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Phytoplankton in the ecological status assessment of European lakes – advantages and constraints

Fitoplankton w ocenie stanu ekologicznego jezior europejskich - zalety i ograniczenia

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Abstract
Although the phytoplankton indices describing the response of phytoplankton to the eutrophication have been developed and used for many years in the routine lake monitoring programme in some countries, the implementation of the Water Framework Directive (WFD) [EC, 2000] stimulated the development and improvement of quite a number of the current WFD-compliant phytoplankton-based methods. This paper is a review of the current phytoplankton-based methods for assessing the ecological status of European lakes. The particular attention was paid to the ways of solving problems arising from the need to reflect the complex and dynamic plankton algal communities on a numerical scale in order to gain reliable information about the state of the ecosystem.

Streszczenie
Pomimo, że w niektórych krajach europejskich wskaźniki fitoplanktonowe, wyrażające odpowiedź fitoplanktonu na eutrofizację, były opracowane i stosowane od wielu lat w monitoringu jezior, to znaczącym impulsem, który zainicjował rozwój i doskonalenie całego szeregu fitoplanktonowych metod było wprowadzenie Ramowej Dyrektywy Wodnej - RDW (EC, 2000). Praca jest przeglądem aktualnych metod oceny stanu ekologicznego jezior, ze szczególnym zwróceniem uwagi na sposoby rozwiązania problemów wynikających z konieczności odzwierciedlenia złożonego i dynamicznego zbiorowiska fitoplanktonu w sposób liczbowy, tak, aby uzyskać wiarygodną informację o stanie ekosystemu.

1. INTRODUCTION

Phytoplankton is a crucial constituent responsible for the production of organic matter in lakes, especially in the pelagic zone. As a result, pelagic algae and cyanobacteria condition the proper function of the food chain and all the changes in phytoplankton assemblage influence the entire aquatic ecosystem. Because of the short generation time of phytoplankton, its response to changes in the aquatic environment, mainly to the enrichment of a lake in nutrients, is rapid and direct. Phytoplankton can be an early warning indicator and, theoretically, can be used to control subsequent changes in aquatic environment. Owing to the constant threat of excessive eutrophication of the aquatic environment, which is the main pressure in many countries, including Poland, the knowledge of planktonic algae and cyanobacteria is gaining in importance. The response of phytoplankton to eutrophication has many negative effects. Usually, it is manifested in its increased abundance and biomass and contributes to greater turbidity of waters. As a result, a large number of secondary effects are observed, for example, changes in the taxonomic composition of phytoplankton, excessive development of cyanobacteria and filamentous green algae, decreased colonisation depth of macrophytes and even their complete withdrawal. Finally, intense cyanobacterial blooms (including those of toxic species) occur [Huisman et al. 2005, Søndergaard et al. 2011]. In turn, the abovementioned changes cause socio-economic consequences: a deterioration of the value of recreational lakes, a ban on swimming (because of blooms), the death of fish (caused by anoxia) or the unsuitability of fish for consumption as a result of their content of toxins, the harmful impact on drinking water and the decline of the natural values of protected areas.

Phytoplankton research to determine lake productivity changes has a long tradition. Thienemann [1918] and Naumann [1919] were the best known pioneers in the classification of lakes based on the trophic conditions. The authors made the distinction between oligotrophic lakes, with low productivity and high water clearness, and eutrophic water bodies with high productivity and turbid water. The well-known model focused on chemical compounds as a primary cause of increased trophy is the Vollenweider system [1968]. Total phosphorus (TP) was selected as the typical measure of phosphorus concentrations in lakes because it is relatively easy to measure. Although inorganic soluble forms of phosphorus are more available for algae, they are more difficult to measure accurately because of its rapid absorption and release by organisms [Lampert, Sommer 2001].

However, the trophy classification based on one criterion only is not possible. Trophic conditions are biological phenomena that require consideration of biological indicators; for example, the primary production expressed as algal biomass and/or the chlorophyll *a* concentration in water. Another indirect indicator of progressive eutrophication is the level of water transparency measured by the Secchi disc (SD) visibility. One of the most commonly known mathematic models that combines the main physical, chemical and biological symptoms of trophy is Carlson's Trophic State Index (TSI) [Carlson 1977], which connects algae biomass with three variables (TP concentration, SD and chlorophyll *a* concentration) as the most important elements of lake classification in respect of trophy. The modified Carlson-type TSI [Kratzer, Brezonik 1981] links another limiting nutrient, nitrogen, with Carlson's TSI. Similar to this approach, apart from chemical parameters, SD and concentration of chlorophyll *a* were also used as measures of plankton biomass in the trophy-based classification of Polish lakes [Hillbricht-Ilkowska, Kajak 1986] and in water quality assessments [Kudelska et al. 1983]. The simplicity, objectivity and relatively small data requirements made such a kind of indices very popular.

Pearsal [1932] was one of the first scientists who proved that the diversity of the trophic types of lakes corresponded to distinct and typical algal assemblages. He found the sub-domination of Desmidiales and chrysophytes in plankton in nutrient-poor lakes, the co-domination of diatoms in mesotrophic lakes and a domination of blue-green algae in hypertrophic water bodies in England. Many authors worked on the seasonal variability of the composition of the phytoplankton community in lakes with different levels of the trophic state [Hutchinson 1944; Järnefelt 1952; Spodniewska 1978; Willén 1979; Hörnström 1981; Rosén 1981; Reynolds 1980, 1984; Rott 1984; Trifonowa 1989]. They pointed out the occurrence of specific phytoplankton assemblages and functional groups depending on water productivity. This research and many other studies provided a scientific basis for the development of phytoplankton-related trophic indices that were used to examine water productivity based on the phytoplankton community. The most common indices include Thunmark's Index [1945], Nygaard's Index [1949], Järnefelt's Index [1956] or Hörnström's Index [1981], which can be used to monitor lake water quality.

Although in some countries the phytoplankton indices representing the response of phytoplankton to the eutrophication pressure have been developed and used for many years in the routine lake monitoring programme, the implementation of the Water Framework Directive (WFD) [EC, 2000] stimulated the development and improvement of quite a number of national methods. One of the main objectives of the WFD is to ensure that different categories of waters achieve 'good ecological status'. Good ecological status should deviate only slightly from the biological, structural and chemical characteristics that could be expected under undisturbed (reference) conditions. Just as for other water categories, the ecological status of lakes should be estimated first of all on the basis of biological elements, that is, assemblages of organisms that live in water. This approach is based on the belief that water is not only a resource used by people but also an element of an ecosystem and its quality should be evaluated with consideration of its ecological role. Therefore, we do classify not only the water quality but also the ecological state of the whole ecosystem [Vallentyne, Beeton 1988, Kudelska et al. 1997]. The organisms that are recommended for the assessment of the status of water ecosystems include phytoplankton (as one of the elements, in addition to macrophytes, phytobenthos, benthic invertebrates and ichthyofauna).

All the EU countries are obliged to prepare methods for phytoplankton sampling and a laboratory analysis strategy, as well as to develop a phytoplankton-based method for the ecological status assessment of water, expressed in numerical terms. According to Annex V of the WFD, the water classification system must take into account fundamental variables of phytoplankton such as (i) biomass or abundance; (ii) composition and (iii) frequency and intensity of blooms.

A sampling strategy and an assessment method must be specific for the abiotic type of a lake; it should refer to the reference conditions and should also be based on numerical indices enabling the calculation of the EQR (Ecological Quality Ratio), ranging between 0 and 1. The EQR represents the ratio between the index for a specific water body and its reference value that characterises this water body.

The response of phytoplankton to the eutrophication pressure makes it possible to define the boundaries between five ecological status classes (High, Good, Moderate, Poor and Bad). The method should not be time consuming and costly and should be possible to use it in routine monitoring. A phytoplankton-based monitoring method can be developed only when an extensive database comes from all the biocenotic types of lakes and covers the whole spectrum of water quality. Thus, the first methods of ecological status assessment appeared in countries that had for long many years carried out comprehensive and unified monitoring of this element in lakes, for instance, in the Scandinavian countries, the Netherlands or Germany. In the Scandinavian countries, the regular monitoring of phytoplankton in lakes started in the late 1970s [Fölster et al. 2014]. The sampling frequency varied from 4 times in the season in Norway, through a monthly survey in Finland and Sweden, up to 16 times per year in Denmark. On the basis of long-term data series, in these countries, a relatively fast development of the new methods of water evaluation in accordance with the WFD could take place. However, over several years of the WFD implementation, in most European countries, many new phytoplankton-based lake assessment methods were also elaborated [Birk et al. 2012, Lyche Solheim et al. 2013].

The aim of this paper is to review the current phytoplankton-based methods developed and applied in European countries for assessing the ecological status of lakes. Particular attention was paid to their approach to the ways of solving problems arising from the need to reflect the complex and dynamic plankton algal communities on a numerical scale in order to gain reliable information about the ecosystem. In order to compare the sampling procedures and the methods for phytoplankton-based assessments of lakes, the review covered 16 methods that emerged over past several years in European countries (Table 1). In this review, consideration was given to the sampling method, including its frequency, recommended deadlines and sampling sites as well as the types of indices applied. This overview does not contain detailed descriptions of assessment procedures (which the reader will find in the cited literature) but will make it possible to compare and discuss the advantages and disadvantages of each solution. Because of the continuous development and modification of the existing methods, the list of methods given below cannot be considered complete.

2. THE OVERVIEW OF THE EUROPEAN PHYTOPLANKTON-BASED METHODS FOR LAKE ECOLOGICAL STATUS ASSESSMENT

2.1. Sampling strategy

Temporal variation

The appropriate schedule of sampling is a basic problem for the effective monitoring of lakes. The development of a sampling strategy requires the determination of the number of sites and sample replicates and the frequency of surveys needed to make an assessment with sufficient precision. It should be planned in time and space with consideration given to phytoplankton ecology in order to obtain representative samples reflecting the ecological status of the ecosystem.

In the temperate zone, the main factor of the phytoplankton variability is the seasonal variation of solar radiation during the year and the associated temperature changes [Kawecka, Eloranta 1994]. Seasonality affects the mixing of water and its stratification and, indirectly, the concentrations of nutrients and algal biomass and composition. The annual development of the phytoplankton taxonomic structure in lakes is driven not only by a seasonal change of weather conditions but also by direct autogenic forces, often following a predictable organism succession as described, for example, in the Plankton Ecology Group (PEG) model [Sommer et al. 1986].

The WFD provides guidelines for the frequency for monitoring of biotic and physicochemical variables in lakes; in the case of phytoplankton, six samples per year are suggested (taken once a month in the vegetation period). These guidelines are designed to address the seasonal variability and to ensure that with given sampling frequencies, inter-annual changes are effectively monitored. Nevertheless, there is the dilemma for a national monitoring programmes to make a trade-off between ideal survey frequencies, logistical constraints and cost-effectiveness. The question arises as to what is the minimum number of inter-annual samples that are needed to reliably evaluate the ecological status of a lake. The sampling frequency should give the most proper information about algal communities, independently of their seasonal variability; however, it should be realistic in terms of its feasibility. In trying to meet these requirements, the sampling strategies, in particular methods, are different in the particular countries and geographical regions, but generally they cover the growing season, including the spring, the early and late summer and the autumn, which means a minimum of four sampling campaigns per year (Table 1). In addition, for the calculation of selected phytoplankton metrics, such as the biomass of cyanobacteria, samples should be taken in the most stable, late summer period [Phillips et al. 2013; Hutorowicz, Pasztaleniec 2014]. The composition of the phytoplankton changes throughout the annual cycle; however, it reaches the maximum species richness and stability in the late summer period (August–September). At that time, the composition of phytoplankton best reflects the physical and chemical conditions in the lake [Eloranta 198]. Moreover, the examination of phytoplankton samples in consecutive years shows the variation in its quantity and quality, often resulting from the impacts of different weather conditions. In view of this fact, many of the phytoplankton assessment systems (in Austria, Ireland, Sweden, the United Kingdom and Hungary) include sampling in three consecutive years as an obligatory condition enabling the classification (Table 1).

Spatial variation

Another question is the representative number of sites and samples taken in the vertical profile and the horizontal space. The phytoplankton distribution in a lake is uneven. Its spread can be very patchy as a result of the spatial variations of biological processes, such as growth, grazing, regulated buoyancy, and vertical migration, and abiotic factors, such as the wind or heavy rainfall [Reynolds 2006]. The distribution of phytoplankton in the vertical profile is quite uniform in the period of spring and autumn water mixing, whilst in the summer, the highest density of plankton algae can be noted in the epilimnion; however, for high light availability, the peak of phytoplankton abundance can appear in the metalimnion layer. The phytoplankton-based methods avoid the problem of the vertical distribution by taking integrated samples of the entire water column (in shallow lakes) or at 1-m intervals of the epilimnion or the euphotic zone.

The horizontal plankton heterogeneity is mainly driven by water movements: wind-induced water currents and inflow intrusions determined by the morphometry of the lake and the surrounding topography [Blukacz et al. 2009]. Greater differences between phytoplankton communities can be found within lakes with inlets and peninsulas, especially if a lake is composed of two or more parts with significantly different depth. It is advisable to analyse in such cases the integrated samples consisting of samples taken in the different parts of a water body. Borics et al. [2013] indicated the risk of high uncertainty in lake quality assessments caused by the horizontal distribution. However, the analysis of the variability of three different metrics (chlorophyll *a* concentration, cyanobacterial biomass and the taxonomic composition index PTI) between open water stations within the lake that was carried out by Carvalho et al. [2013a] showed low variance of these metrics (ca. 5–10%). The lake morphometry is probably the essential factor that may determine the horizontal differentiation of phytoplankton to a greater extent in shallow lakes than in deep ones. The sampling procedures in the individual programmes are uniform, but there are reports that in specific cases, such as shallow lakes with a particularly large surface area (e.g. Lake Balaton or Lake Peipsi), the sampling strategy should be adapted to the individual character of a lake in terms of its frequency and the distribution of sampling points [Nõges, Nõges 2006, Honti et al. 2007].

In the Polish method for phytoplankton-based ecological status assessment [Hutorowicz, Pasztaleniec 2014], when recommending the sampling dates and frequency, an attempt was made to take into account the climatic conditions and morphometric factors affecting lakes. In general, four sampling cycles are recommended in the period from March to October; furthermore, in order to calculate the metric expressing the cyanobacteria biomass in polymictic lakes, at least two sampling cycles in the period from 4 June to 30 September should be performed, whereas in stratified lakes, at least one sampling cycle in the period from 15 July to 15 September should be performed [Hutorowicz, Pasztaleniec 2014].

Table 1. The overview of selected phytoplankton methods used in Europe for the assessment ecological status of lakes.

Country	Frequency of sampling	Periods of sampling	Procedure of sampling	Abundance/ Taxonomic composition metrics	References
Austria	At least four times per year	Spring circulation, beginning of the summer stagnation, peak of the summer stagnation, beginning of the autumn circulation; three subsequent years' data for classification	Integrated sample of epilimnion or euphotic layer from the deepest part of the lake	Chlorophyll *a* Total phytoplankton biovolume Modified Brettum index	Brettum 1989; Dokulil et al. 2005; Wolfram et al. 2011; Wolfram et al. 2014
Belgium	Monthly	April–October	Eight to 16 sites (respectively, small and large lakes), integrated samples from entire water column (shallow lakes) or epilimnion (deep lakes)	Chlorophyll *a* Proportion of harmful cyanobacteria	Phillips et al. 2014
Denmark	One to two times per month	May–September	Integrated sample of euphotic layer from mid-lake station	Chlorophyll *a* Proportion of cyanobacteria Proportion of chrysophytes Phytoplankton taxa indicative for nutrient conditions	Søndergaard et al. 2011
Estonia	Four times per year, in each year	May, July, August, September	Depends on stratification of two to three samples (epi-, meta-, hypolimnion)	Chlorophyll *a* Evenness – modified Pielou index J Nygaard's modified compound quotient (PCQ) Description of the community	Nygaard 1949; Ott, Laugaste 1996; Ott et al. 2005
Finland	Usually three to six times per year but ranges from 1 to 12 One to nine times per year	May–September, more than three samples should be used for assessment	Integrated samples from the depth of 0–2 m at the deepest part of the lake	Chlorophyll *a* Total phytoplankton biovolume TPI (Trophic Plankton Index) Percentage of harmful cyanobacteria	Willén 2007; Vuori et al. 2010; Lyche Solheim et al. 2014
France	Four times per year	At least three samples between May and October	Integrated sample of euphotic layer from the deepest part of the lake	Chlorophyll *a* Specific Composition Metric based on indicator taxa	Menay, Laplace-Treyture 2011; de Hoyos et al. 2014
Germany	Six times per year	May–September	Integrated sample of epilimnion or euphotic layer from the deepest part of the lake	Chlorophyll *a* (mean and maximum value) Total phytoplankton biovolume Algal class metric Phytoplankton Taxa Seen Index (PTSI)	Mischke et al. 2008; Nixdorf et al. 2010

Continued Table 1. The overview of selected phytoplankton methods used in Europe for the assessment ecological status of lakes.

Country	Frequency of sampling	Periods of sampling	Procedure of sampling	Abundance/ Taxonomic composition metrics	References
Hungary	Four times per year	May–October three subsequent years' data for classification	Integrated sample of euphotic layer (deep lakes) or of entire water column (shallow lakes) from the deepest part of the lake	Chlorophyll *a*	Padisák et al. 2006
				Hungarian Lake Phytoplankton Index	
Ireland	Four to 12 times per year	January–December	Sub-surface samples from mid-lake stations	Chlorophyll *a*	Free et al. 2006
	Two times a year	1st June to 7th of September, at least three years' data for classification		Phytoplankton Composition Index	
Italy	Six times per year	One sampling at each period: January to mid-March; April to mid-May; mid-May – mid June; July – August; September; mid-October – November	Integrated sample of epilimnion or euphotic layer from the deepest part of the lake	Chlorophyll *a*	Dokulil, Teubner 2006; Salmaso et al. 2006; Wolfram 2009
				Total phytoplankton biovolume	
				Phytoplankton Trophic Index (PTIot)	
Norway	Usually six times per year (monthly), but ranges from 4 to 24	May–October	Integrated samples of euphotic water column from mid-lake stations	Chlorophyll *a*	Ptacnik et al. 2009; Lyche Solheim et al. 2014
	At least six times (monthly)			Total phytoplankton biovolume	
				Phytoplankton Trophic Index (PTI_{NO})	
				Cyanobacteria maximum biovolume	
Poland	Four times per year	March–May (1 sampling), June–September (2 samplings), October (1 sampling)	Integrated sample of 5 m layer (shallow lakes) or epilimnion (deep lakes) or euphotic layer from the deepest part of the lake	Chlorophyll *a*	Hutorowicz, Pasztaleniec 2014
				Total phytoplankton biovolume	
	At least one time a year	15 July–15 September (stratified lakes), 4 June–30 September (polymictic lakes)		Biovolume of cyanobacteria	
Slovenia	Four times per year	One sampling during spring homeothermic period is obligatory	Integrated sample of euphotic layer from the deepest part of the lake	Chlorophyll *a*	Brettum 1989; Dokulil et al. 2005; Wolfram et al. 2011; Wolfram et al. 2014
				Total phytoplankton biovolume	
				Modified Brettum index	
Sweden	Once a year	July–August, but at least three years' data for classification	Upper layer (approximately 75% of the epilimnion)	Chlorophyll *a*	Willén 2007; SEPA 2010; Lyche Solheim et al. 2014
				Total phytoplankton biovolume	
				Number of species	
				Proportion of cyanobacteria	
				TPI (Trophic Plankton Index)	
The Netherlands	Four to six times per year at regular intervals	April–September	One-meter layer in shallow lakes, integrated sample of epilimnion in deep lakes	Chlorophyll *a*	Phillips et al. 2014
				Bloom metric – based on blooming species and density criteria of blooming	
The United Kingdom	Monthly	January–December, but at least three years' data for classification	Lake outflow (or from the shore)	Chlorophyll *a*	Phillips et al. 2013; Phillips et al. 2014
		July–September, but at least three years' data for classification		Biovolume of cyanobacteria	
				PTI (Phytoplankton Trophic Index)	

2.2. Phytoplankton variables

Biomass or abundance

In general, the trophy level increase is accompanied by an increase in the biomass and abundance of phytoplankton taxa, as a result of which the summer abundance peaks are higher than those in the other periods and sustained [Kawecka, Eloranta 1994]. Hence, in order to classify a lake, that is, to determine the stage of its trophy development as well as its ecological status, it is necessary to determine the amount of planktonic algae. It can be done by directly counting the phytoplankton specimens to determine the total phytoplankton density by measuring the biovolumes of the individual species that enables calculations of the total biomass and, alternatively, by applying the proxy of phytoplankton biomass – the concentration of the key photosynthetic pigment chlorophyll *a*, which occurs in all the algal and cyanobacterial taxonomic groups. The latter parameter has been widely used in lake monitoring and classification schemes as a quick and easy-to-measure indicator of trophy (e.g. Carlson 1977, OECD 1982), and it is still the most common element of ecological status assessment methods (Table 1). In recent years, the boundaries for chlorophyll *a* concentration for five ecological classes were established in most European countries and were successfully compared in the 'intercalibration' process to ensure standardised values for lake types across several geographical regions of Europe [Poikane et al. 2010]. However, there is evidence to the problematic usefulness of chlorophyll as the only metric of phytoplankton abundance. First, in different plankton groups, the quantity of chlorophyll *a* in cells related to their physiological states varies, and therefore, in many cases, it is also supplemented by other types of chlorophyll or other pigments [Reynolds 2006]. In humic lakes, it is possible to be misled into the belief that the phytoplankton biomass is lower than it is, if indicated solely by chlorophyll analyses. That is because in these lakes, the biomass of phytoplankton can in varying degrees consist of poorly pigmented mixotrophic plankton organisms [Lyche Solheim et al. 2014]. Moreover, the proportion of chlorophyll *a* per unit of biomass is inversely related to cell volume; therefore, a given biomass unit of 'small' algal cells is likely to contain more chlorophyll than does the same amount of 'big' phytoplankton cells [Kasprzak et al. 2008]. It was found that the share of picoplankton in the total phytoplankton biomass, in lakes where the value of chlorophyll *a* was lower than 10 mg/l, might exceed 70%, whilst in lakes where the chlorophyll concentration was higher than 100 µg/l, the share was usually lower than 10% [Vörös et al. 1998].

The relation between the concentration of chlorophyll *a* and phytoplankton biomass depends on the taxonomic composition of algal communities, the availability of light and temperature [Reynolds 2006]. A particularly large discrepancy between the concentration of chlorophyll and the biomass and the lack of their overlap during the peaks of these two parameters were observed when dinophytes dominated (those with large cells) or so did big colonial species of Chlorophyta from the Volvocales order [Felip, Catalan 2000]. This is due to the fact that the large cells generally contain lower amounts of chlorophyll per unit volume than the small forms [Malone 1980]. On the other hand, in some cases, the reverse situation can be observed, when a high concentration of chlorophyll is found for relatively low biomass. This is usually caused by an abundance of very small forms, usually phytopicoplankton or autotrophic bacterioplankton, and relates primarily to the lower trophic state of the lake. In some water ecosystems, autotrophic picoplankton species are important constituents of the community and play a leading role in the primary production [Callieri, Stockner 2012]. Moreover, in shallow lakes, a relatively large proportion of the primary production can be shared by benthic organisms such as periphytic algae or higher vegetation [e.g. Sand-Jensen, Søndergaard 1981].

Abundance expressed as the number of cells/colonies/filaments per volume of water provides reliable information about the development of the population of particular taxa. It is difficult, however, to use this parameter in assessment methods without considering the taxonomic composition at the same time, because it does not reflect the state of the ecosystem precisely enough. It is obvious, for example, that the same total number of phytoplankton can be noted in lakes with different nutrient concentrations, which is related to differences in size between organisms.

Phytoplankton biomass is generally measured as the sum of the biovolumes of all the counted specimens and included in many European phytoplankton-based methods (Table 1). The inherent weakness of this parameter results from a lack of information on smaller-sized phytoplankton groups (picophytoplankton), because they are frequently overlooked in analyses carried out using the inverted microscope techniques [Utermöhl 1958]. The need to use a microscope with epifluorescence is an additional difficulty related to the assessment of the biomass of such small organisms. Another important constraint on biomass estimation is the fact that microscopy techniques enabling the evaluation of the composition abundance and biomass of the whole phytoplankton community are time consuming and may not be cost-effective.

Recently, information emerged about the possibilities of algal density measurements offered by new technologies (e.g. fluorescence measurements, flow cytometry and remote sensing), which can be considered as supplements to the evaluation of phytoplankton [Domingues et al. 2008].

To sum up, the chlorophyll *a* concentration, phytoplankton biomass and abundance are three different variables, providing information about the phytoplankton community in different aspects. Despite the abovementioned constraints of these three phytoplankton biomass and abundance measures, a linkage between these parameters and TP, total nitrogen concentrations or SD visibility was confirmed [e.g. Kufel 1999, Søndergaard et al. 2011]. These relationships are stronger within a certain range of trophic conditions and depend on the abiotic lake type. For example, in highly eutrophic waters, for high algae densities, self-shading restricts the development of phytoplankton in spite of the considerable fertility of water. Research on the Great Masurian Lakes, done by Kufel [1999], showed the existence of strong correlation between chlorophyll *a* and TP or SD in deep, stratified lakes, whereas such a relationship was not found in shallow macrophytes-dominated lakes. The decoupling of chlorophyll and nutrient concentrations was reported by other authors [i.e. Dokulil, Padisák 1994]. There is the opinion that measurements of chlorophyll *a* concentration ensure a good overview of the total phytoplankton biomass in a water body; however, this method can be used only as an indication of the current situation. The method can be used for screening and indicating possible changes in the phytoplankton biomass in a water body. In case of doubts, a complete phytoplankton analysis should always be carried out to verify results. In the Polish method, the Phytoplankton Multimetric for Polish lakes (PMPL) integrates three parameters concerning phytoplankton, two of them quantitative (chlorophyll *a* concentration and total biomass) and one combining abundance and taxonomy – the total cyanobacteria species biomass in the summer period [Hutorowicz, Pasztaleniec 2014].

Composition and detection of blooms

The WFD requires the classification of the ecological status of phytoplankton, which includes an assessment of its taxonomic composition. Long-term studies have shown that in some lakes, certain species dominate regardless of short-term changes in the amount of nutrients. This means that the qualitative nature of the changed phytoplankton requires a longer period (at least one year) and increased nutrient supply [Hörnström 1981]. The rapid response of phytoplankton (by multiplication) to the supply of nutrients is an advantage because it indicates the early symptoms of increasing eutrophication. At the same time, this is also a drawback, because such a response may be due to a temporary and local increase in fertility. During the temporary increase of phytoplankton biomass, its taxonomic structure can remain unchanged. The species composition of phytoplankton may represent the true nature of the fertility status of the lake. In this case, the measurements of the chlorophyll concentration and the total number of phytoplankton can be less reliable, because they only reflect the situation just before the sample is taken. However, taxonomy-based indicators are probably more problematic for calculations than the quantity-based ones. In addition to being time consuming, the identification of individuals at the species level is not an easy task, especially for some groups of cyanobacteria and algae, and many mistakes can occur during microscopic analysis [Carvalho et al. 2013a].

The most commonly used indicators of phytoplankton taxonomy are the cyanobacteria percentage share in the total phytoplankton biomass (used in Belgium, Denmark, Finland, Norway, Poland, Sweden and the United Kingdom), the share of other algal groups in total biomass or the mutual relations amongst different algal groups (the PCQ index in Estonian method or the ‘algal classes metric’ index in Germany), trophic indices based on the preferences of the species or higher taxa for phosphorus (as in Austria, Finland, Ireland, Germany, Norway, Slovenia, Sweden, Hungary and the United Kingdom) and biodiversity (the evenness index in Estonia) (see Table 1).

Consideration of the cyanobacterial biomass as an indicator of the eutrophication pressure has obvious advantages. In the scientific literature, there are many reports about the increasing abundance of cyanobacteria and, in consequence, the emergence of blooms in lakes with advanced eutrophy [e.g. Søndergaard et al. 2011]. Cyanobacteria-related measures can be considered a proxy measure of the risk posed by toxic algal blooms [Carvalho et al. 2013b]. In addition, there is a practical reason, because the cells of cyanobacteria as prokaryotic ones can be relatively easily distinguished from eukaryotic algae, enabling the calculation of the index by less experienced phycological researchers. The indices which also cover other phytoplankton groups can pose more problems, because similar phytoplankton assemblages may be encountered in lakes representing very different trophy states and, conversely, lakes deemed to exhibit a similar trophy level may differ in terms of the structure of the species which they support [Reynolds 2000]. The composition of phytoplankton is determined not only by the availability of nutrients but also by many other factors, including temperature, light regime and the way in which waters mix [Moss 2007]. Nevertheless, some species or assemblages can be expected in specific trophy conditions, but we should remember that as Reynolds [2000] concluded ‘… patterns determined rely upon the presence of certain algae indicating a given trophic state rather than the trophic state determining which algae might be there’.

The occurrence of phytoplankton species in the narrow niche of environmental variables is rather rare and the majority of species consist of common, widespread and tolerant taxa. In spite of this, indices applying the trophy preferences of species, higher taxa or functional phytoplankton groups can be found amongst WFD-compliant methods [Free et al. 2006; Padisak et al. 2006; Ptacnik et al. 2009, Phillips et al. 2013; Wolfram et al. 2011]. Indices based on the trophic preferences of taxa take into consideration both the taxonomic composition of the phytoplankton community and its density. This makes them very useful for water status assessment. All the trophic indices exhibit significant relationships with TP and some do so with total nitrogen. A controversial issue is the validation of methods against the TP, the parameter that is also used in most methods for the determination of boundary values and the development of indices. However, as Ptacnik and co-authors [2009] pointed out in the case of Norwegian lakes, the trophy index is excellent in distinguishing between reference and non-reference lakes, giving their natural ‘breakpoint’. Phillips et al. [2013] attempted to develop a multimetric combining the trophy index and the chlorophyll *a* concentration. The multimetric obtained demonstrated higher sensitivity to eutrophication, expressed by the coefficient of its correlation with the TP concentration, than the trophic index considered separately. The usefulness of phytoplankton indicators depends on their accuracy, generality and robustness. Multimetrics reduce data to a single number; however, this simplification enables the management of water resources.

It is difficult to estimate the frequency and intensity of phytoplankton blooms as the indicator of the eutrophication pressure. In Europe, there is no uniform definition of this phenomenon, although its characteristic features include a high phytoplankton density in the summer, its longer lifetime, the domination of one or two species or the presence of potentially toxic species [Mischke et al 2011]. The sampling strategy, that is, its frequency in time and the locations of sampling sites, is of key importance for the monitoring of blooms. The biomass of cyanobacteria, the group that is mainly responsible for summer blooms, may be a variable parameter, in the course of the year and between the individual years when surveys were carried out [Søndergaard et al. 2011] and in spatial terms [Pobel et al. 2011]. Therefore, according to Pobel et al. [2011], it is impossible to work out a sampling strategy that would be suitable for all water bodies in terms of the monitoring of blooms.

As mentioned earlier, the biomass of cyanobacteria or their percentage share in total biomass is an indicator that is often applied in phytoplankton-based methods for ecological status assessments (Table 1) and cannot be considered a metric that indicates the risk of blooms. However, it should be borne in mind that the intensive growth of cyanobacteria may be affected not only by the availability of nutrients but also by other factors, including favourable weather conditions (warm and dry summers, windless weather), and, in turn, their growth is constrained by intensive water mixing, low temperatures, self-shading or grazing [Dokulil, Teubner 2000]. Thus, the correlation between the percentage share of cyanobacteria in the total biomass of phytoplankton and the TP concentration is not strong, and it is difficult to determine the threshold values [Søndergaard et al. 2011]. An interesting solution is the integrated diversity bloom metric, which was developed and tested by Mischke et al. [2011]. This indicator takes into account two parameters that indicate the emergence of blooms: the chlorophyll *a* concentration (substantially exceeding the seasonal average) and the species-specific differentiation (evenness) [Mischke et al. 2011].

The bloom metric included in the Dutch method is an attempt to use the phytoplankton abundance in ecological status assessments [Phillips et al. 2014]. This bloom metric distinguishes between different bloom types, ranging from a massive bloom of *Planktothrix agardhii*, through blooms of, for example, *Scenedesmus*, *Anabaena*, *Botryococcus*, to blooms of *Dinobryon* and *Peridinium*. Blooms are defined by a bloom-specific density criterion, expressed by the number of cells, filaments or colonies. A specific metric is assigned to each bloom, ranging from 0.1 to 0.7, depending on its prevalence in relation to eutrophication [Phillips et al. 2014]. Because the Dutch bloom metric is focused on the autecology of selected taxa that are common dominants, an increase in their number of cells per volume of water can indicate sufficiently well the bloom risk.

Traditionally, phytoplankton biodiversity indices, Shannon–Wiener Index and the evenness index, are used in ecology for water quality assessment. However, the observed taxonomic richness is usually an underestimate of the true taxonomic richness and depends on the sampling strategy and the counter's skills. Moreover, the species diversity changes in a specific way along the eutrophication gradient and the highest diversity is observed at the medium disturbance level, in the context of eutrophication. The low species richness and biodiversity can be noted in nutrient-poor ecosystems, because these deficit conditions are sufficient enough for living only for few taxa, as well as in very fertile habitats where also only very few species which are best adapted to many stress factors (i.e. light and oxygen deficits) can survive. Nevertheless, these patterns can be seen well only when the full trophy spectrum, from the ultraoligotrophic to the hypertrophic, is available. In a relatively narrow productivity range, the species richness increases with increasing lake eutrophication. Various other factors also play a role. It is generally believed that high predation or grazing pressure results in the loss of diversity of prey organisms. As Dodson et al. [2000] noted, both the phytoplankton species richness and the Shannon–Wiener Index are unimodally related to TP, whereas the importance of lake depth and lake area varies: diversity increases with lake area and species richness grows with lake depth. For these reasons, the phytoplankton community richness does not seem to be a useful metric in monitoring programs, and except for Estonia, it is not an element of ecological status assessments as well in Poland as in other European countries (Table 1).

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