Eesti Maaülikool
Estonian University of Life Sciences

PHYTOPLANKTON AS ECOLOGICAL QUALITY INDICATOR OF LAKES

FÜTOPLANKTON JÄRVEDE SEISUNDI INDIKAATORINA

KAIRI MAILEHT

A Thesis
for applying for the Doctoral degree of Philosophy
in Environmental Sciences and Applied Biology

Väitekiri
filosoofiadoktori kraadi taotlemiseks keskkonnateaduste ja
rakendusbioloogia erialal

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LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following papers, which are referred to by Roman numerals in the text.

I **Maileht, K.**, Nõges, T., Nõges, P., Ott, I., Mischke, U., Carvalho, L., Dudley, B., 2013. Water colour, phosphorus and alkalinity are the major determinants of the dominant phytoplankton species in European lakes. Hydrobiologia, 704: 115–126.

II Carvalho, L., Poikane, S., Lyche Solheim, A., Phillips, G., Borics, G., Catalan, J., De Hoyos, C., Drakare, S., Dudley, B., Järvinen, M., Laplace-Treyture, C., **Maileht, K.**, McDonald, C.M, Mischke, U., Moe, J., Morabito, G., Nõges, P., Nõges, T., Ott, I., Pasztaleniec, A., Skjelbred, B., Thackeray, S.J., 2013. Strength and uncertainty of phytoplankton metrics for assessing eutrophication impacts in lakes. Hydrobiologia, 704: 127–140.

III Thackeray, J.S., Nõges, P., Dunbar, J.M., Dudley, J.B., Skjelbred, B., Morabito, G., Carvalho, L., Phillips, G., Mischke, U., Catalan, J., de Hoyos, C., Laplace, C., Austoni, M., Padedda, M.B., **Maileht, K.**, Pasztaleniec, A., Järvinen, M., Lyche Solheim, A., Clarke, T.R., 2013. Quantifying uncertainties in biologically-based water quality assessment: A pan-European analysis of lake phytoplankton community metrics. Ecological Indicators, 29: 34–47.

Author's contribution to the articles (*):

	I	II	III
Concept	*		
Design	*		
Data collection	*	*	*
Data analyses	*	*	*
Statistical analyses	*		
Manuscript preparation	*	*	*

ABBREVIATIONS

AP	Alkaline phosphatase
AIC	Akaike Information Criterion
BQE	Biological Quality Elements
CCA	Canonical Correspondence Analysis
Chl *a*	Chlorophyll *a*
DIC	Dissolved Inorganic Carbon
EC	European Commission
EiE hypothesis	Everything is everywhere hypothesis
EU	European Union
GIG	Geographical Intercalibration Group
CB-GIG	Central-Baltic Geographical Intercalibration group
M-GIG	Mediterranean Geographical Intercalibration group
N-GIG	Nordic Geographical Intercalibration group
TN	Total nitrogen
N	Nitrogen
TP	Total phosphorus
P	Phosphorus
REML	Restricted Maximum Likehood
WFD	Water Framework Directive
UV	Ultraviolet radiation
PTI	Phytoplankton Trophic Index
SPI	Size Phytoplankton Index
MFGI	Morpho-Functional Group Index
FTI	Functional Traits Index
J	Evenness index

INTRODUCTION

The European Water Framework Directive (WFD, EC, 2000) required that all lakes should be at least in "good" ecological status by the year 2015. Still, this goal was not reached for all water bodies and the deadline was postponed to 2027 at the latest. The EU WFD provides for using different biological quality elements (BQE) for assessment of the ecological status of lakes. BQEs should be sensitive to eutrophication and habitat modification. Among biotic groups in lakes, phytoplankton is known as the most sensitive indicator of water quality, it has rapid replication rate, direct response to physical and chemical environmental factors, and quick changes in species or functional groups reflecting changes in the surrounding environment.

According to WFD Annex V three phytoplankton based BQEs should be used: phytoplankton biomass or abundance, phytoplankton composition and intensity and frequency of blooms. EU Member States are using in their assessment systems many different phytoplankton parameters, some only one, some a combination of several ones. For assessing the ecological quality of European lakes, it is important to use rigorous metrics that respond to nutrient enrichment and eutrophication as the dominant pressures for inland waters.

The efficiency of water body assessment depends on many things, but crucially on typology of lakes and selection of right metrics (Nõges et al., 2009). The implementation of the WFD induced extensive search for new biological metrics to measure the anthropogenic impact on water bodies. Seven phytoplankton metrics in use were tested across a large scale of conditions in different countries.

To test the metrics in use or work out new, it is important to study species-based indexes and environmental factors that determine the distribution of phytoplankton species. One paper **(I)** of this thesis is focusing on the impact of hydrochemical, climatic and morphometric factors of dominant phytoplankton species over a broad geographical scale. Focusing on dominant species is important for several reasons: the stability of communities often depends on the dynamics of dominant species, and the dominant species give a hint on resources availability. As community equilibria occur most prominently during summer, it is most promising from the domination point of view to study summer phytoplankton. Many dominant species are nuisance species that gives a high priority to this research topic.

In the two other papers, the focus is on phytoplankton composition, bloom metrics and their strength in relation to eutrophication pressure. The studies were based on uncertainty analysis of temporal and spatial variability in metric scores. For these studies, large number of data across Europe was gathered using strict sampling design. As the main outcome of this study, the most suitable metrics for assessing the ecological status of EU lakes were identified, and recommendations given for representative numbers of samples for assessments.

1. REVIEW OF THE LITERATURE

1.1. Ecological quality of lakes and reference conditions

According to the WFD, "surface water status" is the general expression of the status of a body of surface water, determined by the poorer of its ecological status and its chemical status (EC, 2000). Member States have to achieve at least "good" status of water. Where "good" status already exists, it should be maintained. EU Member States have to monitor the ecological quality of their aquatic environments. With this, WFD has given a new impetus of freshwater ecological studies at species and community level and the need for comparisons over broad geographical ranges is shifted to the forefront of research (EC, 2000).

To assess ecological status of surface waters, besides physico-chemical parameters, Biological Quality Elements (BQE), which are sensitive to eutrophication should be used. For lakes, BQEs are phytoplankton, macrophytes and phytobenthos, benthic invertebrates and fish. Lake phytoplankton is known world-wide as a sensitive indicator of water quality. Phytoplankton derives its nutrients from the water column and has a short generation time, thus it is a direct and earliest indicator of the impact of changing nutrient conditions (Lyche Solheim et al., 2013). Different phytoplankton species, functional groups and indexes are used to determine the ecological quality of lakes (Willen, 2007; Mischke et al., 2008; Ptacnik et al., 2009; Phillips et al., 2010, 2012; Järvinen et al., 2013; Laplace-Treyture & Feret, 2016; Gebler et al., 2020).

One of the biggest challenges was to elaborate type-specific reference conditions for water bodies. The WFD defines type-specific corresponding reference conditions as those normally associated with a water body type under undisturbed conditions, i.e. when no, or only very minor, anthropogenic alterations of the physico-chemical, hydromorphological, and biological quality elements are present. Another challenge has been finding biological metrics to be sensitive to anthropogenic pressures and showing deviations from those conditions, also to be comparable across EU Member States. In practice, reference conditions are most often based on measurements from reference sites with a minimum level of anthropogenic pressure (Nõges et al., 2009).

Finding these reference conditions minimizes the uncertainties related to broad scales of conditions that exist across different types.

Phytoplankton and water quality assessment may raise many questions: Does phytoplankton reflects adequately the ecological status of lakes; What determines the geographical distribution of different species; How does phytoplankton respond to growth limiting conditions; Do phytoplankton adaptations compromise its indicator value; Does climate change interfere to eutrophication processes? The next chapters give a brief excursus to phytoplankton ecology to answer these questions.

1.2. Phytoplankton parameters to assess water quality

Each of the BQEs has different characteristics that are used to assess the ecological quality. For phytoplankton these are abundance and biomass, taxonomic composition, and bloom metrics; for macrophytes and phytobenthos – taxonomic composition and abundance; for benthic invertebrates – taxonomic composition, abundance, and diversity; for fish – taxonomic composition, abundance, and age structure (EC, 2000 Annex V). Many of these characteristics, sensitive to human pressures, have been used for water quality assessment prior to the WFD. The WFD stimulated the development and improvement of national methods and created need to test and elaborate methods that remain functional along biogeographical gradients (Lyche Solheim et al., 2008; Birk et al., 2012; Brucet et al., 2013).

EU WFD Annex V outlines three features of the phytoplankton to be considered important in assessment of lakes. These parameters are: (i) phytoplankton biomass or abundance and its effect on water transparency; (ii) phytoplankton composition and (iii) bloom frequency and intensity.

The most widely used phytoplankton parameter is chlorophyll *a* (Chl *a*), a proxy for phytoplankton biomass (Nõges et al., 2009). Using Chl *a* can be problematic, because its concentration depends on algal community composition and physiological state (Reynolds, 1984b). Still Chl *a* as the simplest quantitative phytoplankton parameter has been used as a benchmark indicator, its scale has been intercalibrated among EU Member States and standardized in different lake types across geographical regions of Europe (EC, 2008; Poikane et al., 2010).

Phytoplankton composition metrics use changes in phytoplankton species or genera along nutrient gradient (Ptacnik et al., 2009). Several authors have created phytoplankton functional and taxonomic groups against different requirements of N and P and other elements or factors. For example, chrysophyta are characteristic of nutrient poor lakes (Maileht et al., 2012; Järvinen et al., 2013), many cyanobacteria species are supported by increasing nutrient conditions. Margalef (1978) pioneered approaches by using the responses of different taxonomic and functional groups of phytoplankton to nutrients and turbulence to predict their occurrence along these environmental gradients. Grime (1979) studied among terrestrial flowering plants adaptive strategies against stress and disturbance. These strategies are applied also to freshwater phytoplankton and adapted by Reynolds. Reynolds (1984a,b; 1988; 2006; Reynolds et al., 2002) developed a detailed classification of different functional groups based on phytoplankton morphological and physiological traits to be tolerant or sensitive against different pressures. Padisak (2003) complemented Reynolds classification. Functional groups are based upon species ecological requirements that often correlate with their morphological attributes such as colonial or unicellular type, occurrence of flagella, and size classes.

The composition of cyanobacteria is often adopted as a "bloom metric". Definition of phytoplankton "bloom" may be interpreted differently. Usually it is associated with visible surface scums of cyanobacteria (McGowan et al., 1999), although biomass of other algal classes, for example, diatoms or dinoflagellates, may increase similarly. In the EU WFD context, "bloom" is defined as persistent domination of summer cyanobacteria (Poikane, 2009).

Quantitative relationships between many species, genera or groups and environmental conditions are described by multimetric indexes (Moss, 2007). Using combinations of different metrics (taxa richness, diversity, sensitive and tolerant species, functional groups, trophic structure) in ecological status assessment is more reliable compared to using a single parameter or metric (Hering et al., 2006). Using highly sensitive individual species for assessment of ecological quality is limited because of their incidental occurrence. On the other hand, using dominant species may be less indicative, because of their wide tolerance (Nõges et al., 2009).

As shown above, different phytoplankton parameters, including functional groups, shed light on various environmental gradients, but first of all, phytoplankton is used to trace the changes in lakes resulting from eutrophication pressure. Phytoplankton abundance parameters are sensitive to changes in nutrient availability whereas species composition reflects ecological status for a longer period compared to biomass, Chl *a* or abundance. All BQE should complement each other and together give an adequate assessment of the ecological status of lakes.

1.3. Distribution of phytoplankton and factors affecting its dynamic

1.3.1. "Everything is everywhere, but the environment selects" or "Everything is endemic"

One of the oldest doctrines in microbiology "Everything is everywhere, but the environment selects", known also as the EiE hypothesis, was postulated by Dutch professor Lourens Gerhard Marinus Baas Becking (Baas Becking, 1934; de Wit & Bouvier, 2006). The first part of the hypothesis states that microorganisms are very small and, therefore, their germs are passively dispersed by air everywhere without barriers, i.e. that all microorganisms are cosmopolitan. As their densities in each particular site are below detection limit, most of the microbial biodiversity is hidden. The second part of the hypothesis "…but the environment selects" underlines that in each location, microorganisms are susceptible to different environmental pressures. Local conditions either suppress or support the reproduction potential of microorganism given them by unique features such as resting cells and asexual or parthenogenetic reproduction for quick procreation. As a result, different microbial communities reflecting the environmental settings are observed at different sites and, hence, can be used as indicators of the environment (Baas Becking, 1934; de Wit & Bouvier, 2006).

There are many counterarguments to this hypothesis regarding geographic barriers, extreme environmental conditions, and specific attributes of microorganisms (spores). The EiE hypothesis was formulated in the era of traditional morphology-based taxonomy. New molecular taxonomy based on DNA sequencing has demonstrated a high degree of previously unknown diversity within phytoplankton species and strains supporting the existence of spatial distribution patterns also for microorganisms (Naselli-Flores & Padisák, 2016; Creer et al., 2010; Marchant et al., 2011). There are different distributions of microorganisms: holoendemic – cosmopolitan, euryendemic – broad continuous or contiguous distribution, stenoendemic – restricted but continuous distribution, and rhoendemic – disjunct distributions that are the basis on their endemism (Myers & de Grave, 2000; Williams, 2011). This controversy between distribution patterns discernable using morphology-based and molecular taxonomy lead Williams (2011) to paraphrase the EiE hypothesis as "Everything is endemic" that in the light of present knowledge has become more meaningful. The approach "Everything is endemic" means that a particular organism lives only in a particular area or areas, and nowhere else.

But does the environment still select as Baas Becking proposed? The answer is "yes". Changes in the environment provoke certain responses in phytoplankton quantity and composition. These responses are trait specific rather than taxon specific meaning that ecologically similar taxa behave similarly as a group. This idea forms the foundation of the functional group concept (Reynolds 1988; 2006; Reynolds et al. 2002; Padisak et al., 2003) that is nowadays widely used in ecological status assessment of geographically distant water bodies.

1.3.2. Limiting nutrients and eutrophication

Phytoplankton species are adapted to live in stress or disturbance conditions. The intensity of stress and disturbance varies markedly among and even within lakes. One of the factors limiting phytoplankton growth is the availability of phosphorus (P) and nitrogen (N). Besides phytoplankton, also bacteria, periphytic algae and aquatic macrophytes require P and N for growth and, therefore, compete for this resource (Cotner & Wetzel, 1992).

The growth rate of phytoplankton depends on internal nutrient concentration and uptake is affected by external nutrient concentration (Droop 1973; Grover 1991; Filiz et al., 2020). Uptake and growth differ significantly across species and major taxonomic groups (Litchman et al., 2007; Shuter 1978; Smayda, 1997). Sommer (1984) proposed three major strategies of nutrient acquisition in phytoplankton: (i) velocity-adapted species that are able to utilize nutrient pulses and grow fast, (ii) storage-adapted species, and (iii) affinity-adapted species that are advantageous under nutrient limitation. Species with high maximum growth rates and high requirement of nutrients (e.g. *Chlamydomonas*) often dominate in eutrophic lakes while slow-growing chrysophytes – in oligotrophic lakes (Reynolds, 1984a; Reynolds, 2006).

P reaches waterbodies mainly through inflows and leaves the lake's water column through the outflow and by sedimentation. Lake sediments may become a secondary source causing so-called internal P loading. Phytoplankton uses mainly dissolved inorganic form of P (soluble reactive phosphorus; SRP), but in P-limited conditions some species use alkaline phosphatase (AP) to break down exogenous organic P compounds to utilizable inorganic forms (Bentzen & Taylor, 1991; González-Gil et al., 1998; Rengefors et al., 2003).

N has many forms such as nitrate (NO_3^-), nitrite (NO_2^-), nitric acid (HNO_3), ammonium (NH_4^+), ammonia (NH_3), gaseous nitrogen (N_2), nitrous oxide (N_2O), nitric oxide (NO), nitrogen dioxide (NO_2). Due to many different forms of N, including gaseous forms exchanged with atmosphere, nitrogen cycle is more complex compared with that of P. N enters water bodies mainly through human activities: from atmosphere, point and diffuse sources. In addition, some cyanobacteria able to fix atmospheric N_2 may bring nitrogen into the water body (Wetzel, 2001). Phytoplankton uses dissolved inorganic and organic nitrogen. Dissolved organic N forms are used in case of N limitation (Palenik & Henson, 1997).

The average assimilation ratio of N and P by phytoplankton (16:1 by atoms and 7:1 by mass), the so called 'Redfield ratio' (Redfield, 1934), has been considered as a criterion to estimate which of these elements potentially limit phytoplankton growth. Several authors (Schindler, 1977; Smith et al., 1995; Huisman & Hulot, 2005) showed that low N/P ratios at high nutrient supply supported the development of N_2-fixing cyanobacteria species, many of which cause water blooms. Nowadays the understanding has been revisited as P alone (Håkanson et al., 2007; Arvola et al., 2011), N alone (Downing et al., 2001; Dolman et al., 2012) or a combination of N, P and water temperature (Beaulieu et al., 2013; Filiz et al., 2020) have been found to be better predictors for cyanobacteria biomass than the N/P ratio.

Besides N and P, dissolved inorganic carbon (DIC) may potentially limit phytoplankton growth in shallow eutrophic and hypertrophic lakes in agricultural areas where N and P levels are high enough while the shallowness of water avoids the onset of light limitation (Riebesell & Wolf-Gladrow, 2002).

Dissolved organic compounds (primarily of terrestrial plant origin) influence phytoplankton metabolism by interacting with macro- and micronutrients (Wetzel, 2001).

As eutrophication is the central phenomenon of anthropogenic pressures on water bodies, the quantitative and qualitative changes in phytoplankton occurring along the trophic gradient of increasing N and P are generally well known and widely used in assessment systems (Phillips et al., 2013; Poikane et al., 2010; Mischke et al., 2010). In particular cases, these regularities may be disturbed by confounding factors if other elements or compounds start limiting or inhibiting phytoplankton growth (e.g. toxins).

1.3.3. Other nutrients: iron (Fe), silicon (Si) and microelements

Iron (Fe) is needed for phytoplankton for nitrogen metabolism. Fe content is high in nitrate and nitrite reductase (Hoffmann et al., 2007). When Fe is limited, then nitrate uptake rate is reduced and nitrate reductase activity is lower (Price et al., 1994; Timmermans et al., 1994). Besides nitrate, phytoplankton cells can directly take up ammonium and incorporate it into amino acids without using iron containing enzymes. Phytoplankton cells which use nitrate as N source have higher Fe demand compared to cells that use ammonium as N source (Maldonado & Price, 1996). Fe also affects the efficiency of the photosynthetic apparatus and thus probably carbon uptake. Fe limitation may cause a decrease in chlorophyll concentration and photosynthetic efficiency (Hoffmann et al., 2007).

Silicon occurs in fresh waters mainly as silicon dioxide or silica (SiO_2) (Wetzel, 2001). Silica is usually moderately abundant and relatively unreactive. Among phytoplankton groups, silica is a significant component of diatoms and chrysophytes. Diatom utilization of silica modifies its flux rates in lakes.

Auxotrophic phytoplankton species need for growth organic micronutrients, especially vitamins B_{12}, thiamine and biotin. In low concentrations vitamins are found in water body and they enter to aquatic ecosystems mainly mainly through bacterial activity. Concentrations of these vitamins vary within a lake and diurnally. More vitamins are found from the littoral zone and higher concentrations in the morning (Hutchinson, 1961; Wetzel, 2001).

Also bioactive trace metals may limit physiological processes of phytoplankton in water bodies (McKay et al., 2001). Availability of microelements such as zinc (Zn), manganese (Mn), nickel (Ni), copper (Cu), cadmium (Cd), molybdenum (Mo), cobalt (Co), boron (B), selenium (Se), vanadium (V) may limit the growth rate of phytoplankton (Hutchinson, 1961; Scharek et al., 1997; Twining & Baines, 2013; Zhang et al., 2019). Contrariwise, high concentrations of some microelements like Cu, Zn, Cr (Vignati et al., 2010) may be lethal to green algae and cyanobacteria. Also, Co and Ni may be toxic (Chakraborty et al., 2010).

During extreme nutrient limitation, some phytoplankton species are able to use other elements in their biomolecules. For example, picocyanobacterium *Prochlorococcus* uses sulfur instead of phosphorus (van Mooy et al., 2006).

Limiting and toxic effects of microelements on phytoplankton are complex and need full attention if there is a known risk of entering these elements from the catchment. A curious example of heavy contamination impacts on the whole lake ecosystem is the pollution history of Lake Orta in northern Italy where, as a consequence of subsequent pollution episodes with ammonium sulphate, Cu, Cr, Ni and Zn over a 60-year period, most of the native species perished, while several new species unsuccessfully attempted to colonize the lake (Calderoni et al., 1992). Nowadays, when the pollution is stopped and several restoration measures applied to the lake, the community structure of different biotic groups has not yet recovered (Riccardi et al., 2016) and the biological monitoring has a highly site-specific character.

1.3.4. Adaptive strategies

Constantly changing environment poses various constraints to growth and survival of phytoplankton species. To overcome these constraints, species have developed certain physiological and morphological plasticity that increase their fitness in a changing environment. If the extent of changes exceeds the adaptation range of a species, other species with a different adaptation range may take over that ultimately will be seen as a succession of the community composition. But, do adaptations in phytoplankton compromise its indicator value or perhaps increase it? Both options are possible and the outcome depends on the ecological competence of the investigator to select and observe the right metrics. For example, the abundance of light-limited phytoplankton may become insensitive to nutrient conditions that compromises their use as indicators of eutrophication. At the same time, the cromatic adaptation reflected in ratios of photosynthetic pigments (Falkowski & Raven, 2007; Stomp et al., 2004) or success of low light adapted species (O´Farrell et al., 2007) gives a clear indication of the onset of light limitation that gives valuable information about the environmental change and can be utilized in assessment systems.

1.3.4.1. Environmental constraints and adaptations

One environmental constraint upon phytoplankton growth is nutrient limitation, discussed above. Other environmental constraints are: light energy, ultraviolet (UV) radiation, seasonal fluctuations on day length, temperature, wind action, frontal activity, rainfall intensity, hydraulic flushing, water colour and transparency which importance for phytoplankton development and implications for lake status assessment will be opened up in paragraphs below.

1.3.4.1.1. Seasonal succession and phytoplankton equilibrium

Algal communities consist of holoplanktonic species, that spend their whole life cycle in the water column, and meroplanktonic species, that periodically have diapause in resting stages.

Seasonal succession of phytoplankton is related to changes in the solar cycle that affects meteorological and hydrological factors (irradiance, temperature, precipitation, wind) and thermal stratification of the water column. These factors and their impacts differ considerably among climatic zones implying that the extent of seasonal changes in phytoplankton biomass in temperate and polar fresh waters may be thousandfold, while in tropical waters only fivefold (Fogg, 1965). As a general pattern for temperate zone, small flagellates of chrysophytes and cryptophytes, adapted to live in low light and temperature, dominate in winter followed by diatoms in early spring and green algae later. Summer population depends on water body trophic status and may show another diatom peak or increase in cyanobacteria.

In conditions of constant low or moderate nutrient loading, phytoplankton biomass remains rather unchanged from year to year (Wetzel, 2001).

Some algal classes or species, like the cyanobacterium *Gloeotrichia ecinulata*, have akinetes – dormant cells that store various essential substances to survive unfavourable conditions and are important to regulate seasonal succession between the benthic and pelagic life stages (Karlsson-Elfgren et al., 2004).

Morphological variations of phytoplankton species assemblages reflect annual periodicity of physical and chemical changes. Therefore, morphological traits are suitable indicators of regularities in seasonal patterns, showing besides regular periodicities also perturbations or disturbances (Naselli-Flores, 2007).

According to the paradox of the plankton, the question is, how it is possible for many phytoplankton species to coexist on limited resources in an apparently homogeneous environmetn (Hutchinson, 1961). In stable conditions, species with even small advantages in resource use efficiency would ultimately outcompete the others that would lead to their monodominance. But, because of the changing conditions in nature over time and space, complete exclusion of phytoplankton species only rarely occurs and, therefore, species with similar demands can coexist.

The equilibrium and non-equilibrium hypotheses highlight the influence of disturbance on diversity. Algal succession is influenced by disturbances that prevent progress towards equilibrium. Equilibrium conditions can be characterized by the following criteria: (i) no more than three dominant species with a share in the total biomass more than 80%; (ii) dominance should persist more than 1-2 weeks; (iii) no significant changes in the total biomass (Sommer et al., 1993; Naselli-Flores et al., 2003).

Seasonal succession complicates the use of phytoplankton in lake assessment as both the abundance and taxonomic composition vary seasonally over broad ranges. Therefore, a seasonal sampling programme is advisable that would enable to estimate this variation. With the development of technology, automated high frequency measurements of phytoplankton related variables such as the fluorescence of phytoplankton pigments (Havskum et al., 2004; Schlüter et al., 2018) or even taxonomic composition with automatic plankton image analysis (Culverhouse et al., 2006; Sosik & Olson, 2007) have become a reality.

1.3.4.1.2. Water movements and wind action

Gravity and wind cause water movements. Lakes are like large mechanical oscillators with wind giving rhythmic oscillations at the surface of water that dissipates internally to deeper layers. Water movements cause turbulence and this has major significance for the biota and productivity of the lake causing changes in water temperature, dissolved gases, sediment disturbance, water transparency, nutrients, and distribution of plankton (Wetzel, 2001). Wind has a horizontal and vertical effect on phytoplankton distribution in lakes.

Changes in wind as they affect phytoplankton abundance through light and nutrient availability, can interfere with lake status assessment. Storm surges change lake light, temperature and nutrients conditions and therefore may affect phytoplankton horizontal and vertical position, frequency and prevalence of cyanobacterial blooms, changes in biomass and abundance, community and functional composition, processes of nutrient uptake, etc. (Stockwell et al., 2020). But also atmospheric stilling – a phenomenon observed over large areas in the Northern Hemisphere (Woolway et al., 2019) has been shown to affect phytoplankton abundance and composition overruling the efforts made in reducing nutrient loads (Janatian et al., 2019).

1.3.4.1.3. Light and ultraviolet radiation (UV)

Plankton communities depend on the availability of light energy to sustain photosynthesis (Reynolds, 1988). Adaptation to move or stay in the illuminated near-surface layer is therefore an important functional feature. The amount and spectral composition of direct solar radiation that reaches the water body surface depends on many factors such as altitude, latitude (angle of sun rays impinging on the water), season, diurnal dynamics, and meteorological conditions (atmospheric transparency, cloudiness, humidity; Wetzel, 2001).

Not all solar radiation reaching water surface penetrates into the water body, some part is reflected. The reflected amount depends on the disturbance of the water surface, and on the occurrence of ice and snow. The proportion of light penetrating the water surface is partly scattered by particles and partly absorbed that together cause vertical light attenuation. The absorption of light in water depends on dissolved organic compounds and differs along wavelength. Also, other factors like salinity affects the absorption (Wetzel, 2001).

UV radiation is the electromagnetic radiation of wavelength between 10 to 400 nm. The UV wavelength range is divided into UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (<280 nm). Nearly all UV-C is absorbed by stratospheric gases and water. Global UV radiation has increased in consequence of a thinning ozone layer. Increasing UV radiation has several impacts on water bodies: enhancing injury to organisms, altering the energy of protective pigments, and altering rates of biogeochemical cycling of organic compounds. UV-B is highly energetic and photoactivating in water. UV-A is less energetic, but penetrates more deeply than UV-B.

Phytoplankton species have a diverse set of pigments to capture different parts of the spectrum of visible light. The main photosynthetic pigment is chlorophyll: chlorophyll *a*. Accessory pigments as chlorophyll *b*, *c* and *d*, phycobilins and carotenoids increase the range of usable spectral range and several carotenoids may function as photoprotective substances against excess light and UV radiation. Mycosporine-like amino acids and photoprotective carotenoids are widespread among lake phytoplankton assemblages, but besides UV explosure other environmental factors such as temperature, N/P ratio, and pH probably also influences their synthesis (Laurion et al., 2019).

Pigment composition is different among phytoplankton species (Falkowski & Raven, 2007; Stomp et al., 2004). Some species can select the parts of the spectrum they utilize by adjusting their pigment composition, this phenomenon is known as chromatic adaption. For example, filamentous cyanobacterium *Tolypothrix tenuis* is able to adjust the ratio of its phycocyanin to phycoerythrin while the total amount of both pigments remains constant (Ohki et al., 1985). Pigment composition is one trait to determine functional groups along the spectral gradient associated with depth and different water colour (Stomp et al., 2004). Differences in spectral use of light gives opportunities for different species to coexistence (Stomp et al., 2004, Stomp et al., 2007).

Algae adapt to changes in light quantity and quality. For example, the number of aerotopes of the cyanobacterium *Cylindrospermopsis raciborskii* increases in dim light to modify the buoyancy of the trichomes (O´Farrell et al., 2007). Light-limited phytoplankton may become insensitive to nutrient conditions that compromises their use as indicators of eutrophication (Luimstra et al., 2020). In particular cases, when light conditions deteriorate because of other factors than eutrophication, e.g increased amount of humic substances, phytoplankton may increase its cellular pigment content as an adaptive response to overcome light limitation (Nõges et al., 2011). Such increase can be easily mixed up and erroneously attributed to eutrophication if the assessment environmental bases on chlorophyll content only.

1.3.4.1.4. Temperature

Seasonal changes of abundant phytoplankton species and compositional differences among lakes at different latitudes are assumed to be related to changes in temperature (Reynolds, 1988). Temperature is entangled and handled together with daylength, light penetration, thermal stratification, and ice regime.

Temperature affects intracellular transport and assimilation of photosynthate and other nutrients into proteins. During winter and early spring when the temperature and light levels are simultaneously low, small cells are more affected by light, large cells and colonies by temperature (Reynolds, 1988). The most suitable temperature range for most phytoplankton is between 10 to 20 °C (Butterwick et al., 2005).

Over ice-free periods, air temperature has a direct effect on water temperature. In changing climate conditions, air and water temperature are important factors influencing the composition of phytoplankton community, its biomass and distribution of alien species.

Global warming affects the rate of numerous processes in the nature including the elemental cycles and physiology of organisms. In water bodies, global warming and eutrophication may mutually reinforce the symptoms they express (Moss et al., 2011) that makes it difficult to disentangle them and, hence, creates additional challenges to assessment systems based on biological groups.

1.3.4.2. Morphological traits and adaptations

Salmaso & Padisak (2007) analysed phytoplankton composition in terms of morphofunctional groups and had very good results with the changes in the species composition during seasonal cycle. In lake assessment system other authors have later also used phytoplankton morphofunctional groups (Phillips et al., 2010; 2013; Mischke et al., 2008; **II**; **III**).

In dynamic aquatic environment phytoplankton species are adapted to change their morphological characteristics: cell and colony size, mucilage formation and filament coiling. Morphological properties of phytoplankton influence its efficiency of light harvesting and utilization, the capacity to absorb and store nutrients, respond to temperature fluctuations, modify sinking rate and withstand to grazing pressure (Lewis, 1976; Reynolds, 1988; Naselli-Flores, 2007).

1.3.4.2.1. Cell and colony size and shape

The ratio of surface area (SA) to volume (V) influences phytoplankton light harvesting, nutrient uptake and loss or admission of useful components and toxins. Therefore, natural selection and competition influence the SA/V ratio (Lewis, 1976). The size and volume differ largely among phytoplankton species making SA/V to vary within three orders of magnitude (0.01-4 μm^{-1}; Reynolds, 1984b). If mucilaginous colonies are excluded, the SA/V range narrows to 0.2-4 μm^{-1} (Reynolds, 1988). High values of SA/V are common for small sized plankton species and are related to a better nutrient flux per unit volume and higher photosynthetic efficiency (Naselli-Flores, 2007). On the contrary, mucilage sheaths lower the SA/V ratio (Lewis, 1976).

Light-limited environment is commonly suitable for small unicellular, non-flagellated organisms, thin filaments and small tabular colonies.

Well illuminated environment is the best suitable for flagellated and larger species. Light availability affects most phytoplankton assemblages in eutrophic and hypertrophic lakes (O'Farrell et al., 2007).

Cell size is important for nutrient uptake. The rate of absorption is correlated with the effective SA/V ratio of the protoplast (Sourina, 1982). Inside cells nutrients must be diffused or to be transported to the sites of metabolic assembly. Larger cells have greater capacity to divert the nutrient influx into intermediate storages like polyphosphate granules or protein bodies. These storages may be utilized during nutrient deficiency of and, once again, topped out during abundance of nutrient (Reynolds, 1988). These species are defined as *storage-adapted* species, which are the most suited to fluctuating environment (Sommer, 1984; Reynolds, 1988).

Cell shape is also an important factor for photosynthesis. Phytoplankton with filamentous shape have good light antennae and photosynthetize capacity at low light is high (Reynolds, 1988). These are some of the features to elaborate taxa according to their tolerance or sensitivity. Designation of sensitive and tolerant phytoplankton species is used to set class boundaries and in indexes (Phillips et al., 2010; 2013; Mischke et al., 2008).

1.3.4.2.2. Grazing, sinking, mucilage, coiling

Phytoplankton cell size and shape change when algae are confronted with a strong grazing pressure (Stoyneva et al., 2007). To avoid grazing, phytoplankton may increase cell or colony size or develop rigid cell walls, protuberances and spines (von Elert & Franck, 1999; Padisák et al., 2003).

Some phytoplankton species have the ability to avoid grazing by producing toxins or repellents. For example, *Microcystis aeruginosa* has short term toxic effects on *Daphnia magna* (Nizan et al., 1986; Liping et al., 2011). In addition, mucilage protects against grazing. Mucilage production also helps to sequester and process nutrients, defend against heavy metals and maintain a reducing microenvironment around the cells (Naselli-Flores, 2007). Mucilage sheaths also avoid sinking (Lewis, 1976).

The foodweb structure is an important driver of water quality and a whole range of techniques exist under the common name of biomanipulation where the foodweb structure is modified in order to achieve some water quality goals. For example, removing planktivorous fish supports zooplankton grazing on phytoplankton that ultimately may improve the water quality (Horppila et al., 1998; Benndorf et al., 2002). Changes in foodweb structure may sometimes result from environmental disasters, outbreaks of deseases or invasions of alien species causing mass mortality of species at some trophic level. For example, in Estonian Lake Uljaste massive fish kill occurred in summer 1993 mainly due to toxication by ammonia. Water transparency improved and phytoplankton biomass decreased in following years (Ott, 1994).

Green algae from genus *Scenedesmus* form coenobia to avoid grazing (Lürling & van Donk, 1996; von Elert & Franck, 1999). Larger colony size and formation of spines are effective defense against grazing by smaller sized zooplankton (Mayeli et al., 2004).

Another morphological feature to avoid grazing is coiling used e.g. by *Dolichospermum* (*Anabaena*) species (Padisák et al., 2003).

Phytoplankton cell and colony size influence its sinking rate. Padisák et al. (2003) showed experimentally that straight filaments have greater form resistance to sinking (Φ) and therefore better floating properties than coiled ones. However, straight filaments are more affected by grazing (Fabbro & Duivevdoorden, 1996). Star-like algae such as *Asterionella formsa* and coenobia with spines such as *Tetrastrum* species have high Φ because of symmetry of a cell or a group of cells. Species which have spines (*Scenedesmus*) sink more slowly than those without spines. Having flagella is also an important feature to avoid sinking. There are some species, such as *Ceratium hirundinella,* having numerous advantages for surviving in lake environments: they are very good swimmers, have clear diurnal migration, can migrate to nutrient rich layers and are too big to be consumed by many grazers. Some cyanobacteria have ability to regulate the buoyancy by their gas vesicles or aerotopes (Padisák et al., 2003).

Some algae have developed chemical defense mechanisms against grazing. These defenses can be divided into constitutive and inducible. In case of constitutive defense chemicals are present all the time, for example, in dinoflagellates that form toxic "red tide". In case of inducible defense, chemicals (for example phlorotannins in brown algae) are produced under grazing pressure (Lee, 2008).

2. AIMS AND HYPOTHESES OF THE STUDY

The aims and hypothesis of this study were:

1. Ascertain the main environmental factors determining distribution and biomass of dominant phytoplankton species in EU lakes (I, II).

   Hypotheses:

   a. Besides natural conditions, anthropogenic factors are important in determining the dominant phytoplankton species in lakes.

   b. Domination of phytoplankton algal classes is different in Northern and Southern Europe.

3. Analyze the suitability and strength of phytoplankton parameters and metrics for estimating the ecological status of lakes in EU (II, III).

4. Exemplify the evaluation of phytoplankton metrics through uncertainty analyses of their response to eutrophication (II, III).

   Hypotheses:

   a. National phytoplankton based assessment systems contain a number of robust metrics to assess the impact of eutrophication pressures.

   b. Single open water sampling location is generally sufficient for assessment of ecological quality of lakes.

3. MATERIAL AND METHODS

3.1. Databases

Lake data were collected during the EU 7th Framework Programme project WISER (Moe et al., 2013). The database contains data on more than 1500 lakes from 20 (**I**) to 21 countries (**II, III**) collected between 1972 and 2009, but more than half of data originates from years 2000-2009 (**I, II, III**).

National assessment methods have been gathered to online database (Birk et al., 2010; Birk et al., 2012) and their suitability to detect the impacts of eutrophication pressure was tested later during the WFD Intercalibration process to ensure the comparability of these assessment systems, especially, that "good" ecological status represents the same level of ecological quality everywhere in Europe (Poikane, 2009, Poikane et al., 2011; **II**).

3.2. Sampling and sample analyses (II, III)

In order to find out the sources of phytoplankton metrics variability, phytoplankton data were analyzed after field survey in 2009 covering 32 European lakes located in 11 countries. Samples were gathered from common lake types elaborated for Alpine, Northern, Central/Baltic and Mediterranean Geographical Intercalibration Groups (GIGs) during the WFD Intercalibration process (WISE, 2008). Lake characteristics and trophic status varied widely (**III, table 1**).

Keeping in mind the planned nested random effects statistical model for uncertainty analysis, phytoplankton samples were collected from three stations in each lake:
(1) at the deepest point,
(2) station located at the mean depth of the lake, and
(3) station at intermediate depth between maximum and mean depths.

Two samples were taken at each station. Three sub-samples were taken from the first sample and one sub-sample from the second sample (**III, figure 1;** Thackeray et al., 2010). In thermally stratified lakes, samples were gathered from the euphotic layer (2.5 * Secchi depth) whereas in mixed lakes, integrated samples covered the whole water column from 0.5 m depth to 0.5 above sediment.

Only two sub-samples were taken for chlorophyll *a* (Chl *a*), because due to the standard methodology (ISO 10260, 1992) used, the effect of the analyst was considered negligible.

Also, the concentration of total phosphorus (Murphy & Reilly, 1962) and total alkalinity were measured.

Two experts counted the samples according to a strict scheme based on the Utermöhl's technique (CEN EN 15204, 2006). Later, the counting results were entered into an Excel spreadsheet. The taxonomy was harmonized among analysts using a common phytoplankton list of about 2300 taxa (Mischke et al., 2012).

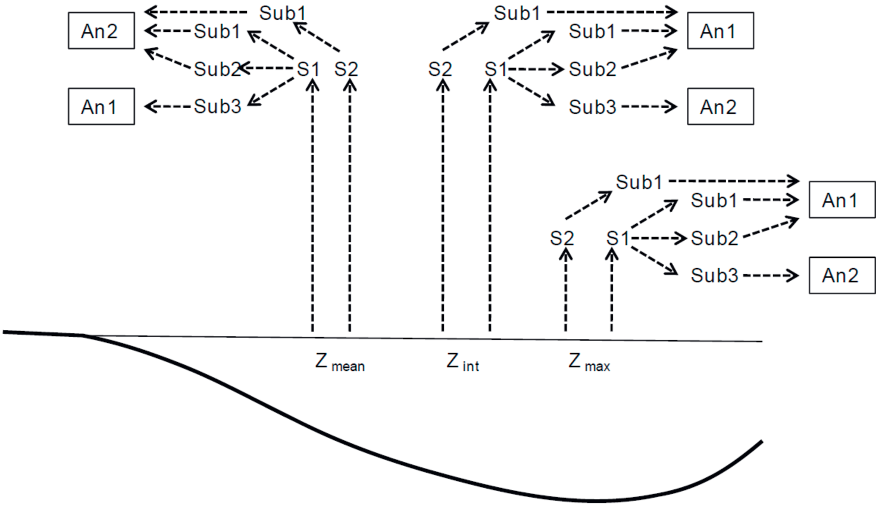

Figure 1. Phytoplankton sampling design. Z_{max} – samples from above of the deepest point of lake, Z_{mean} – samples from the mean depth of lake, Z_{int} – samples from intermediate depth of maximum and mean depth. Two samples (S1, S2) were collected at each station and three sub-samples (Sub1, Sub2, Sub3) taken from S1 and one sub-sample from S2. Samples were processed by two analysts (An1 and An2) according to the design (Thackeray et al., 2010).

3.3. Phytoplankton metrics (II, III)

Three groups of phytoplankton metrics – abundance, composition and bloom metrics, were used in statistical analyses.

3.3.1. Abundance metrics

Chlorophyll-a concentration (Chl-*a*) (II, III) was used as a measure of phytoplankton abundance to assess the ecological status of lakes.

3.3.2. Composition (sensitivity/tolerance) metrics:

Phytoplankton Trophic Index PTI (II, III) was elaborated as a common metric in several GIGs for countries lacking composition metrics in their national assessment system. PTI uses "trophic scores" of each phytoplankton taxon along the eutrophication gradient (Phillips et al., 2010). Phytoplankton species are divided into four groups: very sensitive, sensitive, tolerant, and very tolerant. The PTI increases with increasing lake trophic state.

Size Phytoplankton Index SPI (II, III). Phytoplankton taxa are divided into size classes (e.g. $\leq 0.5\ \mu m^3$, $0.5–1.0\ \mu m^3$, $1.0–2.0\ \mu m^3$, $2.0–4.0\ \mu m^3$ (Kamenir & Morabito, 2009). The SPI increases with increasing lake trophic state, due to a shift towards dominance of larger phytoplankton (Phillips et al., 2010).

Morpho-Functional Group Index MFGI (II, III). Phytoplankton taxa are divided into morpho-functional groups, based on their morphological attributes such as presence or absence of flagella, colonial size and structure etc (Salmaso and Padisák, 2007). The MFGI increases with increasing lake trophic state, due to an increase in the dominance of colonial cyanobacteria, large diatoms, chlorophytes, conjugatophytes, and unicellular, colonial chlorococcales (Phillips et al., 2010).

For final calculation of SPI and MFGI two parameters are needed:

(i) a trophic score, indicating the trophic position of a size class of Morpho-Functional Group across the trophic spectrum and
(ii) an indicator value, estimating the "power" of each size class or Morpho-Functional Group as biotic indicator.

Functional Traits Index FTI (III) is the arithmetic mean of the SPI and MFGI and includes information on the size spectrum and morpho-functional traits of the phytoplankton community.

3.3.3. Bloom (richness/diversity) metrics

Evenness J (II, III)

J is Pielou's evenness index, expresses the ratio between the Shannon diversity and the maximum possible value of the Shannon diversity index (Pielou, 1969, 1975). Evenness has been shown to decline under bloom conditions in more productive lakes, due to an increase in the dominance of a small number of tolerant species with high growth rates (Mischke et al., 2010).

Cyanobacterial biomass (II, III)

This is the total cyanobacterial biovolume (mg/l) and is expected to increase with increasing lake trophic status (Mischke et al., 2010). Cyanobacterial biomass may be used also as abundance metric.

3.4. Statistical analyses

I. Canonical Correspondence Analysis (CCA) CANOCO 4.5 (Ter Braak & Šmilauer, 2002) was carried out to determine the relationships between the distribution of dominant species and environmental variables (surface area, mean and maximum depth, latitude, longitude, altitude, water colour, alkalinity, water temperature and concentration of total phosphorous and nitrogen).

II, III. For statistical analyses, R versions 2.12.1 and 2.1.3.1 (Pinheiro et al., 2010; Warnes, 2010; Barton, 2011; Bates et al., 2011; R Development Core Team, 2011) and STATISTICA 8.0 (Weiss, 2007) were used.

II. Linear mixed-effects (LME) models were used to resolve temporal aspects of metric variation (between months and years), and to compare this variation to that apparent between lakes that span a wide-pressure gradient. Separate analyses were carried out on lake data from three geographical regions (GIGs). Based on this analysis, recommendation of minimum sampling frequencies for TPI, total cyanobacterial biovolume and Chl *a* have given.

3.4.1. Statistical modelling (III)

The above listed seven phytoplankton metrics from the 32 lakes sampled in 2009 were tested for their variance partitioning within and between lakes. Three questions that are introduced below, were asked for testing the metrics.

1. **Do metrics show greater variability among lakes than within lakes or as a result of differences in sample processing?**

These analyses aimed to resolve whether metrics had the potential to be sensitive to variations in the intensity of environmental pressures at lake level. Also, we aimed to identify aspects of sampling campaigns that might be modified to improve the precision of ecological status assessments.

A nested random effects statistical model structure was used. Lake was nested within country, sampling station within lake, sample within station and sub-sample within sample was modelled implicitly as the lowest level "residual" variability. As one analyst could not process sub-samples from all samples or all stations or all lakes while some analysts processed samples from more than one country, the model factor "Analyst" was included (except for Chl *a*). To estimate the separate variance components, the mixed model function in R was used.

Restricted maximum Likehood (REML) models with fixed and random hierarchical and/or crossed factors were used to cope with unbalanced dataset with unequal replication at some levels, providing the sampling design gives some subsets of information within the data which enable the REML algorithm to distinguish and estimate each variance component (Crawley, 2007; Clarke, 2012).

2. **Do metrics differ significantly along a gradient in lake nutrient status, after accounting for within-lake and sample-processing variation?**

These analyses aimed to resolve whether relationships between phytoplankton metrics and measured morphometric, chemical and geographical features of lakes could be detected against the “background” of methodological variation.

For statistical analyses, pure random effect “null model” was used with no environmental variables included. Test included environmental variables such as total phosphorous, alkalinity, mean lake depth, latitude, longitude and altitude as fixed effects and fitted as linear mixed effects models. To estimate the proportion of the among-lake variation in metric scores that could be “explained” by the selected environmental variables, we compared the residual variance of the optimal model with the corresponding “null model”. During modelling, the random effect structure was simplified to retain effects of “Lake” and “Analyst”.

3. **Do metrics show systematic changes in their level of variability along gradient in physical, chemical and geographic attributes of lakes?**

We examined whether metric scores became more or less variable in the gradient of predictor variables, such as total phosphorus or mean depth. For this we added additional variance structures (that allowed for residual “spreading” with respect to each of the explanatory variables) to previously fitted models and compared them with models without these additional structures. The most optimal solution was found by comparing the Akaike Information Criterion (AIC) values of each of these models using REML estimation.

4. RESULTS AND DISCUSSION

4.1. Distribution of dominant phytoplankton taxa among regions in Europe (I)

We recorded 151 phytoplankton taxa as dominants in 5 or more samples. Among them 130 were identified to species level and 21 to genus level. Many of the dominant taxa were shared by only 2-4 countries (**Figure 2**).

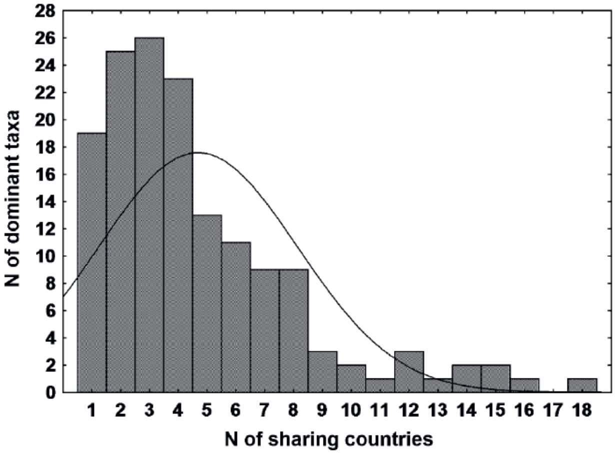

Figure 2. The number of dominant phytoplankton taxa by increasing numbers of European countries

The most widespread taxa were *Ceratium hirundinella*, *Cyclotella* sp., *Aulacoseira granulata* and *Cryptomonas* sp. occurring as dominants in 15 or more countries. We found 132 taxa that dominated in northern lakes (Northern Geographical Intercalibration Group; N-GIG) and 126 in Central and Southern European lakes, whereas the distribution of only 29 and 16 taxa, respectively, was specifically restricted to these areas. About two-thirds of dominant taxa were found among dominants in both geographical areas. Therefore, local lake and catchment specific factors (lake depth, alkalinity) are more important determining the dominant species than broad geographical scale gradient (climate, day length, length of growing season).

The division of dominant taxa between algal classes in the two parts of Europe (**Figure 3**), showed more diatom, chrysophyte and cryptophyte taxa in northern Europe. In terms on frequency of occurrence, chrysophytes occurred thrice and cryptophytes nearly twice as frequently, whereas cyanobacteria and dinophytes half as frequently, among dominants in the N-GIG as in the central and southern part of Europe. The single representative of Raphidophyta, *Gonyostomum semen,* dominated in northern lakes 5 times more frequently than in central and southern parts of Europe. In Central and Southern Europe, cyanobacteria and dinoflagellates are more abundant.

Differences between dominating algal classes in Northern, Central and Southern Europe reflect the broad distinction between dominant lake types in these regions. Northern lakes are generally larger and shallower. Northern lakes have smaller catchment areas, lower alkalinity, pH and conductivity and less nutrients and more dissolved organic compounds than southern lakes (Nõges, 2009). Chrysohytes prefer soft water lakes with low pH, where productivity is low to moderate (Nicholls & Wujek, 2003). Our analyses confirmed the forementioned findings (**In I figure 3**). Cryptophytes are common in many lake types and with widespread distribution, but our study revealed that they dominate in summer and autumn in humic lakes. In Southern Europe cyanobacteria and dinophytes dominated, this is explained by the distribution of lakes of higher nutrient concentration and higher alkalinity in this region.

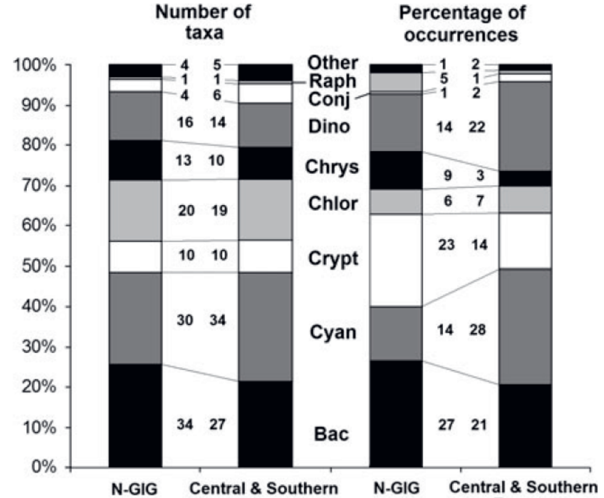

Figure 3. Distribution of dominating phytoplankton taxa among algal classes and their relative frequency of occurrence between countries in Nordic Geographical Intercalibration Group (N-GIG) and in Central and Southern part of Europe. Bac–bacillatophyta; Cyan–cyanobacteria; Crypt–cryptophyta; Chlor–chlorohpyta; Chrys–chrysophyta; Dino–dinophyta; Conj–conjucatophyta; Raph– raphidophyta *Gonystomum semen*; Other–other algae classes.

4.2. Environmental parameters explaining taxa ordination (I)

The CCA showed that significant parameters explaining taxa ordination were depth, latitude, alkalinity, colour, longitude, altitude, total phosphorus (TP), total nitrogen (TN) and surface area ($p \leq 0.024$). The only non-significant parameter was water temperature (p=0.242).

Factors describing lake morphometry (depth, surface area) were strongly intercorrelated and positively related to altitude and negatively to TP and TN. Water colour increased strongly with increasing latitude and longitude and was negatively related to alkalinity. Water temperature, which was the weakest of the explanatory variables tested, increased with decreasing lake size and depth and showed no relationship with geographic location (latitude and longitude).

4.3. Distribution of dominant phytoplankton taxa along environmental parameters (I)

Gradients of water **colour, alkalinity and TP** most strongly determined the distribution of the dominant taxa in the ordination multispace producing a cloud of elongated shape. *Crucigenia tetrapedia*, *Peridinium umbonatum* var. *goslaviense* and *Urosolenia longiseta* **associated with high water colour**, while *Aphanizomenon aphanizomenoides*, *Cryptomonas curvata* and *Staurastrum pingue* **with high alkalinity**. *A. aphanizomenoides* and *Cylindrospermopsis raciborskii* showed strong relationships with **water temperature, TP and alkalinity**.

Longitude, latitude and colour gradients in European lakes are strongly correlated. As longitude and latitude describe the location, then these three parameters are discussed together. Many Scandinavian lakes have coloured and acid water. Common phytoplankton species in high productivity lakes prefer also higher humic content (Arvola et al., 1999). Arvola et al. (1999) and our studies showed special dominant species among different algal classes in brown coloured lakes. Most of these species (except *Trichormus catenula* and *Synura* sp.) dominate in Norway, Finland, United Kingdom, Estonia, Denmark and Spain. Another species, *Gonyostomum semen*, is also known to occur as dominant in Northern European, Scandinavian and Baltic soft water lakes (Willén, 2003; Figueroa & Rengefors, 2006; Trigal et al., 2011). In polyhumic lakes, phytoplankton species are adapted to low light and large fluctuations and gradients of oxygen and temperature. Higher biovolume of species is often associated with higher humic content (Arvola et al., 1999; Carvalho et al., 2008, 2009). In polyhumic lakes this trend stops (Arvola et al., 1999), because there is not enough mineral nutrients and carbon compared with low colour lakes. Light climate in brown coloured waters also does not favour phytoplankton development.

Water temperature, total phosphorus and alkalinity showed strong relationship with many cyanobacteria. Beside *Chroococcus limneticus,* all above mentioned species are tolerant or very tolerant to eutrophication pressure according to Phillips et al. (2012), known to live in meso- and eutrophic water bodies and form water blooms (Mischke & Nixdorf, 2003; Nixdorf et al., 2003; Reynolds et al., 2002; Cronberg & Annadotter, 2006; Willén, 2007). *Euglena* sp. showed also strong relationship with water temperature and TP. This taxon has usually high biovolume in small lakes and ponds (Padisák et al., 2003). *Aphanizomenon gracile* and *Ceratium furcoides* had strong relationship with alkalinity. According to Reynolds et al. (2002), these species are tolerant to low carbon concentrations. *Aphanizomenon aphanizomenoides* and *Cylindrospermopsis raciborskii* showed strong relationships with **water temperature, TP and alkalinity.** Both cyanobacteria species have shown their response to global warming (Briand et al., 2004; Stüken et al., 2006). *C. raciborskii* has rapidly increased its distribution area from tropical to temperate zone (Alster et al., 2010; Kokociński et al., 2010; Moisander et al., 2012). *C. raciborskii* is a tolerant species, successful in lakes of different trophic status, also at high concentrations of dissolved minerals and variable salinity, but affected most by temperature. *A. aphanizomenoides* is also a widespread species and our study showed its dominance in Germany and Spain.

Another cluster of species including *Planctonema lauterbornii*, *Dictyosphaerium subsolitarium*, *Cyclotella ocellata*, *Mougeotia* sp., *Coenochloris fottii* and *Cryptomonas erosa*, had their peak biovolume in southern countries, most of all in Spain. One of these species, *P. lauterbornii*, has shown strong relationship with **temperature** in other studies (Gomes et al., 2004).

The diatoms *Asterionella formosa*, *Tabellaria fenestrata* and *Cyclotella comensis* were strongly related to **lake size** and **depth** and diatoms had generally a stronger dependence on lake morphometry compared to other algal classes. These species dominated in deep lakes with large surface area at higher altitudes in Spain, Italy, Norway and Sweden. The average maximum depth of these lakes is relatively high (164.7 m). These species use their long, thin, belt and star-like structure to stay in illuminated water column.

Chrysophytes instead had a strongly skewed distribution towards increasing **latitude** and **water colour** (**Figure 4**).

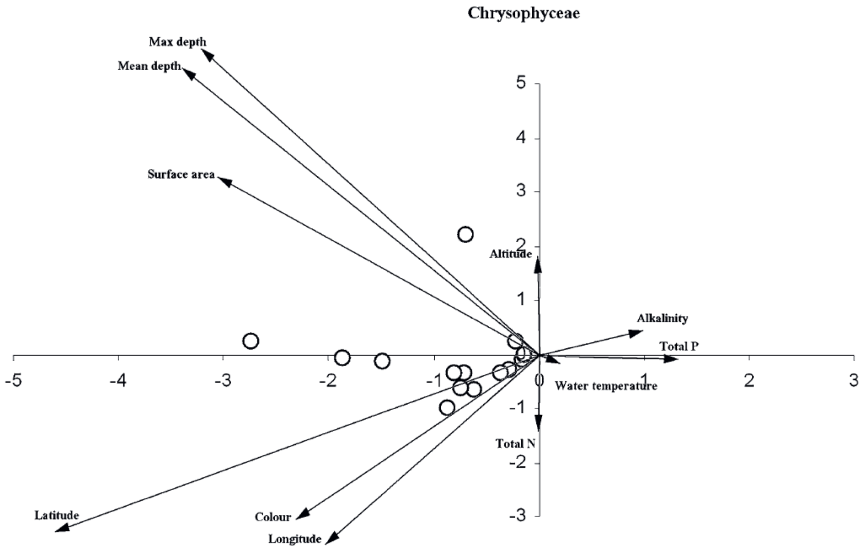

Figure 4. Chrysophytes (○) distribution along different factors in European lakes

4.4. National metrics (II, III)

4.4.1. Variability of national phytoplankton metrics (II)

Phytoplankton is widely used as an early warning and highly sensitive indicator of water quality. According to WFD, EU countries must use phytoplankton parameter(s) to assess ecological status of lakes. Reflecting phytoplankton abundance, composition and blooms, phytoplankton metrics should be robust to quantify the response of phytoplankton communities to nutrient pressure.

Twenty four European countries presented 26 lake phytoplankton assessment methods comprising different metrics. Most of the national methods consist of 2-5 metrics and only Swedish national method contains only one metric. Almost half (46%) of the metrics measure phytoplankton abundance with Chl-*a* being the most popular one. Composition metrics can be divided into two types, those based on sensitive *vs* tolerant taxa (26%) and those using abundance of specific taxa (15%). Almost all countries used some sensitivity/tolerance metrics. Richness/diversity metrics were less used (8%). Cyanobacteria biovolume was the only "bloom metric" used in the European assessment schemes (**Table 1**).

Table 1. Overview of the phytoplankton metrics used in European Union Member State assessment schemes for the Water Framework Directive

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

4.4.2. Strength of metrics, uncertainty and sampling guidance (II, III)

Six phytoplankton metrics (Chl-*a*, PTI, SPI, MFGI, J and cyanobacteria biovolume) were tested against TP concentration (**II**). In another study besides these six metrics, an additional metric, FTI, was tested (**III**). TP has been found to be more powerful predictor compared to TN, but this may depend on the availability of these two nutrients in a certain lake (Brown et al., 2000; Phillips et al., 2008; Sondergaard et al., 2011). In our study, TP had strongest relationships with PTI, Chl *a* and cyanobacterial biovolume. Other studies have also shown strong relationships of TP with Chl *a* (phytoplankton biomass) (Reynolds, 2006), and cyanobacterial biomass (Smith, 1985; Watson et al., 1997; Elliott et al., 2006). PTI increases with increasing TP and biomass of cyanobacteria (Phillips et al., 2010), some groups of chlorophytes and diatoms. Our analysis strongly supports using both Chla *a* and PTI, and additionally also cyanobacterial biovolume in lake status assessment.

All seven metric scores were distributed along a pressure gradient and varied more among lakes than within lakes. Differences between sampling points over the lake and sampling and analytical variability showed relatively small proportion of the variance in metric scores. Chl *a*, PTI and cyanobacterial biovolume had the lowest within-lake variance and were robust to differences between open water sampling points within the lake. Our study did not examine the differences with other sampling points located in the littoral or in the river mouths. A higher number of sampling points in the lake increased the station level effect. Spatial heterogeneity can be greater in lakes with large surface area (10 km^2) or lakes with separated bays. In the littoral, the influence of zooplankton and macrophytes to phytoplankton communities may be different compared to in deeper open water areas. According to the WFD, parts of large lakes with different morphology and nutrient pressure should be assessed as separate water bodies. Using many sampling points is time consuming while the comparability of data collected during long surveys drops. One opportunity to get comparable data for water quality over large lake areas is the use of high resolution, multi-spectra satellite imagery, using parameters such as Chl *a* and cyanobacterial biovolume (Hunter et al., 2010, Tamm et al., 2015). Also DNA-based identification can make assessment procedures more faster and cost-effective (Hering et al., 2018).

Based on our study, we can argue that in most cases a single open water sample is enough to adequately assess the ecological status. Increasing the number of open water sampling stations or number of samples does not considerably improve the precision of assessment of these phytoplankton metrics. The variability of these three metrics among lakes is significantly related to differences in TP concentration meaning that these metrics are sensitive to eutrophication pressure.

Cyanobacterial biovolume represents summer blooms, but not bloom frequency. According to WFD, persistent blooms may occur in lakes of moderate status during summer months. To ascertain persistent blooms, lakes should be monitored more frequently. The latter is not practical for traditional methods because of increasing counting burden, but feasible with technologies based on fluorometry or satellites.

Major within-lake variance components were related to the analyst and sub-sampling. From these two components, sub-sampling was more important (**II, III**). Within-lake analytical variability was low and the reasons for that could be the use of standardized sampling methods and skills of the analysts harmonized at training workshops on counting. This study indicated that using standardized procedures minimizes sampling and analytical variability.

We found weakest relationships with TP and highest within-lake variance for the Evenness index (J; **II**) and the non-taxonomic morpho-functional metrics SPI and MFGI (III). The reason for weak relationships of those metrics compared to species or genera-based metrics may be the smallest number of indicator groups. Another reason may be the greater importance of biovolume estimates in the size-based metrics. The uncertainty may be reduced by better standardizing of counting protocols or applying automated methods (like flow-cytometry) for analyzing algal size-classes (Garmendia et al., 2013). Except Evenness index J, the lake's mean depth had an effect on all metric scores. The mean depth covaries with many physical and chemical features of lakes such as maximum depth, thermal stratification, flushing rate, underwater light availability and internal nutrient loading (Kalff, 2002). Detectable increase in Chl *a*, cyanobacteria biovolume and PTI metrics was observed in shallower lakes and with higher phosphorus concentrations. The higher cyanobacterial biovolume and Chl *a* concentration may be related to a higher nutrient supply in these systems. In shallow lakes frequent mixing of bottom sediments causes internal nutrient loading but also settled phytoplankton may be resuspended back into water column contributing to the Chl *a* values. In deeper lakes, mixing may limit light availability and because of this phytoplankton biomass is lower (Phillips et al., 2008).

MFGI, SPI and FTI were also higher in shallow lakes and in lakes at higher altitudes. These patterns in functional composition metrics show systematic changes in the community structure and trait representation with changing depth. In shallower lakes, high value of MFGI indicates increasing biomass of large colonial buoyant Chlorococcales and Nostocales. In deep lakes with low MFGI, non-motile Xanthophytes, small pennate and centric diatoms or Oscillatoriales dominate. Relationships between the mean depth and MFGI may be driven by the trophic preference of these functional groups of phytoplankton.

Metric scores were more variable in some limnological contexts than in others. AIC between models was lower in the case of SPI and MFGI, higher for Chl *a*, PTI, J and cyanobacteria biovolume. Chl *a* and J were more variable at lower P concentration, cyanobacteria biovolume had reverse pattern. Residual Chl *a* was more variable at greater mean lake depths, PTI and MFGI were less variable in these deeper lakes. Residual SPI and PTI were more variable in higher altitude lakes (**III**).

The availability of environmental data for each composition and bloom metric differed among lakes. Some variables (grazing, flushing, water colour, silica, dissolved nitrogen, turbidity) were registered inconsistently to include their effect in calculations. Unexplained among lake variability may be due to the temporal variability of phytoplankton. Phytoplankton community responds quickly, but changes in community are affected by previous environmental interactions. Therefore, phytoplankton communities and metrics characterize within year temporal variation and water body assessments vary accordingly.

4.4.3. Temporal variability

Phytoplankton community is very dynamic between years and within a year. Using phytoplankton as a parameter of quality assessment, the effect of seasonal variability associated with changing biological and physical structure of water column should be minimized and phytoplankton response to nutrient pressure should be strong. National assessment methods used in European countries differ greatly in sampling frequency varying from once in the summer period to monthly sampling throughout the year (Poikane, 2009). These differences affect the final result of assessment, for example, (chlorophyll) standards set by growing seasonal means or on annual means. Over large geographical regions such as Europe, the growing season differs, but, as the review of methods highlighted, a common period for sampling is from July to September.

For temporal variability, three metrics, Chl *a*, PTI and cyanobacteria biovolume, were examined. Based on this analysis, minimum sampling frequencies for these metrics in different geographical groups could be proposed. For example, due to the higher level of temporal variability in the Central-Baltic GIG, Chl *a* should be sampled three months between April to September for four years (12 samples altogether) to achieve comparable levels of uncertainty with other geographical regions. Samples for PTI should be taken from July to September in two months for four years or, alternatively, in one month for six years (**Table 2; II**).

Cyanobacteria biovolume metric has a different uncertainty pattern than Chl *a* or PTI. According to summer sampling scheme (July to September), the inter-annual variability of Cyanobacteria biovolume was much larger than monthly variability within the summer. Therefore, a more representative picture of cyanobacteria biovolume would achieved if sampling is extended to several years. The reason of this uncertainty pattern is not clear, but may be due to the use of only a single algal class. Also, cyanobacteria are known as being sensitive to many factors, for example temperature. Another reason for that may be that lakes differ by their cyanobacteria dominance. In some lakes, cyanobacteria are more common or dominating, while they do not live in others (e.g. low alkalinity lakes). Lakes with little cyanobacteria have low seasonal or interannual variability in cyanobacteria. Our analysis suggests that in cyanobacteria dominated lakes, variability is between years rather between summer months, although weather conditions during summer months are more stable than between years. Future analyses should include additional climate-related factors (for example annual flushing rates) to strengthen the relationship of cyanobacteria biovolume with eutrophication pressure.

The uncertainty analyses showed, that sampling variance in the Nordic Geographical Intercalibration Group can be markedly reduced with increasing the number of months sampled and sampling multiple years (based on Chl *a*). In the Central-Baltic and Mediterranean Intercalibration Group the more samples are needed to achieve the same reduction in sampling variance (**II**).

Table 2. Minimum recommended sampling frequencies for Chl *a*, PTI and cyanobacteria biovolume. CB-GIG – Central-Baltic Geographical Intercalibration group, M-GIG – Mediterranean Geographical Intercalibration group, N-GIG – Nordic Geographical Intercalibration group.

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

If it turns out unfeasible to study a larger number of lakes in the future, it would be possible to assess the robustness of among lake gradients in the variability of metrics used in this study. We suggest considering the phytoplankton metric variability reported in the present paper while designing future monitoring programmes, especially in Europe.

CONCLUSIONS

According to Water Framework Directive (WFD), phytoplankton abundance, composition and bloom metrics should be used in lake ecological quality assessment. Phytoplankton is highly sensitive to environmental changes and can serve as an early warning parameter in water quality assessment. To quantify the response of phytoplankton communities to nutrient pressure, the metric should be robust. Still numerous confounding factors and even the adaptational plasticity of phytoplankton can compromise its indicator value and increase the uncertainty of assessment results. Therefore, the final assessment of the ecological quality needs to be based on multifaceted analysis of phytoplankton ecology. EU countries use different numbers of phytoplankton parameters in their assessment schemes, the most popular being chlorophyll *a* (Chl *a*) and indexes based on indicator species.

The aims of this study were to ascertain the main environmental factors determining the distribution and biomass of dominant phytoplankton species in the EU lakes (**I, II**), to analyze the suitability and strength of phytoplankton parameters and metrics for estimating the ecological status of lakes in EU lakes (II, III), and to exemplify the evaluation of phytoplankton metrics through uncertainty analyses of their response to eutrophication (**II, III**).

According to the aims of study four hypotheses were posed:

a. Besides natural conditions, anthropogenic factors are important in determining the dominant phytoplankton species in lakes.
b. Domination of phytoplankton algal classes is different in Northern and Southern Europe.
c. National phytoplankton based assessment systems contain a number of robust metrics to assess the impact of eutrophication pressures.
d. Single open water sampling location is generally sufficient for assessment of ecological quality of lakes.

Substantial effort was made within the EU 7th Framework Programme project WISER to collect consistent phytoplankton data across European lakes. These data was used in the present study to elaborate the best sampling procedures for lakes, test metrics in use, develop a common metric and study parameters which influence the distribution of dominant phytoplankton species and algal classes.

The CCA ranked **water colour, total phosphorous (TP) and alkalinity as the most important factors determining the large-scale distribution patterns of lake phytoplankton dominants in European lakes.** Besides trophic conditions, hydrochemical variables have important role in determining phytoplankton community composition in lakes. **The study showed that chrysophytes and cryptophytes dominate more coloured and less alkaline lakes in Northern Europe whereas in cyanobacteria and dinophytes dominate lakes in Southern Europe**, which is explained by higher trophic state and higher alkalinity of lakes in this region.

During this study, strict sampling procedure was tested, wherein appeared, that **single open water sampling location is generally sufficient for assessment of ecological quality of lakes.** Phytoplankton community structure may change quickly within sampling period. To overcome the dynamism of phytoplankton community, either frequent monthly sampling or restricted seasonal window (e.g. only summer month samples) for metrics should be used.

The WFD emphasizes the need for consistent classification schemes to describe the health and functioning of water bodies. Metric strength is commonly associated with TP, but some metrics which do not have strong relationship with TP may have a complementary value. For example, cyanobacteria biovolume does not always have a strong relationship with TP, but is widely used to characterize water blooms. WFD stipulates that lakes in good ecological status should not have persistent blooms in summer, but there is no requirement to have a relationship between phytoplankton metric and TP. **In this study three robust metrics, Chl *a*, Phytoplankton Trophic Index and cyanobacteria biovolume, are provided to assess the impact of eutrophication pressures.**

In future, it is essential for freshwater ecological quality assessment to examine temporal variability and the extent to which uncertainty in lake assessment may vary systematically among lakes because of their physico-chemical and ecological features.

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SUMMARY IN ESTONIAN

Euroopa Liidu Veepoliitika Raamdirektiivi (VRD) nõuete kohaselt tuleks veekogude ökoloogilise seisundi hindamisel kasutada fütoplanktoni näitajaid, mis iseloomustaksid tema hulka ja koosseisu ning veeõitsengute sagedust ja intensiivsust. Fütoplankton on tundlik ja reageerib kiiresti veekogus toimuvatele muutustele. Survetegurite mõju hindamiseks peaks nende seos fütoplanktoni näitajatega olema piisavalt tugev ja stabiilne. Mitmed kõrvaltegurid ja koguni fütoplanktoni kohastumisvõime võivad seda seost häirida, mis suurendab määramatust ja vähendab fütoplanktoni indikaatorväärtust. Seetõttu peab järve seisundile antav lõpphinnang tuginema igakülgsele fütoplanktoni ökoloogia arvestamisele. Euroopa Liidu (EL) liikmesriigid kasutavad järvede hindamisel erinevaid fütoplanktoni parameetreid. Kõige enam kasutatavad parameetrid on klorofüll *a* kontsentratsioon ja indeksid, mis põhinevad liikide indikaatorlusel.

Doktoritöö eesmärgiks oli välja selgitada peamised tegurid, mis mõjutavad fütoplanktoni dominantliikide levikut EL järvedes (I, II), analüüsida fütoplanktoni parameetrite sobivust ja tugevust hindamaks järvede ökoloogilist seisundit (I, II), ja testida eutrofeerumist iseloomustavaid fütoplanktoni näitajaid (II, III).

Vastavalt doktoritöö eesmärkidele, püstitati neli hüpoteesi:

a. Nii looduslikud kui antropogeensed faktorid on olulised fütoplanktoni dominantliikide levikul veekogudes.
b. Vetikarühmade domineerimine on erinev Põhja- ja Lõuna-Euroopas.
c. Euroopa riikide fütoplanktonil põhinev veekogude hindamise süsteem on piisavalt jõuline selleks, et hinnata eutrofeerumisest tulenevaid mõjutusi.
d. Järvede ökoloogilise seisundi hindamiseks piisab ühest avavee proovivõtupunktist.

EL 7. raamprogrammi projekti WISER raames koguti Euroopa järvedest kindla juhendi alusel suurel hulgal uusi fütoplanktoni andmeid. Nende andmete põhjal töötati käesolevas töös välja parim veeproovide kogumise kord, testiti näitajaid, mis olid juba varasemalt kasutusel, töötati välja ühine indeks ja uuriti dominantliikide, perekondade ja muude rühmade esinemist mõjutavaid tegureid.

Üle-euroopalistel andmetel põhinev kanooniline korrespondentsanalüüs näitas, et **kõige enam mõjutavad fütoplanktoni dominantliikide esinemist üldfosfori sisaldus vees, vee värvus ja** üldaluselisus**.** Fütoplanktoni kooslus sõltub suuresti vee abiootilistest näitajatest. Selle töö käigus selgus, et **koldvetikad ja neelvetikad domineerivad peamiselt pruuni- ja pehmeveelistes Põhja-Euroopa järvedes. Lõuna-Euroopas domineerivad sinivetikad ja vaguviburvetikad**, kes eelistavad suuremat troofsust ja karedamat vett.

Selle uurimuse käigus töötati välja kindel proovivõtu protokoll (II, III), mille käigus jõuti järeldusele, **et üks avavee proov järves on tavaliselt hinnangu andmiseks piisav.** Fütoplanktoni koosluse struktuur võib proovivõtu perioodi jooksul kiiresti muutuda. Selleks, et neid kiireid muutusi registreerida, võiks kasutada igakuist proovivõttu või kindlat aastaajalist vahemikku, näiteks igakuist proovivõttu suvel.

VRD lähtub põhimõttest, et terve ja funktsioneeriva veekogu jaoks on vaja elustikul põhinevat klassifikatsiooni. Näitaja või indeksi tugevust on sageli seostatud üldfosforiga, aga mõnedel indeksitel, millel see puudub, võib ökosüsteemi seisundi hindamisel olla muu indikatiivne väärtus. Näiteks sinivetikate biomass ei seostu alati tugevasti üldfosforiga, kuid annab olulist informatsiooni veeõitsengu kohta, mida heas ökoloogilises seisundis ei tohiks esineda. Selles töös pakume välja **kolm surveteguritest oluliselt sõltuvat indeksit**, mida võiks kasutada veekogude seisundi hindamisel. Nendeks on: **klorofüll *a*, fütoplanktoni troofsusindeks (PTI) ja sinivetikate biomass**.

Tulevikus on oluline uurida järvede ökoloogilise seisundi hinnangute ajalist varieeruvust ja ulatust, mis võib muutuda järvede füüsikalis-keemiliste ja ökoloogiliste näitajate muutumise tõttu.

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I

ORIGINAL PUBLICATIONS

Maileht, K., Nõges, T., Nõges, P., Ott, I., Mischke, U., Carvalho, L., Dudley, B., 2013. Water colour, phosphorus and alkalinity are the major determinants of the dominant phytoplankton species in European lakes. Hydrobiologia, 704: 115–126.

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WATER BODIES IN EUROPE

Water colour, phosphorus and alkalinity are the major determinants of the dominant phytoplankton species in European lakes

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Abstract Analysis of phytoplankton data from about 1,500 lakes in 20 European countries has revealed that two-thirds of the species that dominate lakes during the summer are dominant right across Europe. Using Canonical Correspondence Analyses, we have examined how both habitat conditions within lakes and environmental factors over broad geographical scales explained the distribution of the 151 most common summer dominant species. The distributions of these species were best explained by water colour and latitude, although alkalinity and total phosphorus also appeared to be important explanatory factors. Contrary to our original hypothesis, summer water temperatures had a negligible impact on the distribution of dominants, although, due to the restricted summer season we examined, only a limited temperature gradient was present in the dataset. Cryptophytes occurred more frequently among dominants in Northern Europe whereas cyanobacteria and dinophytes dominated more in Central and Southern Europe. Our analyses suggest that besides nutrient concentrations, other water chemistry variables, such as alkalinity and the content of humic substances, have at least as important a role in determining the distribution of the dominant phytoplankton species in European lakes.

Keywords WISER Project · Geographical gradients · Nutrients · CCA · Water temperature

Introduction

Despite continuous efforts of generations of algologists studying individual lakes, the biogeographical distribution of freshwater phytoplankton and its driving factors are still largely unknown. Padisák et al. (2003) pointed out that this may because taxonomic and floristic work has had a stronger focus in small lakes, the bulk of our knowledge on the ecology of phytoplankton is derived from relatively large lakes. The major problem in biogeographical studies has

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Table 1 Examples of broad-scale studies on phytoplankton community composition

Scope of study	Selection of lakes	References
Gradients in phytoplankton community structure with increasing lake trophic status	165 lakes in Florida	Duarte et al. (1992)
Patterns in phytoplankton taxonomic composition across lakes of differing nutrient status	91 temperate lakes	Watson et al. (1997)
Responses of three major phytoplankton classes to eutrophication	850 lakes from Scandinavia and the United Kingdom	Ptacnik et al. (2008)
Relationship between phytoplankton species richness and productivity including six major taxonomic groups	33 well-studied lakes on different continents	Dodson et al. (2000)
Geographic gradients in phytoplankton biodiversity at species-level over a continental scale	540 lakes and reservoirs on the continental U.S.	Stomp et al. (2011)
Community structure of summer phytoplankton	73 nutrient-poor Swedish lakes	Willén et al. (1990)
Dominant species and functional assemblages in late summer phytoplankton	80 Hungarian small shallow lakes	Padisák et al. (2003)
Type-specific and indicator taxa of phytoplankton as a quality criterion for assessing the ecological status	55 Finnish boreal lakes	Lepistö et al. (2004)

been the absence of phytoplankton data of a comparable resolution and harmonized taxonomy covering broad continental scales. Variability in phytoplankton composition and abundance is driven by local environmental factors, such as lake morphometry, as well as broad latitudinal, longitudinal and altitudinal gradients. Phytoplankton abundance and taxa richness may display some regularity in spatial distribution, but these patterns are often blurred because of regionally different taxonomic resolution, counting routines and traditions in taxonomic work. Nevertheless, a number of important studies have been carried out on a relatively large numbers of lakes, generally at a national level (Table 1).

In Europe the implementation of the Water Framework Directive (Directive, 2000) has given a new impetus to freshwater ecological studies at species and community level and the need for comparisons over broad geographical ranges shifted to the forefront of research. Chemical and biological data from more than 5,000 lakes in 20 European countries were compiled into databases within the EU REBECCA Project (Moe et al., 2008) and complemented by new data during the EU WISER Project (Hering et al., 2012; www.wiser.eu). This database is now the largest combined dataset on phytoplankton composition in Europe.

It is always challenging to study phytoplankton community responses to changes in the environment due to the high variability of phytoplankton species structure. Common ways to cope with this complexity is to “boil it down” to major taxonomic groups (e.g. Duarte et al., 1992; Ptacnik et al., 2008), functional groups (Reynolds et al., 2002, Padisák et al., 2003) or strategist groups (Grime, 1979; Reynolds, 1988) or to calculate various indices to characterize different aspects of the community structure, such as diversity (Shannon, 1948; Simpson, 1949; Margalef, 1958) or evenness (Pielou, 1975). For many purposes even the simplest parameter, the number of species (Hill, 1973), may be the most useful measure of local or regional diversity. In our study, we follow a different approach and focus on the dominant species. Studying the dominants is interesting for several reasons. Firstly, the stability of ecological communities often depends greatly upon the population dynamics of the dominant species (Grime, 1998; Flöder et al., 2010); and secondly, as the winners of competition for resources, the dominants can give a robust picture of resource availability. In this respect, studying the summer phytoplankton is most promising, as community equilibria occur most prominently during summer when higher growth rates and less flushing allows competitively stabilized associations to develop (Padisák et al., 2003). Many of the dominants tend to be nuisance species, so the distribution and understanding of their controlling factors remains a high priority research topic. Finally, selecting just to examine the dominant species should guarantee reduced taxonomic uncertainty, as researchers tend to pay more attention to abundant species and their high abundance in the sample should minimize misidentification errors as sufficient material is observed to cover phenotypic variability in the species.

A recent analysis of the 1,337 lakes included in the European Environment Agency (EEA) database (Nõges, 2009) showed that lakes at higher latitudes are larger but shallower and have smaller catchment areas. Northern lakes have lower alkalinity, pH and conductivity, and also lower concentrations of nitrogen and phosphorus while the concentration of organic matter is higher compared to southern lakes. Several gradients in lake environments were found also along longitudinal and altitudinal scales. As Europe extends from arctic to sub-tropical areas, and from maritime to continental climates, the temperature and ice regimes of inland waters vary within a wide range. The present study aims to assess the impact of these hydrochemical, climatic and morphometric factors on the dominant taxa of lake phytoplankton and their functional attributes, over broad geographical scales. As differences in humic matter content and alkalinity have been shown to be the major factors modifying phytoplankton response to eutrophication within the Nordic countries (Ptacnik et al., 2008), we hypothesize that expanding the geographical range to the south and west, and focusing on dominant species, we should see an even stronger impact of these factors. We also hypothesize that the effect of water temperature will be clearly manifested in the occurrence of different phytoplankton dominants.

Materials and methods

Twenty countries provided lake data to the EU 7th Framework Programme project WISER (Moe et al., 2012). Data were gathered spanning a long time period (1972–2009). Data from samples taken only in July, August and September were selected for analysis, comprising a total of 6,120 samples from 1,558 water bodies from Belgium (BE) 11, Cyprus (CY) 7, Germany (DE) 217, Denmark (DK) 64, Estonia (EE) 46, Spain (ES) 135, Finland (FI) 156, France (FR) 5, Greece (GR) 1, Hungary (HU) 13, Ireland (IE) 40, Italy (IT) 14, Lithuania (LT) 36, Latvia (LV) 58, The Netherlands (NL) 43, Norway (NO) 401, Poland (PL) 39, Romania (RO) 10, Sweden (SE) 113 and United Kingdom (UK) 149. More than half of these data (62%) originated from the last 10 years.

Phytoplankton and chemical data were largely based on integrated samples from either the epilimnion or euphotic zone. Samples were analyzed according to the Utermöhl technique (CEN EN 15204, 2006). Very seldomly, additional slide preparation for identification of diatom species was carried out in parallel, so the diatom taxa list is based on different analytical approaches. Therefore, for example, the diatom genus *Cyclotella* is identified to species level in only a small proportion of samples. The European WISER phytoplankton list was created as an operational list to merge European data (http://www.freshwaterecology.info/). This list is not kept up to date with new names, but was harmonized based on the status of common determination keys in Europe in 2010.

We focused our study on the most dominant taxa, which we defined as the single species with the largest biovolume from each sampling date. Only species which were recorded as dominants in at least five of the 6,120 samples in the dataset were included in our analysis. We also examined the dominant species in terms of their belonging to 11 algal classes: Bacillariophyceae (Bac), Chlorophyceae (Chlor), Chrysophyceae (Chrys), Conjugatophyceae (Conj), Cryptophyceae (Crypt), Cyanophyceae (Cyan), Dictyochophyceae (Dict), Dinophyceae (Dino), Euglenophyceae (Eug), Prymnesiophyceae (Prym) and Raphidophyceae (Raph).

To study the occurrence of dominant species in Europe, we split the data into two parts—countries belonging to the Nordic Geographical Intercalibration Group (N-GIG: FI, SE, NO, IE, part of UK) and countries located in Central and Southern Europe, belonging to the Central Baltic (CB-GIG), Alpine (AL-GIG), East Continental (EC-GIG) and Mediterranean (M-GIG) Geographical Intercalibration Groups. The GIG boundaries were delineated within the WFD implementation process and reflect the eco-regions which share common types of surface water bodies. This split divided the data into relatively comparable parts with 4,071 samples collected from 859 N-GIG lakes and 2,049 samples collected from 699 lakes located in Central and Southern parts of Europe. The fact that many lakes were represented by a number of samples in which the dominant species could either be the same or different, complicated the calculation of occurrence frequencies of different dominant species: calculation by lakes became impossible whereas calculating by samples would have caused a bias towards lakes for which there were more samples in the dataset. To overcome this, we considered the occurrences of different dominant species in the same lake as different counting units or occasions, but if the same species dominated in all samples from a lake, it was considered as one counting unit. We got 1,897 such counting units for N-GIG and 1,341 counting units for Central and Southern part of Europe that were analyzed for the frequency of dominant species belonging to different algal classes.

The database included the following environmental parameters: latitude, longitude, altitude, alkalinity, maximum depth, mean depth, surface area, colour (Pt–Co scale), total nitrogen (TN), total phosphorus (TP) and water temperature.

For evaluation of the relationships between the distribution of dominant species and the environmental variables, we ran a Canonical Correspondence Analysis (CCA), using CANOCO 4.5 (Ter Braak & Šmilauer, 2002).

Results

In Northern Europe, lakes are generally larger, but shallower, have lower alkalinity, total N and total P and are generally more coloured than in Central and Southern European lakes. A difference in summer water temperatures between the two regions was not so apparent (Table 2).

Altogether 151 phytoplankton taxa were recorded as dominants in 5 or more samples, 130 of which were identified to species level and 21 to genus level. We handled all of them as unique taxa. The occurrence of these dominant taxa by country is presented in Electronic Annex 1.

The frequency distribution of the dominant taxa among the 20 countries had a positive skew (Fig. 1) with many of the dominant taxa only shared by 2–4 countries. The most widespread taxa, occurring as dominant in 15 or more countries, were *Ceratium hirundinella, Cyclotella* sp., *Aulacoseira granulata* and *Cryptomonas* sp. Among dominants, 132 taxa occurred in N-GIG lakes, with only 29 of these being restricted to the N-GIG and 126 dominant taxa occurred in Central and Southern Europe, with only 16 taxa restricted to this area. About two-thirds of the dominant taxa, therefore, dominated in both regions of Europe.

The division of the dominant taxa between algal classes was rather similar in the two parts of Europe (Fig. 2). There were slightly more diatom, chrysophyte and chlorophyte taxa and slightly less cyanobacterial taxa among dominants in the North than in

Table 2 Information of range of maximum and mean depth, surface area, alkalinity, colour, total N, total P and water temperature in two different parts of Europe: Nordic Geographical Intercalibration Group (Nordic GIG) and Central and Southern part of Europe belonging to Alpine (AL-GIG), Central Baltic (CB-GIG), East-Continental (EC-GIG) and Mediterranean (M-GIG) Geographical Intercalibration Group

Parameter	Max depth (m)	Mean depth (m)	Surface area (km^2)	Alkalinity (meq l^{-1})	Colour (mg l^{-1} Pt)	Total N (mg l^{-1})	Total P (mg m^{-3})	Water temp. (°C)
Nordic GIG								
n	3,014	3,817	3,799	1,609	1,758	2,795	2,954	1,463
min	0.7	0.23	0.02	0	0.5	0.05	1	5
max	516	238.7	1,377	3.47	554.5	4.7	872	25.4
med	32	7.2	3.01	0.16	30.8	0.417	11	18.2
avg	74.3	20.9	74	0.29	46.5	0.54	22.4	17.6
stdev	119.9	38.7	183.2	0.42	53.7	0.42	43.2	3.6
Central and Southern part of Europe								
n	1,821	1,922	2,028	417	107	598	1,497	576
min	1	0.28	0.006	0.07	5	0.06	2	6
max	370	178	1,190	20	195	20.1	3,810	30
med	14	5.3	1.46	1.96	25	0.97	43	20
avg	31.6	12.4	10.8	2.18	32.3	1.37	113.8	20.5
stdev	57.5	26	55	2.05	28.5	1.64	229.7	3.5

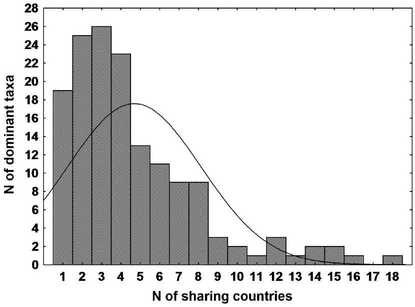


Fig. 1 Histogram of the number of dominant phytoplankton taxa that are shared by increasing numbers of European countries

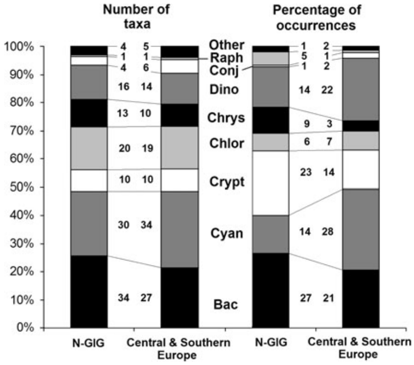


Fig. 2 Distribution of the dominating lake phytoplankton taxa among algal classes (two *left columns*) and their relative frequency of occurrence (two *right columns*) compared between countries belonging to the Nordic Geographical Intercalibration Group (N-GIG) and in Central and Southern part of Europe belonging to Central Baltic (CB-GIG), Alpine (AL-GIG), East Continental (EC-GIG) and Mediterranean (M-GIG) Geographical Intercalibration Group

Central and Southern Europe. The same differences appear much stronger in terms of the frequency of occurrence of dominants between the two regions. Chrysophytes occurred thrice and cryptophytes nearly twice as frequently among dominants in the N-GIG than in Central and Southern part of Europe and cyanobacteria and dinophytes occurred about in about half as many samples. The biggest difference, however, was revealed for *Gonyostomum semen*, the single representative of Raphidophyta, which dominated in N-GIG lakes 5 times more frequently than elsewhere. Our dataset revealed that *G. semen* was a dominant species of soft water lakes (alkalinity between −0.067 to 1.055 meq/l) in DK, EE, ES, FI, LV, NO, SE and UK. The highest biovolumes of *G. semen* were recorded in humic lakes in SE, NO, EE and DK (average water colour of these lakes was 112 mg/l Pt). Our analysis highlighted other species as being capable of dominating dark acid waters, like *Chrysosphaerella longispina* (Dillard, 2008; Trigal et al., 2011), *Botryococcus terribilis* (Trigal et al., 2011), *Peridinium inconspicuum* (Willén, 2003) and *Dinobryon sociale* var. *americanum* (Canter-Lund & Lund, 1995). Species which showed a good relationship with longitude, like *Dinobryon pediforme*, were also a common dominant in acid lakes (Willén, 2003).

Cumulative percentage variance of species and environmental relations in two CCA axes was 36.0. Monte Carlo permutation test showed that the most significant parameters explaining the taxa ordination in rank order were maximum depth, latitude, alkalinity, colour, longitude, altitude, total P, total N, surface area and mean depth ($P \leq 0.024$). The only insignificant parameter was water temperature ($P = 0.242$). The CCA biplot (Fig. 3a, b) revealed a strongly intercorrelated group of factors describing lake morphometry (mean depth, maximum depth, surface area), which was positively related to altitude and negatively to TP and TN. Water colour increased strongly with increasing latitude and longitude and was negatively related to alkalinity. Water temperature, which was the weakest of the explanatory variables tested, increased with decreasing lake size and depth and showed no relationship with geographic location (latitude and longitude).

The cloud of the dominant species had a strongly elongated shape in the gradient determined by water colour, alkalinity and TP. Taxa associated with high water colour were in rank order *Crucigenia tetrapedia*, *Peridinium umbonatum* var. *goslaviense* and *Urosolenia longiseta* and those associated with high alkalinity *Aphanizomenon aphanizomenoides, Cryptomonas curvata* and *Staurastrum pingue. A. aphanizomenoides* and *Cylindrospermopsis raciborskii* showed very good relationships with water temperature, TP and alkalinity.

At low latitudes and longitude, i.e. Southern Europe, there is another cluster of species such as

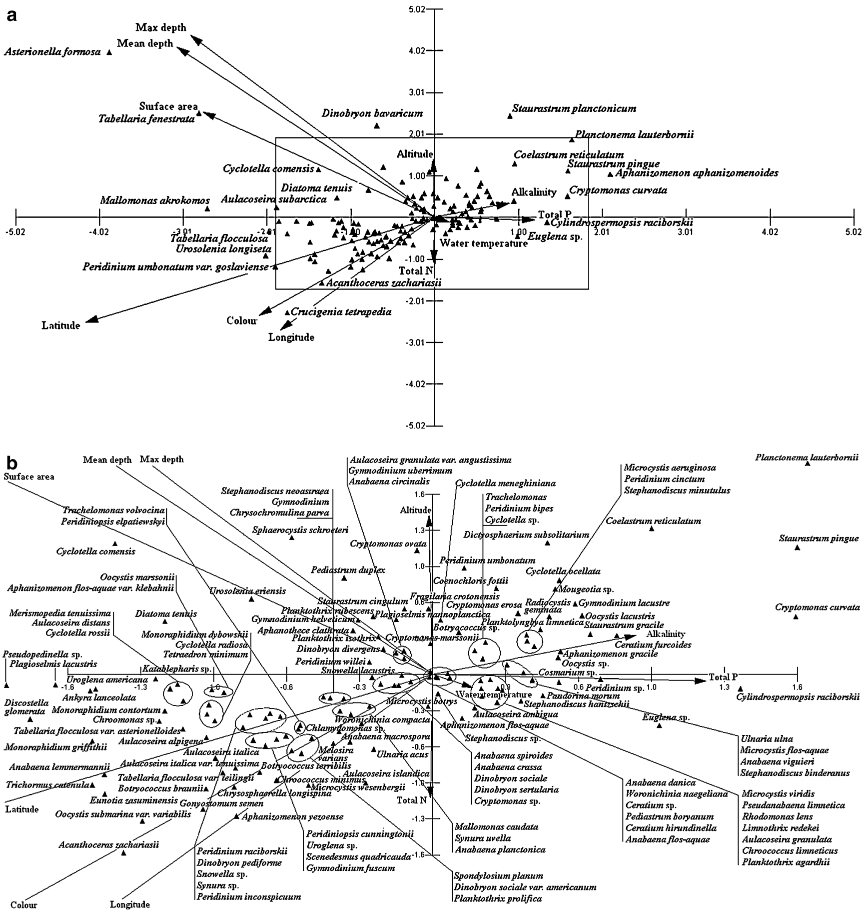
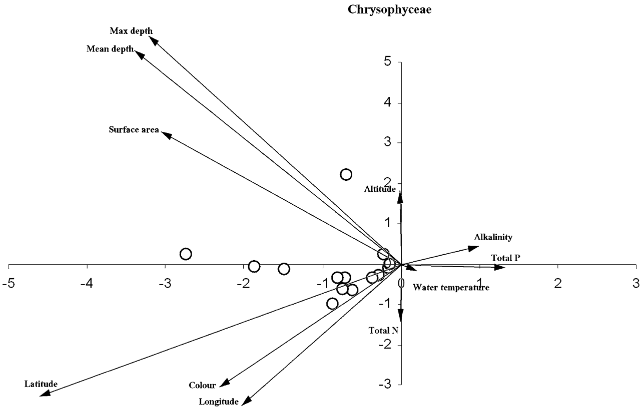


Fig. 3 Biplot of the Canonical Correspondence Analysis (CCA) results on factors determining the distribution of dominant phytoplankton taxa in lakes of Europe. **a** The large picture, **b** the central part magnified. The *arrows* in the biplot representing the environmental variables indicate the direction of maximum change of that variable across the diagram and the length of the *arrow* is proportional to the rate of change. Each point representing a dominant species lies at the centroid of the samples in which it was found

Planctonema lauterbornii, *Dictyosphaerium subsolitarium*, *Cyclotella ocellata*, *Mougeotia* sp., *Coenochloris fotti* and *Cryptomonas erosa*. All these species had their peak biovolume in southern countries, most of these in ES. One of these species, *P. lauterbornii*, has been shown in other studies to have a strong relationship with temperature (Gomes et al., 2004).

The diatoms *Asterionella formosa, Tabellaria fenestrata and Cyclotella comensis* were strongly related with lake size and depth. If plotted by algal

Fig. 4 CCA biplot showing the factors determining the distribution of Chrysophytes in lakes of Europe



class, the stronger dependence of diatoms on lake morphometry compared to other algal classes was expressed in the much broader vertical spread of the cloud (not shown). Chrysophytes (Fig. 4) instead had a strongly skewed distribution towards increasing latitude and water colour.

Discussion

Dominant taxa

The fact that, in general, about two-thirds of the dominant species in Northern Europe were the same taxa that dominated in Central and Southern part of Europe is surprising. This suggests that broad geographical-scale gradients, such as the effects of climate and day length or length of growing season are less important in determining the dominant species than more local lake- and catchment-specific factors, such as depth and alkalinity. Less surprising is that dominant species spanned many algal classes, reflecting the diverse range of lake types and broad alkalinity and nutrient gradients across Europe. The fact that chrysophytes, cryptophytes, diatoms and raphidophytes were more frequently dominant in Northern Europe, whilst cyanobacteria and dinoflagellates more frequently dominated Central and Southern Europe reflected the broad distinction between dominant lake types in these two regions. Northern lakes are generally larger and shallower with smaller catchment areas, lower alkalinity, pH and conductivity and with less nutrients and more dissolved organic compounds than southern lakes (Nõges, 2009). Chrysophytes are common in softwater lakes with low or moderate productivity and lakes with low pH (Nicholls & Wujek, 2003), which is in good correspondence with our analyses (Fig. 3). Cryptophytes are common species with a widespread distribution in many lake types, but our analysis supports individual lake studies that show they often dominate in the summer and autumn in humic lakes (Arvola et al., 1999). The higher frequency of cyanobacteria and dinophytes (Fig. 2) as dominants in Southern Europe is clearly explained by the distribution of lakes of higher nutrient concentrations and higher alkalinity in this region. The impact of these specific individual gradients in geography, morphology and water quality on species is discussed in more detail below.

Colour, latitude and longitude

Latitude, longitude and colour gradients in European lakes are correlated, but the strongest factor was colour, since the other two simply describe location. The majority of Scandinavian lakes have acid and coloured waters in correspondence with catchment areas covered mostly by forests, swamps and mires. Thin soils lie directly on bedrock and buffer capacities are relatively low (Arvola et al., 1999). Hereafter colour and latitude, and to a lesser degree longitude, are discussed together, not as separate parameters.

Many taxa common in highly productive lakes, are also more frequently recorded in lakes of higher humic content (Arvola et al., 1999). Arvola et al. (1999) presented a list of species that occur more frequently in brown coloured lakes. The following species also occurred as the dominant species in our dataset: *Acanthoceras zachariasii*, *Anabaena planktonica*, *Aphanizomenon flos-aquae*, *Eunotia zasuminensis*, *Mallomonas caudata*, *Melosira varians*. *G. semen*, *Botryococcus braunii*, *C. tetrapedia*, *Tabellaria flocculosa, Monoraphidium griffithii, D. pediforme, Synura* sp., *Aulacoseira alpigena, Spondylosium planum, P. umbonatum, U. longiseta* and *Aulacoseira italica*. Additionally, *Anabaena lemmermannii*, known as a characteristic species of soft water lakes (Ott & Kõiv, 1999) was also found as a dominant predominantly in Northern Europe, whereas we observed that *Trichormus catenula* is widely distributed (Zabelina et al., 1951). Most of these species dominate in northern parts of Europe, in countries like NO, SE, FI, UK, EE and DK, with some exceptions like *T. catenula* and *Synura* sp.

Rosen (1981) identified *Oocystis submarina* (Arvola et al., 1999) and small naked chryso- and dinoflagellates as typical of humic conditions. *Anabaena macrospora* and *Woronichinia compacta* are also common in the northern temperate zone (Komárek & Anagnostidis, 1999; Komárek & Zapomelova, 2008).

Gonyostomum semen is a well-known nuisance alga with widespread distribution in Northern Europe (Figueroa & Rengefors, 2006) and has been recorded as increasing in Scandinavian (Willén, 2003; Figueroa & Rengefors, 2006; Trigal et al., 2011) and Baltic soft water lakes (Rakko et al., 2008).

Polyhumic lakes usually have a very specific phytoplankton composition, where dominant species are adapted to low light and large fluctuations and gradients of temperature and oxygen. Higher humic content has been observed to be associated with higher phytoplankton biovolume (Arvola et al., 1999; Carvalho et al., 2008, 2009). In polyhumic lakes (>100 g Pt m^{-3}) this trend stops (Arvola et al., 1999). Moderate content of humic matter seems to positively affect phytoplankton abundance. One explanation of this is that environmental resources are enriched in humic waters. If besides moderate humic matter, the supply of mineral nutrients is enriched, and there is enough carbon resource, phytoplankton have been shown to be richer in comparison with low colour lakes (Ott & Kõiv, 1999).

Water temperature, TP and alkalinity

The wide distribution of *C. raciborskii* and *A. aphanizomenoides* in the temperate zone is widely cited as a response to global warming (Briand et al., 2004; Stüken et al., 2006). *C. raciborskii* is a common species in tropical and pantropical regions (Cronberg & Annadotter, 2006). Our analyses showed that *C. raciborskii* was now a dominant species in samples from ES, HU and NL. This species has rapidly increased all over the world from tropical to temperate zones (Fabbro & Duivenvoorden, 1996; Chapman & Schelske, 1997; Lagos et al., 1999; Shafik et al., 2001; Briand et al., 2004; Valerio et al., 2005; Bouvy et al., 2006; Fastner et al., 2007; Moustaka-Gouni et al., 2009; Alster et al., 2010; Kokociński et al., 2010; Moisander et al., 2012) except Antarctica (Padisák et al., 2003). *C. raciborskii* prefers highly eutrophic waters, when water temperature is high and light conditions are poor (Moustaka-Gouni et al., 2006, 2009), but it can also survive in water bodies with lower trophic status, because of its effective storage capacity for phosphorus. This species can also dominate under varied abiotic conditions, such as high concentrations of dissolved minerals or variable salinity. Temperature appears to be the most important factor. *A. aphanizomenoides* is also recorded from tropical and subtropical regions, but has expanded its distribution into the temperate zone (Stüken et al., 2006). Our database showed that *A. aphanizomenoides* is now a dominant in Germany (DE) and Spain (ES).

Water temperature, TP and alkalinity also showed a strong relationship with many cyanobacteria, such as *Microcystis flos-aquae*, *Anabaena viguieri*, *Aphanizomenon gracile*, *Planktothrix agardhii*, *Pseudanabaena limnetica*, *M. viridis*, *Limnothrix redekei*, *Chroococcus limneticus* and *Anabaena danica*. All these species, except *C. limneticus*, are particularly known from meso- and eutrophic water bodies and may form water blooms (Mischke & Nixdorf, 2003; Nixdorf et al., 2003; Reynolds et al., 2002; Cronberg & Annadotter, 2006; Willén, 2007). Phillips et al. (2012) classified phytoplankton genera into very tolerant, tolerant, sensitive and very sensitive taxa of high nutrient conditions. Many of the cyanobacterial genera were classified as very tolerant or tolerant. Only *Chroococcus* sp. was classified as a slightly sensitive genus of nutrient concentrations. The dominance by *Euglena* sp. also showed a strong relationship with water temperature and TP. This taxon does not generally reach a high biovolume in large lakes, but in small lakes its biovolume can be great (Padisák et al., 2003), as was supported in this study. *Pandorina morum* and *C. curvata* are common in nutrient-rich water bodies (Reynolds et al., 2002; Padisák et al., 2003) and *C. curvata* is tolerant of low light (Reynolds et al., 2002). *Ceratium furcoides* and *A. gracile* dominance showed strong relationships with alkalinity in our study but both species are described by Reynolds et al. (2002) as tolerant of low carbon concentrations, although this may be the case in waters of very high alkalinity.

Lake morphometry (surface area, mean and maximum depth) and altitude

Lake morphometry and altitude appeared important in favouring the dominance of the following species in rank order: *A. formosa*, *T. fenestrata*, *C. comensis* and *Dinobryon bavaricum*. *A. formosa* and *T. fenestrata* showed very strong relationship with mean depth, surface area and maximum depth. Both species are dominant in deep lakes with large surface area. Despite heavy frustules they are best adapted to float in the water column due to long, thin cells, or the belt- and star-like structure of their colonies. *C. comensis* showed a particularly strong relationship with surface area. This species is common in alpine lakes (Zabelina et al., 1951; Hausmann & Lotter, 2001; Scheffler & Morabito, 2003). Our analyses supported this, showing that it was dominant in higher altitude locations of ES, IT, NO and SE (with average altitude 397.6 m). The average maximum depth of these lakes was also relatively high (164.7 m).

Conclusions

We recorded 151 phytoplankton taxa mostly identified to species level, which occurred as the most dominant taxa by biovolume in at least five of the 6,120 samples collected between July and September from 1,558 lakes in 20 countries of Europe.

Two-thirds of the dominant species in Northern Europe (including Finland, Sweden, Norway, Ireland and part of UK) were the same taxa that dominate in Central and Southern regions of Europe. The dominant species spanned all algal classes in both regions reflecting a diverse range of lake types across Europe. There were slightly more diatom, chrysophyte and chlorophyte taxa and slightly less cyanobacteria taxa among dominants in the north than in the south.

Chrysophytes occurred thrice and cryptophytes nearly twice more frequently among dominants in the North European lakes than in Central and Southern part of Europe whereas cyanobacteria and dinophytes occurred about twice less frequently.

The CCA ranked water colour, alkalinity and TP as the most influential factors determining the large-scale distribution patterns of lake phytoplankton dominants in Europe. This suggests that, besides trophic conditions, other hydrochemical variables, have at least an important role in determining phytoplankton community composition in lakes. Water temperature from July to September had only a negligible impact on the distribution of dominants, showing the prevalence of rather homogeneous thermal conditions throughout Europe for this period of year.

Cryptophytes and especially chrysophytes revealed a clear affinity to more coloured and less alkaline waters of Northern Europe. The higher frequency of cyanobacteria and dinophytes as dominants in Southern Europe can be explained by the higher trophic state and higher alkalinity of lakes in this region.

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(IRSTEA) (6). Greece: Maria Moustaka, Aristotle University of Thessaloniki. Central-Baltic GIG (Data manager: Ute Mischke, IGB) Estonia: Estonian University of Life Sciences (EMU) gathered for the state monitoring programme supported by the Estonian Ministry of Environment. Latvia: Sandra Poikane, Latvian Environment, Geology and Meteorology Centre. Lithuania—EPA Lithuania. Denmark: National Environmental Research Institute, University of Aarhus. Belgium: Jeroen Van Wichelen, Ghent University (UGENT). Netherlands: Rijkswaterstaat (RWS). Germany: Data from German water bodies were kindly provided by the following institutions of the German Federal States: Landesamt für Umwelt, Gesundheit und Verbraucherschutz Brandenburg (LUGV; 127), Ministerium für Landwirtschaft, Umwelt und Verbraucherschutz Mecklenburg-Vorpommern (MLUV, Seenprogramm, 65), Landesbetrieb für Hochwasserschutz und Wasserwirtschaft Sachsen-Anhalt (LHW, 5), Landesamt für Landwirtschaft, Umwelt und ländliche Räume Schleswig–Holstein (LLUR, 13), Senatsverwaltung für Gesundheit, Soziales und Verbraucherschutz Berlin (SenGUV, 12), Niedersächsische Landesbetrieb für Wasserwirtschaft, Küsten- und Naturschutz (NLWKN, Sulingen, 1). Poland: Data were provided partly by the Institute of Environmental Protection—National Research Institute. The Inspection for Environmental Protection provided data obtained within the framework of state environmental monitoring. Hungary: Environmental Protection Inspectorate for Trans-Tiszanian Region. Northern GIG (Data manager: Geoff Phillips, EA) Finland: Finnish Environment Institute (SYKE). Sweden: Swedish University of Agricultural Sciences (SLU). Norway: Norwegian Institute for Water Research (NIVA). United Kingdom—Scottish Environment Protection Agency (SEPA) and the Environment Agency for England & Wales (EA). Ireland: Environment Protection Agency (EPA).

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

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Abstract Phytoplankton constitutes 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. 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

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(chlorophyll-*a*, phytoplankton trophic index (PTI), and 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 the 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., 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) and 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

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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., Carlson, 1977; Wolfram et al., 2009), European (EC, 2008) and international (OECD, 1982) lake monitoring and classification schemes. Measurements of chl-*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 (Capblancq & 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 & Padisak, 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 study, we very briefly review the national metrics submitted by the end of the second 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 (Watson et al., 1997; Carvalho et al., 2011). 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, Poikane et al., 2011). Based on existing metric classifications (Karr & 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 >1,500 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 >1,500 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, b). 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 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 =

$$\frac{\sigma_y^2 \times \left(1 - \left[N_{\text{year}}/\text{Max}_{\text{year}}\right]\right)}{N_{\text{year}}} + \frac{\sigma_m^2 \times \left(1 - \left[N_{\text{month}}/\text{Max}_{\text{month}}\right]\right)}{\left(N_{\text{month}} \times N_{\text{year}}\right)}$$

where σ_y^2 is the year-level metric variance from mixed effects model, σ_m^2 the month-level metric variance from mixed effects model, N_{year} the number of years sampled, N_{month} the number of months sampled per year, $\text{Max}_{\text{month}}$ the maximum number of months that can be sampled per year [for total cyanobacteria and PTI, $\text{Max}_{\text{month}} = 3$ (July–September); for Chl-*a*, max $_{\text{month}} = 6$ (April–September)], Max_{year} the 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

Twenty-four 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).

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 three 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, 2005); (3) only two MS used indices based on a functional group approach (Reynolds, 1998) 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 < 100 µg/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) and Mischke et al. (2011).

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.

The analysis of temporal variability only examined three candidate metrics (chl-*a*, PTI, and cyanobacteria biovolume) but highlighted different levels

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	Numbers
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

Table 2 Relationship strength between six WISER phytoplankton metrics and total phosphorus as a proxy of eutrophication pressure

Metric	Metric description	Pressure	r^2	GIG	*P*	*N*
Chl-*a*	Chl-*a* (μg/l)	Eutrophication (Total-P)	0.63	All	<0.001	16,949
PTI	Phytoplankton trophic index	Eutrophication (Total-P)	0.67 (GAM)	All	<0.001	1,500
SPI	Size phytoplankton index	Eutrophication (Total-P)	0.23	CB	<0.0001	122
			0.34	N	<0.0001	77
			0.19	M	<0.05	29
MFGI	Morpho-functional group index	Eutrophication (Total-P)	0.33	CB	<0.0001	122
			0.05	N	<0.05	77
			0.38	M	<0.001	29
J′	Pielou's evenness index	Eutrophication (Total-P)	0.19	N	<0.001	716
			0.07	CB	<0.001	559
Cyanobacteria bloom intensity	Cyanobacteria biovolume (mg/l)	Eutrophication (Total-P)	0.34 (GAM)	All	<0.001	1,710

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

Table 3 Metric precision given as the proportion of total metric variance that occurred between and within-lakes

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 major within-lake variance component is also highlighted. See Table 2 for description of metrics. Data taken from Thackeray et al. (2012b)

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 and 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 chl-*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).

Discussion

Recommendations of metrics for intercalibration and national schemes

Lake phytoplanktons are widely adopted around the world as a highly sensitive, early warning indicator of water quality. European environmental legislation,

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 three summer months (July–September), and for chl-*a* within 6 months (April–September)

	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

For example for NGIG, chl-*a* should be sampled at least once in two different months (Apr–Sep) in each of three different years, or alternatively, once in three different months (Apr–Sep) in each of two different years, meaning six 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

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

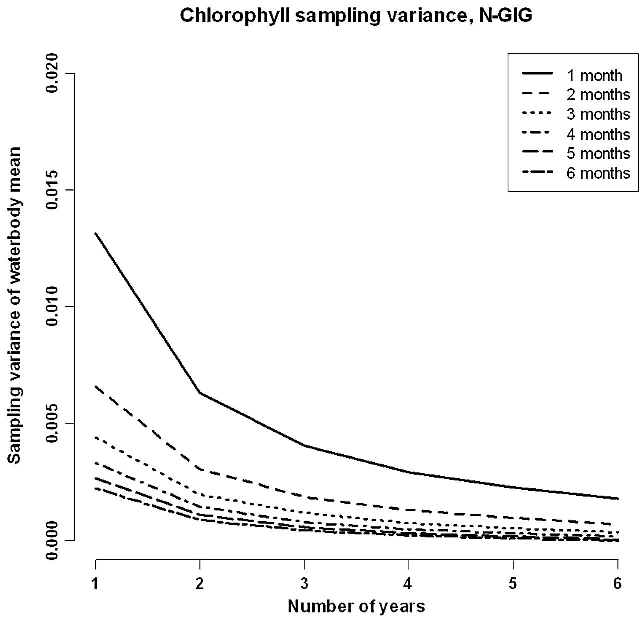

Fig. 1 Changes in temporal sampling variance for chl-*a* in the N-GIG when sampling in different numbers of years and months (Apr–Sep) within years (see Thackeray et al., 2012a for full details)

"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 one 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 6-year reporting period. For this, the WISER temporal uncertainty analysis indicates that generally at least three 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 6 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 6 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–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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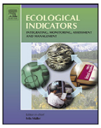

Quantifying uncertainties in biologically-based water quality assessment: A pan-European analysis of lake phytoplankton community metrics

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ABSTRACT

Lake phytoplankton are adopted world-wide as a sensitive indicator of water quality. European environmental legislation, the EU Water Framework Directive (WFD), formalises this, requiring the use of phytoplankton to assess the ecological status of lakes and coastal waters. Here we provide a rigorous assessment of a number of proposed phytoplankton metrics for assessing the ecological quality of European lakes, specifically in response to nutrient enrichment, or eutrophication, the most widespread pressure affecting lakes. To be useful indicators, metrics must have a small measurement error relative to the eutrophication signal we want them to represent among lakes of different nutrient status. An understanding of variability in metric scores among different locations around a lake, or due to sampling and analytical variability can also identify how best this measurement error is minimised.

To quantify metric variability, we analyse data from a multi-scale field campaign of 32 European lakes, resolving the extent to which seven phytoplankton metrics (including chlorophyll *a*, the most widely used metric of lake quality) vary among lakes, among sampling locations within a lake and through sample replication and processing. We also relate these metrics to environmental variables, including total phosphorus concentration as an indicator of eutrophication.

For all seven metrics, 65–96% of the variance in metric scores was among lakes, much higher than variability occurring due to sampling/sample processing. Using multi-model inference, there was strong support for relationships between among-lake variation in three metrics and differences in total phosphorus concentrations. Three of the metrics were also related to mean lake depth. Variability among locations within a lake was minimal (<4%), with sub-samples and analysts accounting for much of the within-lake metric variance. This indicates that a single sampling location is representative and suggests that sub-sample replication and standardisation of analyst procedures should result in increased precision of ecological assessments based upon these metrics.

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For three phytoplankton metrics being used in the WFD: chlorophyll *a* concentration, the Phytoplankton Trophic Index (PTI) and cyanobacterial biovolume, >85% of the variance in metric scores was among-lakes and total phosphorus concentration was well supported as a predictor of this variation. Based upon this study, we can recommend that these three proposed metrics can be considered sufficiently robust for the ecological status assessment of European lakes in WFD monitoring schemes.

1. Introduction

The Water Framework Directive (WFD; EC, 2000) has revolutionised the assessment of anthropogenic impacts upon fresh- and coastal-transitional waters of the member states of the European Union. The central tenet of the Directive is that the assessment of human impacts on the surface water environment, rather than being based solely upon chemical parameters, should be based upon the attributes of key communities (Biological Quality Elements, BQEs). In turn, these BQEs should be sensitive to environmental pressures such as eutrophication and physical habitat modification.

For lakes, the phytoplankton has been identified as a key BQE to be used in ecological status assessment (Carvalho et al., 2012) and is already widely used as an important early-warning indicator of water quality changes. This is because of rapid replication rates (ensuring rapid responses to environmental stressors), direct sensitivity to physical and chemical environmental factors, and high diversity with species and/or functional types showing markedly variable responses to changes in the surrounding environment (Murphy et al., 2002; Reynolds, 2006). Furthermore, sampling of these communities is simple and inexpensive, with minimal impacts on co-existing biota. As a result of these features, phytoplankton was included in the WFD monitoring scheme as a relevant quality element for all surface water categories. As parameters to be studied, the WFD prescribes phytoplankton abundance, composition, and the frequency and intensity of blooms. While phytoplankton community composition and diversity are regulated by a complex interplay of intrinsic and extrinsic drivers such as climate, resource availability, patterns of competition and predation, and dispersal (Reynolds, 2006) they may also act as sensitive indicators of environmental pressures such as eutrophication as a result of increased nutrient loading (Kümmerlin, 1998; Padisák and Reynolds, 1998). Phytoplankton abundance, composition and the frequency/intensity of blooms are all considered to undergo changes along this pressure gradient (Carvalho et al., 2006, 2012). The WFD explicitly requires robust quantitative high-level indicators, or metrics, of the phytoplankton community which can be used to monitor the status of freshwater communities in the face of anthropogenic pressures, and identify improvements to ecological status as a result of management interventions. As part of the EU project WISER (http://www.wiser.eu/) a number of existing, or newly developed, metrics have been considered for this purpose (Mischke et al., 2010; Phillips et al., 2010).

However, there is a WFD requirement to assess the uncertainty in ecological status assessments when using such metrics (Hering et al., 2010). Phytoplankton communities show marked spatial heterogeneity within lakes, over a range of spatial scales, as a result of patterns in lake circulation and mixing, and spatial gradients in flushing, grazing and nutrient availability (Pinel-Alloul and Ghadouani, 2007). In addition, variation in phytoplankton metrics may occur due to differences in the analysts processing samples and sub-sampling procedures (Vuorio et al., 2007). Therefore, it is highly likely that the choice of sampling location within a lake and sample processing will affect the values of metrics based upon phytoplankton community data. Where metric values fall close to ecological status class boundaries, then these variations may fundamentally influence the overall assessment of a waterbody (Clarke et al., 2006b; Clarke, 2012). This has led to suggestions that results of ecological status classification should be given in terms of probabilities (Hering et al., 2010). Analyses of riverine macroinvertebrate community metrics have shown that the level of metric variability due to sampling may itself change with the ecological quality of a site (Clarke et al., 2002, 2006a). If the candidate phytoplankton metrics are to be used to distinguish between lakes of differing ecological quality, then among-lake variations in metric scores must be maximised and variation due to sampling/sample-processing minimised. This would give the best chance for the former to be related to differences in the intensity of key ecological pressures acting upon those lakes. It is also important to know whether these metrics become inherently more or less variable (uncertain) along this pressure gradient.

Until now, there has not been a formal assessment of the multiple sources of uncertainty that are inherent in phytoplankton metrics, even for widely adopted metrics, such as chlorophyll *a*. The statistical tools to make this assessment exist (Carvalho et al., 2006; Clarke and Hering, 2006; Clarke, 2012) but there has been a need for new data, collected according to a sampling design that allows distinction of different and independent sources of variability in metric scores. Knowledge of the relative importance of different sources of metric variability will guide the design of sampling campaigns aimed at ecological quality assessment. For example if a large component to the total variance in a metric is associated with sub-sampling of field samples, then the precision of assessments based upon this metric could be improved by analysing a larger number of sub-samples to derive a more representative average metric score for the lake. Herein, we present the results of a novel analysis of seven established phytoplankton community metrics based on a pan-European field sampling campaign of 32 lakes. Rigorous standardisation of sampling and sample processing procedures, along with a hierarchical sampling design targeted at uncertainty estimation, allow an entirely consistent analysis of sources of variation in phytoplankton metrics within and between European lakes. Specific objectives address the following questions; do candidate phytoplankton community metrics:

Q1: show greater variability among lakes than within lakes or as a result of differences in sample processing?
Q2: differ significantly along a gradient in lake nutrient status, after accounting for within-lake and sample-processing variation?
Q3: show systematic changes in their level of variability along gradients in physical, chemical and geographic attributes of lakes?

2. Materials and methods

2.1. Field survey

The analysis is based upon water samples collected from 32 lakes in eleven European countries during the spring and summer of 2009 (Table 1). These collectively represent lake types found within Member States and Norway comprising the Alpine, Northern, Central/Baltic and Mediterranean Geographical Intercalibration Groups (GIGs; WISE, 2008). All lakes were less than 10 km^2 in surface area, but varied widely in mean depth (3.5–34 m)

Table 1
Lakes sampled in the field campaign. GIG indicates the Geographical Intercalibration Group within which each lake falls: AL: Alpine; CB: Central/Baltic; M: Mediterranean; N: Northern. Only Chl-*a* data were available for lakes marked with an asterisk.

Lake	Country	GIG	Latitude (°N)	Longitude (°W)	Mean depth (m)	Maximum depth (m)	Altitude (m a.s.l.)	Total phosphorus ($mg\,m^{-3}$)	Alkalinity ($mequiv.\,L^{-1}$)
Nordborgsø	Denmark	CB	55.06	9.76	5.0	8.5	20	62.67	2.30
Fussingsø	Denmark	CB	56.47	9.88	12.6	31.0	15	45.67	1.50
Saadjärv	Estonia	CB	58.54	26.65	8.0	21.7	85	14.00	2.53
Viljandi	Estonia	CB	58.35	25.60	5.5	9.5	75	21.50	4.40
Sääksjärvi	Finland	N	62.17	25.73	9.3	15.2	121	12.00	0.23
Vuojärvi	Finland	N	62.41	25.94	4.4	10.2	91	35.5	0.54
Iso-Jurvo	Finland	N	62.60	25.93	8.6	29.6	139	8.00	0.06
Salagou	France	M	43.66	3.40	15.6	49.3	139	21.76	2.77
Caramany	France	M	42.74	2.59	14.5	36.0	170	26.80	2.96
Glindower See	Germany	CB	52.36	12.92	4.9	14.3	24	151.00	2.40
Grienericksee	Germany	CB	53.10	12.89	4.7	11.5	55	19.00	2.20
Roofensee	Germany	CB	53.11	13.02	9.0	19.1	59	18.00	2.00
Alserio	Italy	AL	45.78	9.21	5.0	8.0	243	24.00	2.34
Bidighinzu	Italy	M	40.56	8.66	7.5	21.8	330	65.00	2.24
Candia	Italy	AL	45.33	7.92	5.0	7.5	226	16.50	1.00
Monate	Italy	AL	45.80	8.66	18.0	34.0	266	8.50	0.88
Segrino	Italy	AL	45.83	9.27	3.5	8.0	374	12.50	2.23
Nøklevann	Norway	N	59.88	10.88	19.0	31.0	163	4.00	0.17
Longumvatnet	Norway	N	58.49	8.76	14.0	35.5	34	7.50	0.28
Temse	Norway	N	58.38	8.64	6.0	10.2	15	17.00	0.32
Rumian	Poland	CB	53.38	20.00	6.0	14.0	152	88.00	2.60
Lidzbarskie	Poland	CB	53.26	19.80	10.0	24.0	128	56.50	2.45
Kiełpińskie	Poland	CB	53.35	19.79	5.8	10.0	120	63.50	2.90
Vencías, Las	Spain	M	41.43	−3.96	8.0	14.8	869	20.46	2.43
Vega de Jabalón	Spain	M	38.76	−3.79	6.6	10.8	635	54.65	2.26
Arquillo de San Blas	Spain	M	40.36	−1.21	34.0	38.0	970	6.90	2.80
Fiolen*	Sweden	N	57.08	14.53	3.8	10.0	226	10.00	0.10
Skirösjön*	Sweden	N	57.36	15.38	5.2	8.0	146	45.33	0.63
Västra Solsjön*	Sweden	N	59.08	12.29	12.3	40.0	147	10.00	0.16
Loweswater	UK	N	54.58	−3.36	8.0	14.8	125	9.97	0.22
Grasmere	UK	N	54.45	−3.02	8.4	19.4	61	9.15	0.21
Rostherne Mere	UK	CB	53.35	−2.39	11.5	29.7	27	121.00	2.44

and altitude (15–970 m a.s.l.). The lakes also differed markedly in productivity/trophic status, with wide variation in alkalinity (0.06–4.40 $mequiv.\,L^{-1}$) and total phosphorus concentration (4–151 $mg\,m^{-3}$) at the time of sampling.

Each lake was sampled according to the same standardised protocol. The sampling design allowed the total variability in phytoplankton community structure, as indicated by a range of metrics, to be decomposed into a series of independent variance components, each indicating a potential source of uncertainty. The sampling design was as follows (Fig. 1):

(i) Within each lake, water samples were collected at three stations. These were above the deepest point of the open water zone, and at points representing the mean depth of the lake and a depth intermediate to the mean and maximum depths. This allowed quantification of within-lake spatial heterogeneity in phytoplankton community composition and metric scores, at the basin scale.
(ii) Two water samples were collected at each of the three stations. This allowed quantification of errors associated with repeated sampling at a specific location, as a result of smaller-scale heterogeneity in the phytoplankton community.
(iii) Each sample was sub-sampled in order to quantify variations in phytoplankton metric scores due to sub-sampling errors and differences in the analyst identifying and enumerating phytoplankton in the sub-samples. For analyses of phytoplankton composition, three sub-samples were collected from the first sample. Two of these were processed by the same analyst (revealing sub-sampling error), while the third was processed by a different analyst (to evaluate variability in metric scores due to differences in the approach used by different analysts). This is similar to the sampling design used by Clarke et al. (2002) to separate field replicate sampling variation from operator effects for river macroinvertebrate community metrics. From the second sample, only one sub-sample was collected, to allow comparison with metric scores derived from the first sample. Prior to microscopic examination an aliquot (sub-sub-sample) of each sub-sample was collected and put into a sedimentation chamber. Any variation associated with this sub-sub sampling is of course confounded with sub-sample variation in what follows, as no replication is available at this level of the hierarchy. For chlorophyll *a* (Chl-*a*) analysis, which followed a rigorously standardised spectrophotometric protocol, the effect of the analyst was not addressed and only two sub-samples were taken from the first sample to evaluate the sub-sampling error.

For reasons of cost the hierarchical sampling design was unbalanced at the within-station level: it was not feasible for both analysts to assess every replicate sub-sample of every sample at every station. However, by using appropriate statistical modelling approaches (see Section 2.5) it was possible to use this design to identify elements of field sampling campaigns that, through greater replication or standardisation, could be modified in order to improve the precision of ecological status assessments. For example, would the precision of such assessments be improved if we collected more samples, samples from more stations throughout the lake, processed more sub-samples or standardised taxonomic skills among analysts?

At each station, water samples were collected using an integrated tube sampler. If a lake was thermally stratified samples were taken from the euphotic layer (estimated as 2.5 × Secchi depth). When the water column was mixed samples were collected from throughout the whole water column, down to 0.5 m above the

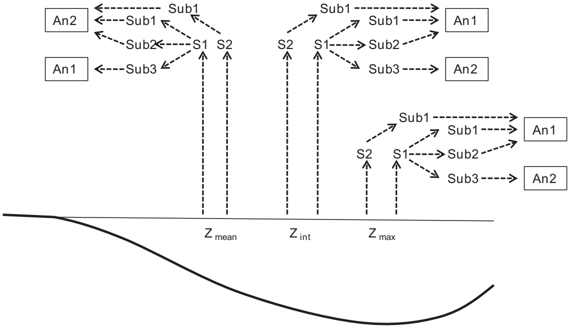

Fig. 1. The sampling design employed in each lake. Samples were collected from three stations, above the deepest point ($z_{\max}$), the mean depth (z_{mean}) and a depth intermediate between the maximum and mean depths (z_{int}). Two samples (S1 and S2) were collected at each station. At each station, three sub-samples (Sub1, Sub2, Sub3) were collected from sample 1 and one sub-sample from sample 2. In each case, two sub-samples from the first sample and the only sub-sample from the second sample were processed by one analyst (An1 or An2), while the third sub-sample from sample one was processed by a different analyst (An1 or An2).

sediment surface. Sub-samples were collected from each sample after thorough mixing. If immediate extraction of Chl-*a* samples was not possible, they were stored in a refrigerator or ice box for as short a time as possible. Samples for microscopic analysis were preserved using a solution of Lugol's iodine (final concentration approximately 0.5% by volume) and stored in the dark.

A further separate water sample was collected at the deepest point of each lake and analysed for alkalinity and concentrations of total phosphorus (TP). TP was measured following sulphuric acid–potassium persulphate digestion of unfiltered samples, according to Murphy and Reilly (1962). For some lakes multiple determinations of each variable were made and these were averaged prior to statistical analyses. Whilst data on total phosphorus concentrations were available for all lakes, alkalinity values were missing for some lakes and so representative values were necessarily derived from data collected under a parallel hierarchical macrophyte survey (Dudley et al., 2010). Secchi depth was also recorded at the deepest point of each lake.

In the following analyses TP concentrations were used to indicate where the sampled lakes fell on a gradient of nutrient enrichment. Latitude, longitude and altitude of each lake were also included, as proxies for broad climatic gradients that might impact upon phytoplankton communities via effects on lake physical processes. Alkalinity and mean lake depth were included in the study as they are the primary determinants of the fundamental lake "types" described in the WFD. Different combinations of high-low alkalinity and mean depth have been used to categorise these lake "types". This captures the fact that lakes show natural variability in their phytoplankton communities, due to their catchment setting and morphometry, irrespective of differences in nutrient enrichment (Pinel-Alloul et al., 1990).

2.2. Sample processing for Chl-a analysis

A fixed volume of water, dependent on the amount and type of seston present in each lake, was filtered through 47-mm GF/F filters and the filter was placed into 10 ml of 96% ethanol for pigment extraction at 4 °C for 24 h. Analysis then followed the International Standard Method ISO 10260 (1992).

2.3. Sample processing for microscopic examination of phytoplankton

Microscopic examination of phytoplankton followed the same standardised protocol across Member States, and was based upon procedures outlined in CEN 15204 (2006), National Rivers Authority (1995) and Brierley et al. (2007). Briefly, samples were examined in sedimentation chambers with an inverted microscope, according to the Utermöhl technique (Utermöhl, 1958). For each sample, a low magnification (40× or 100×) whole chamber count, two intermediate magnification (200× or 250×) transect counts and 50–100 field of view counts at high magnification (400× or greater) were completed. Phytoplankton taxa were identified to the highest possible level. Counts of each taxon were converted to biovolumes by measuring cell/colony dimensions and approximating each taxon to a simple geometric shape (Brierley et al., 2007). Phytoplankton cells were measured using eye-piece graticules, after calibration with a stage micrometer. All subsequent phytoplankton metric calculations were based upon the biovolume data.

2.4. Phytoplankton metrics

Seven candidate phytoplankton metrics are considered herein, a brief description of which is given below. Full details on each metric are provided in Phillips et al. (2010) and Mischke et al. (2010). These metrics have been categorised according to whether they relate to phytoplankton abundance or composition, or to features of blooms.

1. Chl-*a* concentration (abundance metric, in mg m^{-3}) is a measure of phytoplankton abundance, commonly used to represent the ecological status of a lake with respect to eutrophication pressures.
2. Phytoplankton Trophic Index (PTI, composition metric). This has been developed, using an independent data set, from the "trophic scores" of phytoplankton taxa along a eutrophication gradient (Phillips et al., 2010). After a Canonical Correspondence Analysis (CCA) constrained by total phosphorus, taxa optima on the first ordination axis were derived indicating the TP concentration for the mean occurrence of each taxon. For each sub-sample, PTI was calculated as the weighted average of these taxa optima, where

the weighing factor is the proportional biovolume of each taxon. The PTI increases with increasing lake trophic state.

3. Size Phytoplankton Index (SPI, composition metric). The phytoplankton taxa within a sub-sample are grouped into a series of size categories, each one encompassing a doubling of cell biovolume e.g. $\leq 0.5\,\mu\text{m}^3$, $0.5–1.0\,\mu\text{m}^3$, $1.0–2.0\,\mu\text{m}^3$, $2.0–4.0\,\mu\text{m}^3$ etc. (Kamenir and Morabito, 2009). The SPI is then calculated as a function of the size categories and "trophic scores"/"indicator values" for those categories (Phillips et al., 2010). Trophic scores indicate the position of a size class along the trophic spectrum and indicator values estimate the "power" of each size class as a biotic indicator. The SPI tends to increase with increasing lake trophic state, due to a shift towards increased dominance of larger, rather than smaller, phytoplankton (Phillips et al., 2010).
4. Morpho-Functional Group Index (MFGI, composition metric). The phytoplankton taxa within a sub-sample are grouped into a series of categories ("Morpho-Functional Groups") based upon their morphological attributes e.g. presence/absence of flagella, colonial or unicellular, large or small size (Salmaso and Padisak, 2007). The MFGI is then calculated as a function of the Morpho-Functional Groups and the "trophic scores"/"indicator values" for those groups (Phillips et al., 2010). The MFGI tends to increase with increasing lake trophic state, due to an increase in the dominance of colonial cyanobacteria, large diatoms/chlorophytes/conjugatophytes, and unicellular/colonial chlorococcales (Phillips et al., 2010).
5. Functional Traits Index (FTI, composition metric). This is the arithmetic mean of the SPI and MFGI, and thus combines information on both the size spectrum and morpho-functional traits of the phytoplankton community. Phillips et al. (2010) recommend the use of the FTI for water quality assessment.
6. Evenness metric (bloom metric). This is Pielou's evenness index, which expresses the ratio between the Shannon diversity of a sub-sample and the maximum possible value of the Shannon diversity index (Pielou, 1969, 1975). Evenness has been shown to decline under bloom conditions in more productive lakes, due to an increase in the dominance of a small number of tolerant species with high growth rates (Mischke et al., 2010).
7. Cyanobacterial abundance (bloom metric). This is the total cyanobacterial biovolume ($\text{mm}^{-3}\,\text{L}^{-1}$) within a sub-sample, and is expected to increase with increasing lake trophic status (Mischke et al., 2010).

2.5. Statistical modelling

Q1: Do metrics show greater variability among lakes than within lakes or as a result of differences in sample processing?

These analyses aimed to resolve whether metrics had the potential to be sensitive to variations in the intensity of environmental pressures acting at the lake level. This potential was to be estimated by the relative size of the among-lake variance in metric values and the within-lake variance components. Furthermore, we aimed to identify aspects of sampling campaigns that might be modified to improve the precision of ecological status assessments (by comparison of components of within-lake metric variance). A nested random effects statistical model structure was used to emulate the hierarchical nature of the sampling campaign. In this structure, lake was nested within country, sampling station within lake, sample within station, and sub-sample within sample was modelled implicitly as the lowest level "residual" variability. Each analyst could not process sub-samples from all samples or all stations or all lakes, even though some analysts processed samples from more than one country. Therefore the model factor 'Analyst' was included (except for analyses of Chl-*a* concentration) as a random effect which was, in mixed model technical terms, partially crossed with the other factors and variables. However, it was still possible for the mixed model functions in R to estimate the separate variance components. These variance components are (as usual in most mixed models) estimates of the average size of that source of variance averaged over the other factors; it was not feasible to investigate interactions in factor variance components. Our variance estimates provide the best available information on the relative typical (i.e. average) sizes of the different sources of metric total and within-lake variance. More formally, the model structure can be denoted:

$$m_{\text{austlc}} = \beta_0 + \nu_{\text{ustlc}} + \nu_{\text{stlc}} + \nu_{\text{tlc}} + \nu_{\text{lc}} + \nu_{\text{c}} + \nu_{\text{a}} + e_{\text{austlc}} \tag{1}$$

where m_{austlc} is the value of the metric m for analyst a, for sub-sample u, in sample s, in station t, in lake l, in country c. Thus, m_{austlc} is the sum of a series of components that each contribute to the total metric variation about an overall mean β_0. The components of metric variation are modelled as independent, normally distributed, variance components for analyst ($\sigma_{\text{a}}^2 = \text{Var}(\nu_{\text{a}})$), sub-sample ($\sigma_{\text{u}}^2 = \text{Var}(\nu_{\text{ustlc}})$), sample ($\sigma_{\text{s}}^2 = \text{Var}(\nu_{\text{stlc}})$), station ($\sigma_{\text{t}}^2 = \text{Var}(\nu_{\text{tlc}})$), lake ($\sigma_{\text{l}}^2 = \text{Var}(\nu_{\text{lc}})$) and country ($\sigma_{\text{c}}^2 = \text{Var}(\nu_{\text{c}})$).

Sub-sampling variance, being the lowest level in the hierarchical sampling, is estimated implicitly by the fitted model residual variance. Having fitted random effects model Eq. (1) to our data, the relative sizes of the estimated variance components were used to determine the levels of the sampling hierarchy at which each metric's values showed the greatest variability. In particular, the total variance among all lakes is $\sigma_{\text{A}}^2 = \sigma_{\text{c}}^2 + \sigma_{\text{l}}^2$, the average total variance within lakes is $\sigma_{\text{W}}^2 = \sigma_{\text{t}}^2 + \sigma_{\text{s}}^2 + \sigma_{\text{u}}^2 + \sigma_{\text{a}}^2$ and therefore the total variance in all metric values is $\sigma_{\text{T}}^2 = \sigma_{\text{A}}^2 + \sigma_{\text{W}}^2$. The percentage of the total metric variance (σ_{T}^2) occurring at each level in the sampling hierarchy was calculated from these variance parameter estimates (e.g. percentage among lakes $= 100\sigma_{\text{A}}^2/\sigma_{\text{T}}^2$). The hierarchical and crossed random effect models of Eq. (1) were all fitted to the unbalanced datasets using the standard Restricted Maximum Likelihood (REML) method of model fitting in order to give unbiased estimates of the random effects. Whenever subsequent truly mixed effects models with different fixed effects structures (i.e. different combinations of predictors) were compared, models were re-fit using the Maximum Likelihood (ML) method of model fitting (Crawley, 2007). Unlike many traditional ANOVA techniques, REML fitting of models with fixed and random (i.e. variance component) hierarchical and/or crossed factors can cope with unbalanced datasets with unequal replication at some levels, providing the sampling design gives some subsets of information within the data which enable the REML algorithm to distinguish and estimate each variance component (Crawley, 2007; Clarke, 2012). This is the case for our lake sampling design.

Q2: Do metrics differ significantly along a gradient in lake nutrient status, once accounting for within-lake and sample-processing variation?

We investigated whether relationships between phytoplankton metrics and measured morphometric, chemical and geographical features of lakes could be detected against the "background" of methodological variation resolved in stage 1 of the analysis. It is convenient here to refer to the pure random effects models as the "null model" in terms of having no environmental predictor variables. These pure random effect null models were augmented to include the measured environmental variables (TP, alkalinity, mean lake depth, latitude, longitude and altitude) as fixed effects and fitted as linear mixed effects models. Secchi depth was omitted since the direction of causality between this variable and the phytoplankton community is equivocal. In order to explicitly take into account uncertainty and parameter bias due to model selection, arising since both model formulation and parameters are estimated from the sample data, we used multi-model inference (Burnham and Anderson, 2002). For each metric, a "global" linear mixed effects model was constructed containing the same within-lake random effects structure and all the predictor variables (alkalinity, latitude, longitude, altitude, mean depth and TP). These environmental predictor variables have single values for each lake and therefore can only explain aspects of the null model total among lake variance. Models were then run including all possible subsets of these variables, and ranked by the Akaike Information Criterion (AIC). A subset of top models, receiving progressively lower levels of statistical likelihood support from the data, was determined by finding the model with the most optimal combination of environmental predictor variables (i.e. lowest AIC value) and other candidate models with AIC values differing from this "top" model by ≤ 4 (Burnham and Anderson, 2002; Zuur et al., 2009). Model-averaged parameters (with 95% confidence intervals) were calculated using the parameter estimates in models within this top model subset. Maximum Likelihood (ML) estimation was used when fitting models with different combinations of predictor variables.

To estimate the proportion (Prop_e) of the total among-lake variation in metric scores that could be "explained" by the selected environmental variables we compared the residual among-lake metric variance ($\sigma^2_{\text{l,fitted}}$) estimated by the model with the most optimal combination of environmental predictors (i.e. lowest AIC value), with the total among-lake variance ($\sigma^2_{\text{l,null}}$) estimated in the corresponding null model (i.e. with no environmental predictors) thus:

$$\text{Prop}_\text{e} = 1 - \left(\frac{\sigma^2_{\text{l,fitted}}}{\sigma^2_{\text{l,null}}}\right) \tag{2}$$

$\sigma^2_{\text{l,fitted}}$ therefore represents the among lake variation in a metric that cannot be explained by the predictor variables in the top fitted model, while $\sigma^2_{\text{l,null}}$ represents the total among-lake variation in that metric. This approach is conceptually similar to that employed by Clarke et al. (2006b) to compare variance components of invertebrate metric scores gathered from hierarchical sampling designs. Since $\sigma^2_{\text{l,fitted}}$ and $\sigma^2_{\text{l,null}}$ are themselves estimated parameters, and therefore each have a level of uncertainty associated with them, Prop_e must also be considered an estimate with a level of uncertainty. Herein, we do not calculate the uncertainty associated with the estimate of Prop_e and merely use the values as broadly indicative of the explanatory power of the selected predictor variables.

During the model fitting exercise, it was necessary to simplify the random effects structure to retain only crossed effects of "Lake" and "Analyst". Preliminary analyses revealed that the inclusion of the full random effects hierarchy when comparing models with different fixed effect structures resulted in convergence errors, due to high levels of model complexity. Furthermore, fitting of null models (see Section 3) demonstrated that the omitted random effects consistently accounted for little of the total metric variance.

Q3: Do metrics show systematic changes in their level of variability along gradients in physical, chemical and geographic attributes of lakes?

As a final step in the analysis, we examined whether metric scores became more or less variable as a function of between-lake changes in predictor variables, such as TP concentration or mean depth. If metric variability is not constant across lakes with different environmental attributes, then this could mean that sampling campaign design (in terms of sample replication, level of standardisation) might also need to vary between lakes. This was done by adding additional variance structures to previously fitted models that allowed for changes in residual metric variability as a function of the measured environmental predictors. For each metric, we worked with the model with the most optimal combination of environmental predictor variables (lowest AIC) and added these extra variance structures based upon each of the predictors within this top model. These structures took the form (Zuur et al., 2009):

$$\text{var}(\varepsilon) = \sigma^2 e^{2\delta x} \tag{3}$$

so that the residual variance [$\text{var}(\varepsilon)$] was allowed to vary as an exponential function of explanatory variable x and the estimated parameter δ. For each metric, we compared the top fitted model with none of these additional variance structures, with models including structures that allowed for residual "spreading" with respect to each of the explanatory variables present in the top model. So, for example, if the top model for a particular metric included predictors x_1 and x_2, we compared models (i) without structures to capture spreading of residual metric variation, (ii) with residual spreading as a function of x_1, (iii) with residual spreading as a function of x_2 and, (iv) with residual spreading as a function of x_1 and x_2. The most optimal solution was found by comparing the AIC values of each of these models, after fitting using REML estimation.

All analyses were conducted using the *base*, *gplots*, *lme4*, *MuMIn* and *nlme* packages of R version 2.13.1 (Pinheiro et al., 2010; Warnes, 2010; Barton, 2011; Bates et al., 2011; R Development Core Team, 2011) and the Variance Estimation and Precision (VEPAC) package of STATISTICA 8.0 (StatSoft. Inc. 1984–2007).

3. Results

3.1. Sources of metric variability

Exploratory analyses of the metrics data revealed that Chl-*a* and total cyanobacterial biovolume were positively skewed and so, prior to statistical modelling, we $\log_{10}(x + 0.1)$ transformed these metrics in order to reduce the potential influence of the minority of relatively high values in the dataset. Results from null models of all seven metrics (Table 2) suggest that the majority of metric variance occurred between lakes. The Country (σ^2_c) and Lake (σ^2_l) random effects together accounted for between 65% and 96% of the total metric variance, with the majority of this variability found among lakes rather than among Countries. This suggested that metric scores varied more among lakes (which were distributed along a pressure gradient) than within lakes. It is noteworthy that the Analyst (σ^2_a) and Error (sub-sample level, σ^2_u) variance components were the major contributors to the within-lake component. Therefore, metric variation due to analyst differences and sub-sampling exceeded variation due to within-lake spatial heterogeneity in the phytoplankton.

3.2. Relationships between metrics and lake characteristics

The seven metrics varied widely in their relationship to total phosphorus concentration; highlighting different strengths of the metrics for indicating the primary among-lake pressure gradient of nutrient enrichment (Fig. 2). Visual inspection of the data suggested that metric-phosphorus relationships were strongest for the abundance metric Chl-*a*, PTI composition metric and total cyanobacterial biovolume bloom metric. This was confirmed by the structure of the most optimal models for these metrics, which included fixed effects of total phosphorus concentration and mean lake depth (Table 3). Delta AIC values for these models, all ≥ 13.5, indicated a significant improvement in model fit compared to (null) models with no predictors. Therefore a detectable increase in all three of these metrics was observed in lakes with higher phosphorus concentrations, and in shallower lakes. This was observed despite methodological uncertainty arising due to sampling and sample

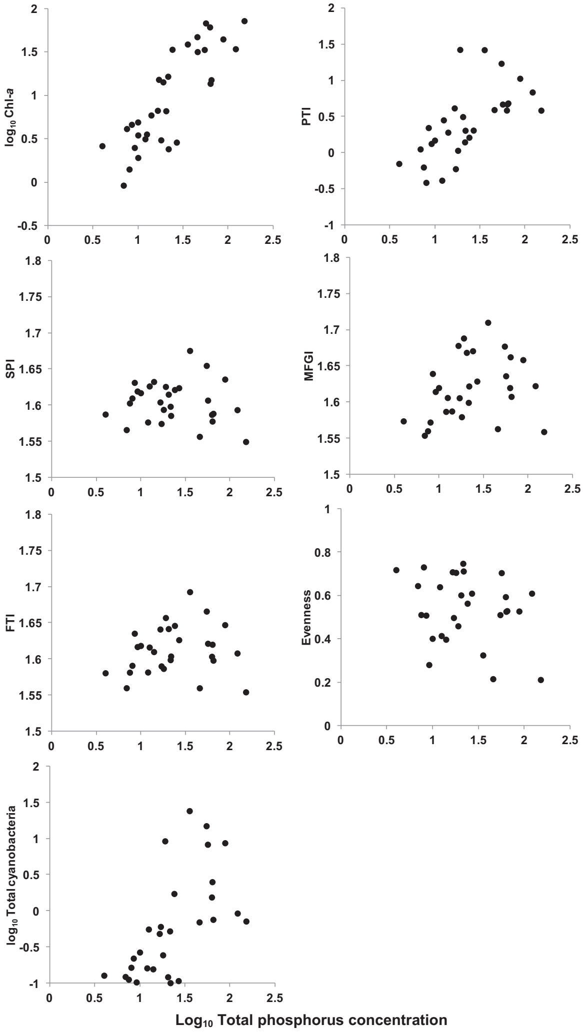

Fig. 2. Scatterplots of lake-averaged values of the seven phytoplankton metrics against $\log_{10}$ total phosphorus concentration.

Table 2
Proportions of metric variance at different levels in the sampling hierarchy, for null models of the seven different metrics. Total among: Country + Lake; Total within: Station + Sample + Analyst + Error (sub-sample). Models fitted using REML estimation.

Metric	Country	Lake	Station	Sample	Analyst	Error (sub-sample)	Total within	Total among
Log_{10} Chl-*a*	0.00	0.96	0.01	0.01	–	0.02	0.04	0.96
PTI	0.00	0.88	<0.01	0.00	0.04	0.07	0.12	0.88
SPI	0.00	0.65	0.03	0.00	0.19	0.13	0.35	0.65
MFGI	0.00	0.86	0.02	<0.01	0.05	0.08	0.14	0.86
FTI	0.00	0.81	0.02	0.00	0.09	0.08	0.19	0.81
Evenness	0.00	0.69	0.04	0.00	0.17	0.10	0.31	0.69
Log_{10} total cyanobacteria	0.09	0.86	0.01	0.00	0.02	0.03	0.06	0.94

Table 3
Relationships between metrics and environmental drivers, in the most optimal linear mixed-effects models for each metric. Shown are the number of estimated model parameters (k), the predictors present in the model, the difference in AIC between the most optimal model and the corresponding null model (ΔAIC_{null}) and the Akaike weight; a measure of the relative level of support for the most optimal model, compared to other candidate models, given the data. For the Akaike weight, values close to 1 indicate overwhelming support for the corresponding model, while lower values indicate the presence of other models with similar levels of support. See Figs. 4–6 for model averaged estimates of the parameters for each metric-lake attribute relationship, based upon all models with similar levels of support for each metric. Note that k includes the global intercept and parameters for both the fitted predictors and the random effects variances. For each predictor, the sign of the corresponding relationship is given as positive (+) or negative (−). Models fitted using ML estimation.

Metric	k	Predictors	ΔAIC_{null}	Akaike weight
Log_{10} Chl-*a*	6	Log_{10} Mean lake depth (−) Log_{10} total phosphorus (+) Latitude (+)	35.5	0.12
PTI	7	Log_{10} Mean lake depth (−) Log_{10} total phosphorus (+) Log_{10} Altitude (+)	13.5	0.11
SPI	6	Log_{10} Mean lake depth (−) Log_{10} Altitude (+)	2.0	0.12
MFGI	6	Log_{10} Mean lake depth (−) Log_{10} Altitude (+)	10.0	0.12
FTI	6	Log_{10} Mean lake depth (−) Log_{10} Altitude (+)	9.0	0.19
Evenness	6	Log_{10} total phosphorus (−) Alkalinity (+)	2.3	0.06
Log_{10} total cyanobacteria	6	Log_{10} Mean lake depth (−) Log_{10} total phosphorus (+)	16.2	0.13

processing. Top models for the three remaining composition metrics (MFGI, SPI and FTI) suggested that all three metrics were higher in shallow lakes and in lakes at higher altitudes. While ΔAIC values ≥ 9 indicated that top models were considerably better supported than null models for MFGI and FTI, this was not the case for SPI ($\Delta AIC = 2$). Similarly the top model for the evenness metric, suggestive of a reduction in this bloom metric with increasing phosphorus concentration and at low alkalinity, represented only a modest improvement on a model with no fitted predictor variables ($\Delta AIC = 2.3$). The majority of the among-lake variance in Chl-*a* concentration was accounted for by the fitted predictors in the top model, as indicated by $Prop_e$ (Table 3 and Fig. 3). For total cyanobacteria and the PTI metric, the amount of among-lake variance "explained" by the fitted predictors in the top model was less, at 43–47%, while for the remaining metrics <40% of the among lake metric variance was accounted for in the fitted models.

However, relatively low Akaike weights for the top models for all metrics (0.06–0.19, Table 3) suggested that the top models did not receive overwhelming support within each model set and that, for each metric, other candidate models collectively received likelihood support from the data. We used a multi-model inference approach to calculate model averaged parameters for the relationships between each metric and the selected environment predictors. This confirmed strong support for an increase in Chl-*a* concentration, PTI and total cyanobacterial biovolume at high phosphorus concentrations, despite methodological metric variation (positive slope parameters, Figs. 4–6). Across many of the metrics there was a support for an effect of mean lake depth on

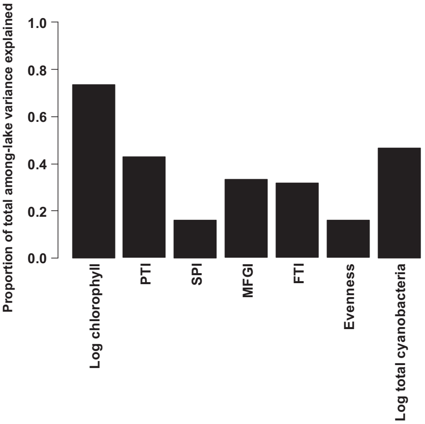

Fig. 3. The proportion ($Prop_e$, Eq. (2)) of the total among-lake variance in metric scores "explained" in top models, with the most optimal combination of environmental predictor variables. REML estimation used in model fitting.

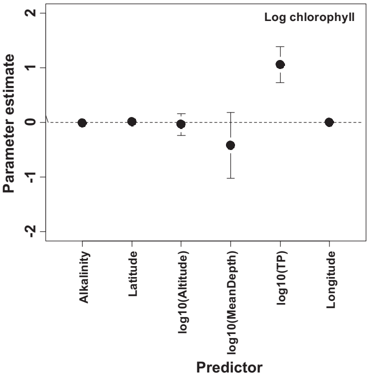

Fig. 4. Model-averaged slope parameters for the relationships between the modelled environmental predictors and the phytoplankton abundance metric ($\log_{10}$ Chl-*a* concentration). Filled circles indicate the model-averaged slope parameter estimate for each metric–predictor relationship, and whiskers indicate the 95% confidence interval for the estimate. Dashed horizontal line indicates zero. ML estimation used in model fitting.

metric scores. With the exception of evenness, all metrics decreased with an increase in mean lake depth i.e. a negative slope parameter for their relationship (Figs. 4–6). For MFGI, FTI and total cyanobacterial biovolume there was strong support for this effect, while for the remaining metrics support for this effect was relatively weaker. With the exception of Chl-*a* concentration there was also consistent, though weak, support for an effect of altitude on metric scores. Tables summarising the model sets used to derive these averaged parameters for each metric can be found in Supplementary Information.

3.3. *Changes in metric variability as a function of among-lake variations in physical, chemical and geographical attributes*

For all but one of the metrics (FTI) the fit of the most optimal statistical model (from Table 3) was improved by allowing residual metric values to vary as a function of certain explanatory variables (phosphorus concentration, lake depth, Table 4). In general, this supported the idea that metric scores were more variable in some limnological contexts than in others. In the case of SPI and MFGI the difference in AIC between models including and excluding these structures (5.7 and 2.7 respectively) was much lower than for Chl-*a* concentration, PTI, evenness and total cyanobacteria biovolume (20.9–44.8). While residual Chl-*a* concentrations and evenness appeared to become more variable at lower phosphorus concentration (negative δ estimates), cyanobacterial biovolume showed the reverse pattern; with residuals being more variable at higher phosphorus concentrations (positive δ estimate). Residual Chl-*a* concentrations also became more variable at greater mean

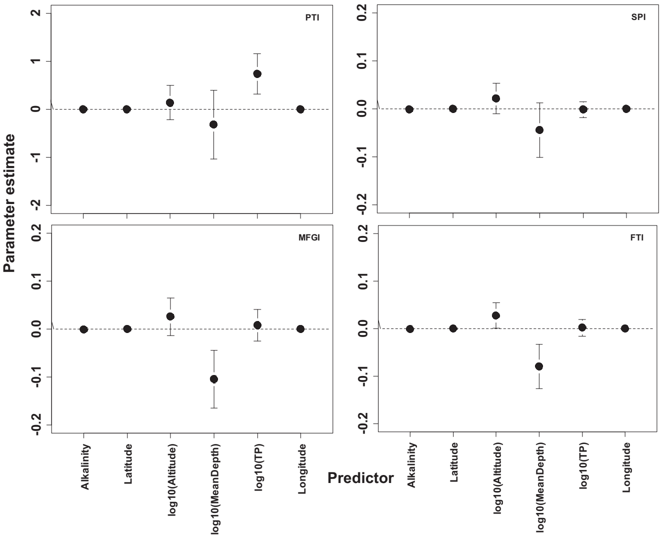

Fig. 5. Model-averaged slope parameters for the relationships between the modelled environmental predictors and the four phytoplankton composition metrics. Filled circles indicate the model-averaged slope parameter estimate for each metric–predictor relationship, and whiskers indicate the 95% confidence interval for the estimate. Dashed horizontal line indicates zero. ML estimation used in model fitting.

Table 4
Models examining metric variability as a function of environmental drivers. AIC comparison of the most optimal linear mixed-effects models for each of the seven phytoplankton metrics (see Table 3) when including/excluding variance structures to account for changes in metric variability (residual metric variance) as a function of the fitted predictors. Shown are the predictors that residual variability is modelled as a function of (predictor), the estimated delta parameter for the exponential function describing the relationship between residual variance and the named predictor (δ) and the AIC for each model. For each metric, the most optimal model is indicated in bold. Models fitted using REML estimation.

Metric	Model No.	Predictor	δ	AIC
Log$_{10}$ Chl-*a*	1	None	–	−195.1
	2	Log$_{10}$ Mean lake depth	0.88	−205.7
	3	Log$_{10}$ total phosphorus	−0.70	−230.7
	4	Latitude	0.02	−198.3
	5	**Log$_{10}$ Mean lake depth**	**0.57**	**−233.8**
		Log$_{10}$ total phosphorus	**−0.65**	
	6	Log$_{10}$ total phosphorus	−0.70	−228.8
		Latitude	<0.01	
	7	Log$_{10}$ Mean lake depth	0.75	−205.3
		Latitude	0.01	
	8	Log$_{10}$ Mean lake depth	0.57	−231.8
		Log$_{10}$ total phosphorus	−0.66	
		Latitude	<−0.01	
PTI	1	None	–	−138.7
	2	Log$_{10}$ Mean lake depth	−0.44	−144.9
	3	Log$_{10}$ total phosphorus	−0.40	−147.9
	4	Log$_{10}$ Altitude	0.66	−180.4
	5	Log$_{10}$ Mean lake depth	−0.53	−156.3
		Log$_{10}$ total phosphorus	−0.43	
	6	Log$_{10}$ total phosphorus	−0.11	−179.0
		Log$_{10}$ Altitude	0.62	
	7	**Log$_{10}$ Mean lake depth**	**−0.39**	**−183.5**
		Log$_{10}$ Altitude	**0.65**	
	8	Log$_{10}$ Mean lake depth	−0.43	−183.1
		Log$_{10}$ total phosphorus	−0.17	
		Log$_{10}$ Altitude	0.59	
SPI	1	None	–	−1682.9
	2	Log$_{10}$ Mean lake depth	0.19	−1682.8
	3	**Log$_{10}$ Altitude**	**0.23**	**−1688.6**
	4	Log$_{10}$ Mean lake depth	−0.06	−1686.7
		Log$_{10}$ Altitude	0.25	
MFGI	1	None	–	−1760.6
	2	**Log$_{10}$ Mean lake depth**	**−0.43**	**−1763.3**
	3	Log$_{10}$ Altitude	−0.12	−1760.7
	4	Log$_{10}$ Mean lake depth	−0.42	−1763.3
		Log$_{10}$ Altitude	−0.12	
FTI	**1**	**None**	**–**	**−1854.2**
	2	Log$_{10}$ Mean lake depth	−0.15	−1853.1
	3	Log$_{10}$ Altitude	0.01	−1852.2
	4	Log$_{10}$ Mean lake depth	−0.19	−1851.3
		Log$_{10}$ Altitude	0.04	
Evenness	1	None	–	−621.7
	2	**Log$_{10}$ total phosphorus**	**−0.51**	**−642.6**
	3	Alkalinity	−0.13	−633.8
	4	Log$_{10}$ total phosphorus	−0.42	−641.6
		Alkalinity	−0.04	
Log$_{10}$ total cyanobacteria	1	None	–	−171.6
	2	Log$_{10}$ Mean lake depth	−0.52	−177.1
	3	**Log$_{10}$ total phosphorus**	**0.71**	**−214.4**
	4	Log$_{10}$ Mean lake depth	−0.23	−214.0
		Log$_{10}$ total phosphorus	0.67	

lake depths (positive δ estimate), while residual PTI and MFGI became less variable in these deeper lakes (negative δ estimates). Both residual SPI and PTI became more variable in higher altitude lakes (positive δ estimates). The model selection process, using multi-model inference to find the most well supported predictors of between-lake variations in each of these metrics, was repeated after including these additional variance structures, although the final parameter estimates for the fixed effects were affected minimally (results not shown).

4. Discussion

Comparison of sources of variation in metric scores showed that among-lake variation was by far the dominant component of variability for all seven metrics. This suggested that, all other things being equal, the capability of the metrics to respond to pressures acting at the lake level should not be limited by sampling variation arising from within-lake spatial variation. Differences in locations around a lake, or sampling and analytical variability,

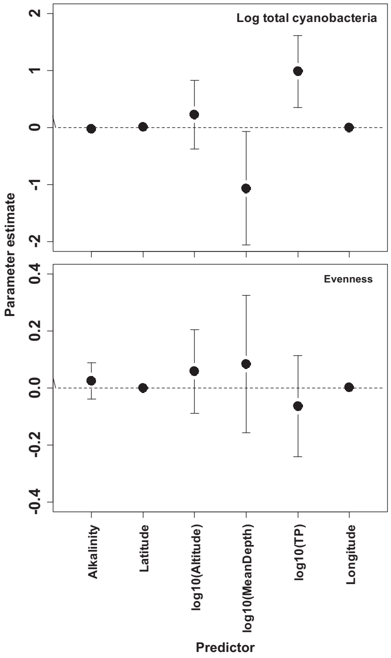

Fig. 6. Model-averaged slope parameters for the relationships between the modelled environmental predictors and the two phytoplankton bloom metrics. Filled circles indicate the model-averaged slope parameter estimate for each metric–predictor relationship, and whiskers indicate the 95% confidence interval for the estimate. Dashed horizontal line indicates zero. ML estimation used in model fitting.

only accounted for a relatively small proportion of the variance in metric scores. These results are especially true for the three candidate phytoplankton metrics adopted by many European Member States: chlorophyll, PTI, and cyanobacterial abundance. For these metrics, 88% or more of the variance in metric scores occurred at the among-lake level of the sampling hierarchy. Between-analyst and between sub-sample variation accounted for most of the within-lake variation. Little variation was attributable to within-lake spatial heterogeneity i.e. differences among lake stations and repeated sampling from each station. This was despite the fact that lake stations were treated as "random" in the modelling approach even though they were selected: which should lead to an over-estimate of the station-to-station variability. Lake stations were selected to represent water columns of mean depth or greater in the present study, and it is plausible that a greater station level effect might have been observed if stations had been selected from a wider range of water depths and/or including from outflow or edge samples. Processes in inshore regions of lakes, such as flushing by influent waters (Mackay et al., 2011), enhanced zooplankton grazing facilitated by structurally complex macrophyte refugia (Schriver et al., 1995) or chemical interactions with macrophytes (Wium-Andersen et al., 1982; Jasser, 1995) may generate differences in phytoplankton communities between these areas and the deeper, open-water, zone. If sampling stations are distributed among the multiple interconnected basins of some lakes, it is conceivable that more station-level metric variation would be observed, but any resulting uncertainty can be minimised by using the facility within the WFD to treat such basins as separate water bodies.

Though within-lake metric variance was relatively low compared to among-lake variance, the relative magnitude of the components of the former indicates potential areas for the refinement of field sampling campaigns, which could improve the precision of ecological assessments of lakes. Increasing the number of open water sampling stations visited, or the number of samples collected at each station, would do little to improve the precision of ecological assessments based upon these phytoplankton metrics. The representativeness of ecological assessments based upon the metrics, with respect to the impact of lake level pressures, could instead be improved by processing greater numbers of replicate sub-samples from each sample and standardising either (i) analyst identity for samples from different lakes, or (ii) taxonomic skills and laboratory procedures among different analysts (e.g. Vuorio et al., 2007). In fact, the majority of analysts had attended workshops that aimed to standardise sample processing techniques and algal identification/enumeration. Furthermore, counters followed standard procedures based upon ISO 10260 (1992), CEN 15204 (2006), National Rivers Authority (1995) and Brierley et al. (2007). It may therefore be that analyst variability was lower than normal. Nevertheless, the results of this study indicate that rigorous standardisation of sample mixing and sedimentation protocols, as well as of taxonomic procedures, can help minimise sampling and analytical variability. In turn, this would permit more meaningful comparisons of ecological status between different lakes.

We should also note that, in the current sampling design, the effects of analyst and sub-sampling variation were crossed. Therefore, it was not possible to compare results derived from different analysts counting exactly the same fields of view from the same sub-sample, or the same analyst counting different fields of view from the same sub-sample. Furthermore, the sub-samples were actually sub-sub-sampled prior to microscopic examination; another source of potential metric variability that was unquantifiable in this study. It is, therefore, difficult to truly isolate the effect of analyst variation upon metric scores in this study. Future studies targeting sources of variation arising from sampling processing and analyst variation alone would allow more accurate assessment of the extent to which metrics are influenced by these factors.

Taking a multi-model inference approach, there was strong support for a response of metric scores to phosphorus concentrations for three of the seven metrics: Chl-*a* concentration, PTI and total cyanobacterial biovolume. This would suggest that these proposed metrics are indeed responsive to the eutrophication pressure gradient apparent across the lakes sampled. Furthermore, this would suggest that such relationships are detectable, despite metric variation arising due to sampling/sample processing decisions. These relationships suggested a general increase in Chl-*a* concentration and cyanobacterial abundance with increased phosphorus availability. The finding that Chl-*a* concentration increases with lake phosphorus concentration is consistent with the idea that the availability of this nutrient determines the supportive capacity of a lake system for phytoplankton biomass (Reynolds, 2006); a relationship embodied in the results of previous empirical (Dillon and Rigler, 1974; Schindler, 1978; Phillips et al., 2008; Sondergaard et al., 2011), and process-based modelling studies (Elliott et al., 2006). Indeed, between lake variations in total phosphorus concentration have been found to be more powerful predictors of phytoplankton biomass than similar variations in total nitrogen concentrations

(Brown et al., 2000; Phillips et al., 2008; Sondergaard et al., 2011), though this difference may be dependent on the relative availability of these two nutrients (McCauley et al., 1989; Brown et al., 2000; Phillips et al., 2008). The observation of increased cyanobacterial biomass at higher phosphorus concentrations is similarly consistent with the findings of previous studies (Smith, 1985; Watson et al., 1997; Elliott et al., 2006). PTI scores were also higher in lakes with higher phosphorus concentrations, as shown by Phillips et al. (2010), due to increases in the biomass of cyanobacteria, and some members of the Chlorophyceae and Bacillariophyceae.

Comparison of results across metrics also revealed consistent support for an effect of mean lake depth, particularly for FTI, MFGI and total cyanobacterial biovolume (though there was also weaker support for this effect for PTI, SPI and Chl-*a* concentration). Mean lake depth acts as a surrogate for a variety of physical and chemical attributes, such as maximum depth, the likelihood of thermal stratification, flushing rate, underwater light availability and the likelihood of internal nutrient loading (Kalff, 2002). Furthermore, inverse relationships between among-lake variations in lake depth and Chl-*a* concentrations/cyanobacterial abundance have been noted in a number of previous studies (Pridmore et al., 1985; Smith, 1985; Smith et al., 1987; Phillips et al., 2008). The fact that lake depth covaries with so many other physical and chemical determinants of phytoplankton production, renders hypothesising the mechanism behind the observed relationships difficult. That depth and total phosphorus concentration co-occur as independent predictors in the top models for Chl-*a* concentration and total cyanobacterial biovolume would suggest that depth offers "unique" explanatory power for these phytoplankton metrics compared to phosphorus availability. The higher observed Chl-*a* concentrations and cyanobacterial biovolumes in shallower lakes could be related to the increased average nutrient supply in these systems. This would occur due to frequent mixing-induced internal nutrient loading. In addition, in shallow lakes sedimented phytoplankton may be resuspended back into the water column. However, it is also true that in deep lakes, simply mixing at times during the summer and subsequent light limitation of primary production may result in a lower phytoplankton/cyanobacterial biomass (Sakamoto, 1966; Berger et al., 2006; Phillips et al., 2008).

Effects of mean depth were also strongly supported in analyses of functional composition metrics (MFGI, FTI), suggesting systematic changes in community structure and trait representation with changes in lake depth. High values of MFGI (such as in shallow lakes) indicate an increasing biomass of large, colonial and buoyant Chroococcales or Nostocales cyanobacteria. Low MFGI values (deep lakes) indicate an increasing biomass of non-motile xanthophytes, small pennate diatoms, small centric diatoms or Oscillatoriales. The inverse relationship between MFGI and depth seems to be driven by the trophic preferences of these functional groups, with the most eutrophic colonial Chroococcales and Nostocales being more abundant in shallow lakes. The results for these trait metrics may therefore suggest that the effect of mean depth is via correlated changes in the frequency of episodic nutrient release, as hypothesised above for Chl-*a* and cyanobacterial biovolume.

However, for each metric, considerable among-lake variation remained unexplained by the available environmental data. This was particularly the case for the composition (PTI, MFGI, SPI, FTI) and bloom (total cyanobacterial biovolume, evenness) metrics. While some of this variation might arise due to measurement errors in some of the environmental variables, this would also suggest the existence of important unmeasured drivers of phytoplankton community structure. Geographic variables were included in the analysis as a proxy for the effects of broad climatic gradients upon community structure, via lake physical processes, but the effects of grazing, flushing, water colour (DOC), silica or even other parameters associated with eutrophication pressure, such as dissolved nitrogen and turbidity, are all likely to be influential. However, these variables were not recorded consistently enough to include their effects in the current analysis.

Unexplained among-lake variability is also likely to arise due to the temporal dimension inherent in phytoplankton-environment interactions. Current phytoplankton community structure is a biological response to previous environmental conditions (Madgwick et al., 2006), with the time lag of the relationship determined by the time-scale over which phytoplankton gather resources and replicate. It is therefore to be expected that phytoplankton communities (and thus metrics) will show within-year temporal variation, and that the results of waterbody assessment will vary accordingly. However, waterbody assessment must ultimately depend upon sampling programmes that produce "snapshots" of this temporal variation. It is therefore important to know the uncertainties associated with such samples if we are to understand how well sample metric scores represent *current* conditions. Once sampling uncertainty is resolved for samples collected at a single point in time (the aim of this study), the next step would be to examine the temporal uncertainties associated with waterbody assessment. To this end, the relationship between metrics and environmental drivers could be resolved by integrating these variables over the growing season. In lakes with suitable time-series data it would, in principle, be possible to model temporal variability in metric scores as a further source of uncertainty, and also include the temporal relationship between metrics and drivers. Explicit consideration of these temporal aspects could not be achieved here due to the sampling design, but this is highly recommended for future research.

For six of the seven metrics there was evidence that not only mean values, but also variability, changed systematically with among-lake variations in physical, chemical and geographical attributes. Residual variability in metrics was not constant with respect to total phosphorus concentration (Chl-*a*, evenness, total cyanobacterial biovolume), mean depth (Chl-*a*, PTI, MFGI) or altitude (PTI, SPI). Furthermore, the association of this variability with specific drivers differed among metrics e.g. increases in total phosphorus concentration led to increased variability in total cyanobacterial biovolume, but decreases in variability in evenness and Chl-*a*. These findings are similar to the observations of Clarke et al. (2006a), who found that the sampling variability of macroinvertebrate community metrics can vary as a function of the overall ecological quality of a site (i.e. the average metric score). Plots of residual metric variability against predictor variables for some of the metrics in the present analysis suggested that a greater spread of metric variation for only a small proportion of the 32 study lakes compared to the rest was sufficient for the inclusion of these variance structures to result in an improvement in overall model fit, as judged by AIC. If a future study were to compile data from a larger number of lakes it would be possible to assess how robust these among-lake gradients in metric variability are. For now, the present results suggest that phytoplankton metric variability, and therefore uncertainty, may differ with attributes of the environment from which the phytoplankton samples were drawn and that this may be an important consideration when planning monitoring programmes.

5. Conclusion

By analysing the results of a unique pan-European hierarchical sampling programme we have shown that seven candidate phytoplankton community metrics, being considered for intercalibration under the Water Framework Directive, show the potential to indicate among lake variations in the effects of environmental pressures. This is particularly true for Chl-*a* concentration, PTI and total cyanobacterial biovolume, which appear to respond to variations in total phosphorus concentration as a proxy of eutrophication. These metrics are clearly also responsive to among-lake variations in other attributes such as mean depth, and other unidentified factors. In order to further assess the performance of such metrics, it is essential to examine the temporal dimension of their variability (Sondergaard et al., 2011) and also the extent to which uncertainty in waterbody assessment may vary systematically among lakes differing in their physico-chemical and ecological attributes. These should be considered priorities for future research into freshwater ecological quality assessment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2012.12.010.

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