it being widely acknowledged as representing important impacts of eutrophication on lake ecosystems, phytoplankton composition has rarely been adopted as a component of modern lake classification schemes. The requirement of expert skills in identification and the complexity of interpretation may have previously limited their routine application. The WFD has changed this. It required metrics for phytoplankton abundance, composition and blooms to be applied in combination. Substantial efforts in collecting consistent phytoplankton data across Europe have allowed robust quantitative relationships to be developed between composition and nutrient pressure, with the PTI metric being of comparable strength to chl-a, the most widely used lake assessment metric. A sufficiently strong metric for phytoplankton blooms, based on cyanobacteria biovolume has also been demonstrated. We have also shown that a single open water sampling location is generally sufficient for characterising a lake's status and that the dynamic nature of phytoplankton communities can be overcome by either frequent monthly sampling where possible (e.g. chl-a) or by restricting the seasonal window that metrics operate in (e.g. summer monthly samples only).

However, there are still issues to resolve. The WFD outlines the need for classification schemes to represent the health of the structure and function of the water body, so metrics need to represent more than just TP, and represent what we believe eutrophication is all about more widely. Metric strength in this analysis and in most published studies (e.g. OECD, 1982) has largely been assessed based on relationships with TP. However, some metrics which show weaker relationships with TP may also be of value. For example, the cyanobacteria biovolume bloom metric did not show such a strong relationship with TP, but it is widely accepted as a major impact of eutrophication on water use for recreation and water supply, and adopting it as a bloom metric makes WFD targets relevant to these ecosystem services that are highly valued by the general public. In fact, Annex V of the WFD (EC, 2000) does not require phytoplankton metrics to indicate changes in TP, but does outline that a lake in good status should not have persistent blooms in summer. Other composition metrics, such as the size-structured and trait-based indices, SPI and MFGI, or diversity and evenness metrics may in fact not just represent impacts of eutrophication, but may indicate the impacts of other stressors, including climate change which affects flushing rates and water column stability (e.g. Tuvikene et al., 2011). These size-structured approaches are also recognised as being useful for understanding the transfer of energy to higher consumers and higher consumer feeding behaviours (Jansson et al., 2007; Woodward et al., 2010). They may, therefore, be more useful in more holistic measures of the health and resilience of lake ecosystems as a whole to multiple stressors. Nevertheless, the WISER research has provided clear recommendations on three robust metrics (chl-a, PTI and cyanobacteria biovolume) for use in specifically diagnosing the impact of eutrophication pressures. These three metrics are not simply structural indicators, but both implicitly and explicitly, represent broad impacts of eutrophication on lake structure and functioning and, importantly, the quality of ecosystem services we derive from them.

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