

# Performance of a new phytoplankton composition metric along a eutrophication gradient in Nordic lakes

Robert Ptacnik · Angelo G. Solimini ·  
Pål Brettum

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**Abstract** A new phytoplankton metric is presented, which is developed from a large dataset of Norwegian lakes (>2,000 samples from >400 lakes). In contrast to previous metrics, this index is not built on selected ‘indicative’ taxa, but uses all available taxonomic information at genus and species level. Taxa optima with respect to lake trophic status (derived from total phosphorus concentrations) are used to calculate a

phytoplankton trophic index (TI) for each sample. Analysis of the TI shows that phytoplankton communities exhibit highly non-linear responses to eutrophication in Norwegian lakes. Reference lakes are characterized by very similar TIs despite having considerable variation in total phosphorus and chlorophyll *a* concentrations. TI exhibits a non-linear distribution along the eutrophication gradient which separates unimpacted from impacted sites in the study area. We further show that TI exhibits smaller seasonal variations than chlorophyll *a*, making it a more reliable indicator for lake monitoring. Implications for its applicability within the WFD are discussed.

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Assessment of the Ecological Status of European Surface Waters

**Keywords** Water Framework Directive · Phytoplankton · Indicators · Eutrophication · Biodiversity · Threshold · Lakes

R. Ptacnik · P. Brettum  
Norwegian Institute for Water Research (NIVA),  
Gaustadalléen 21, 0349 Oslo, Norway

*Present Address:*  
R. Ptacnik (✉)  
Institute of Chemistry & Biology of the Marine  
Environment (ICBM), Carl von Ossietzky University  
of Oldenburg, 26382 Wilhelmshaven, Germany  
e-mail: ptacnik@icbm.de

A. G. Solimini  
Department of Experimental Medicine, Sapienza  
University of Rome, Regina Elena 324,  
00161 Rome, Italy

## Introduction

Among other things, the Water Framework Directive of the European Commission (WFD; Directive, 2000) provides the environmental objectives for the management of European lake ecosystems. The “good” status objective, to be reached by 2015 for all surface waters, is based on both chemical and ecological standards. The ecological status is defined as an expression of the quality of the structure and the

functioning of the ecosystems that should be assessed through the analysis of various ecological characteristics (e.g. indicators) of specific biological quality elements (phytoplankton, phytobenthos, macrophytes, macrozoobenthos or fish). Current challenges are to identify reliable indicators of ecological status and to assess at which levels of environmental pressure (e.g. eutrophication, acidification, hydro-morphological changes) this status is at risk of not meeting the 2015 objective.

Many classification systems of ecological status assessment adopted or developed for the WFD implementation represent qualitative or quantitative expressions of one or several characteristics of community and rely on appropriate metrics (see Solimini et al., 2008 for a recent review). An example of suitable community characteristics are the relative abundance of a taxon or taxonomic group or functional group (e.g. groups of species having similar ecological traits, Reynolds, 1980, 1984), the composition and/or diversity of organisms present in a community, the sensitivity of selected taxa to a given pressure, or a combination of them (e.g. multimetric indexes). However, the complex structure of biological communities allows virtually an indefinite way how the information contained in species' occurrence and abundances may be combined into a mathematical metric. Metrics may either use presence-absence data or quantitative data. In case of phytoplankton, either cell numbers or (proportional) biovolumes (Ptacnik et al., 2008b) were used. Many biological indices split biological elements into indicative (sensitive) and non-indicative, and use only a subset of the biological communities as indicator. Often those indicative taxa are then split into categories with categorical indicator values (e.g. Wolfram et al., 2007; see Table 1.3.1 in Solimini et al., 2008 for an overview of metrics applied in EU member-states).

The shape of the response curves of ecological indicators along a gradient of a variable related to a given pressure is, in principle, suitable for recommending boundaries between different levels of ecological status. Knowledge of the pressure intensity where changes in community composition are observed should be the basis for the definition of high/good and impact status, especially if the changes observed help discriminate stable from unstable and/or desirable from undesirable system status. Such change points (aka thresholds or regime shifts) have

become established as a framework in ecological theory for the understanding of non-linear dynamics (Scheffer & Carpenter, 2003). A change point is defined with respect to an environmental factor or stressor, which modifies a defined system or community. While the system exhibits only minor responses to the factor well below the change point, a sudden shift occurs once the change point is passed (Lyche Solheim et al., 2008).

Phytoplankton is one of the four major biological elements used in the WFD for lake ecological status assessment. Phytoplankton has a long tradition as a biological indicator, and the concentration of chlorophyll *a* (Chl *a*) has been widely used as a measure of algal biomass in European countries because it can be measured more easily and more objectively than phytoplankton biovolume and species composition (personal communication with various experts). However, in the conceptual reasoning of the WFD, an indicator of eutrophication should not simply assess the increase of biomass, but also the deviation in the biological structure and function from those observed in an undisturbed state. The initiation of the WFD has triggered development of new metrics based on phytoplankton composition (e.g. Carvalho et al., 2007; Ptacnik et al., 2008b).

Changes in phytoplankton composition in lakes along the eutrophication gradient have been well-known for several decades in Europe and North America. The most conspicuous responses are increases in cyanobacteria and green algae as well as certain diatom species (e.g. Watson et al., 1997). Such responses have mostly been described for higher taxonomic levels (e.g. Watson et al., 1997; Ptacnik et al., 2008b). However, Brettum and Andersen scored 150 species based on their contribution to the total biomass in relationship to the physical and chemical data (Brettum & Andersen, 2005).

In this article, we analyse the response of phytoplankton communities to eutrophication in a dataset of over 2000 phytoplankton samples from 450 different lakes in Norway. We use weighted averaging in order to estimate species optima along the eutrophication gradient, which is given by the concentration of total phosphorus in each sample. This information is used to calculate a trophic index (TI) for each sample. We test how the TI correlates with proxies of eutrophication (total phosphorus, Chl

*a*) and analyze its dependence on environmental parameters considered in the lake typology of the WFD in reference lakes. We also compare seasonal variability of our TI to that of Chl *a* in order to compare the reliability of these two metrics.

## Materials and methods

### Dataset

The phytoplankton and chemistry data were obtained from a large lake database which has been assembled as part of the EU project REBECCA (Moe et al., 2008). Depth integrated samples over the euphotic zone were taken in the growth season (May to September) in the period from 1988 to 2000 (most samples in 1988). All phytoplankton samples were analyzed by one person (Pål Brettum) using the Utermöhl method (Utermöhl, 1958). Within a given sample, we scanned an appropriately sized area to count at least 100 (usually more) individual cells from each of the abundant taxa. Taxon-specific biovolume was calculated per sample based on approximations of cell shapes by simple geometrical bodies (Tikkanen & Willén, 1992). Water chemistry was analyzed by standard methods. Temperature was not measured in situ, but taken from a regional grid of air temperature, which gives averages for the period 1975–2000 (Mitchell & Jones, 2005). Measurements of water colour ( $\mu\text{g Pt l}^{-1}$ ) were available for a small number of lakes only. We therefore used a categorical variable where colour was estimated being either below or above the threshold separating clearwater from humic lakes ( $30 \mu\text{g Pt l}^{-1}$ ; Directive, 2000). For lakes without colour measurement, this categorical variable was assigned by local researchers at NIVA ('expert knowledge').

### Reference lakes

Within the available data, reference lakes were defined and selected as sites which are not impacted by land-use, hydromorphological manipulations or by acidic precipitation. As a result, our selection of reference sites is very strict and for this reason, our dataset contains numerous oligotrophic lakes not assigned as reference lakes.

### Data analysis

All statistical analyses were based on single samples, except for the analysis of seasonal variability (see below). Species optima and trophic indices were calculated using quantitative phytoplankton data. Initial analysis showed that using the proportional (each taxon's share per sample total biovolume) rather than the absolute biovolume gives better results when estimating species optima and trophic indices. This is in line with ecological reasoning, since the proportional biovolume represents the relative success of a given taxon within the community under given environmental conditions (inc. total phosphorus (TP) concentration). We build our analyses, therefore, on proportional biovolumes.

### Species optima

Ln-transformed TP concentrations were used as a proxy for eutrophication in order to estimate a taxon's optimum relative to eutrophication. For each taxon, a trophic optimum was calculated by weighted averaging TP concentrations from all sites where this taxon occurs, using the taxon's squareroot-transformed proportional biomass as weight. We calculated optima both at the species and at the genus level. In the latter case, species were aggregated at the genus level. A list with taxon-optima is provided as supplemental material.

### Trophic index (TI)

For each sample, a TI was calculated by weighted averaging the optima of all taxa in a given sample with their square root transformed proportional biomass as weights. Here all taxa identified to genus level or higher were used, while unidentified taxa (level of order or below) were excluded. In addition, we used two groups which are important in Norwegian lakes, being small unidentified algae 2–4  $\mu\text{m}$  as well as unidentified chrysophyte flagellates, the latter being separated in two size classes,  $< 7 \mu\text{m}$  and  $\geq 7 \mu\text{m}$ . These are characteristic taxa in oligotrophic lakes.

In an initial analysis, we also compared the performance of a TI calculated from selected taxa only. We ranked taxa by their niche width, or

tolerance, with respect to their occurrence along the eutrophication gradient, and selected among all taxa those with the smallest niche width, using various quantiles. However, TIs calculated from reduced taxa lists were generally noisier compared to the TI obtained from using all taxa.

### Quantile regression

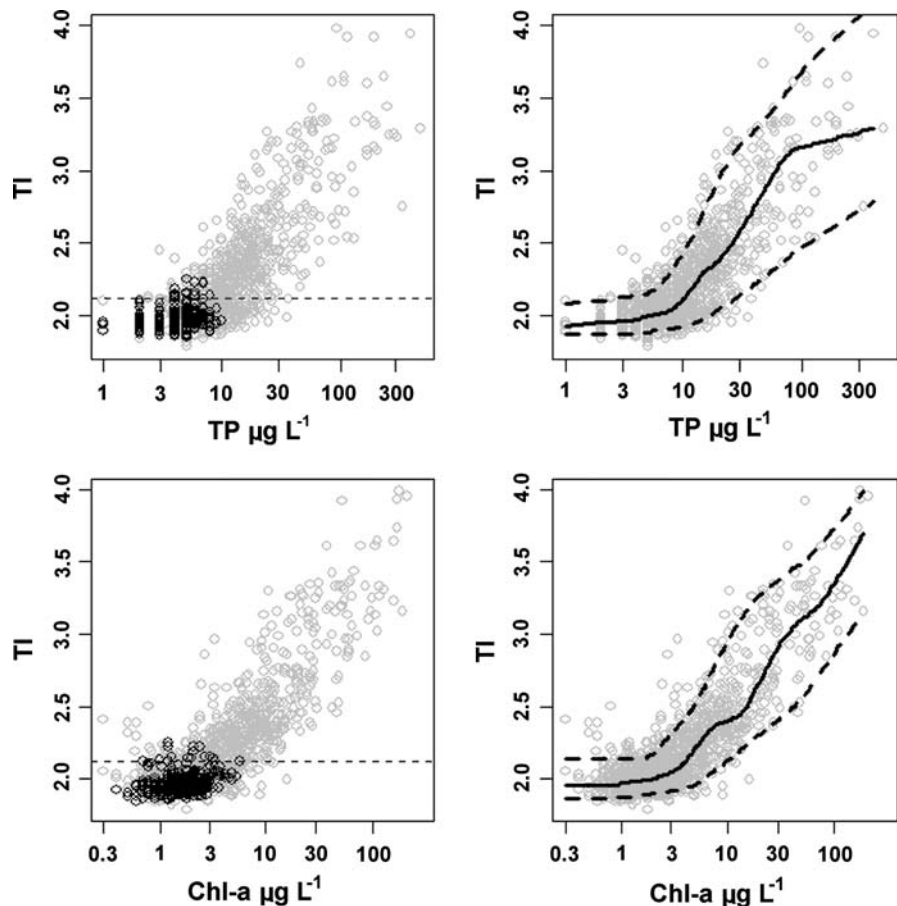
Visual inspection of the relationship between TI and proxies of eutrophication (TP, Chl *a*) indicated non-linear responses of TI. We visualize these relationships using quantile regressions, since percentiles (e.g. median) are not affected from extreme observations, allowing a more ‘objective’ analysis of the mean trend compared to least square-based regression methods. Moreover, inspection of outer percentiles allows direct assessment of the variability in the data.

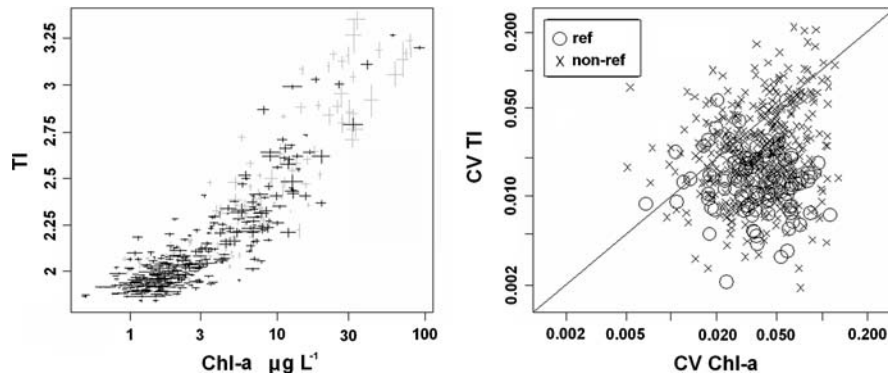
Quantile regressions were done using the *quantreg* package in *R* (Koenker, 2008).

### Seasonal variability of Chl *a* and TI

In order to compare the reliability of different indices, we calculated the coefficient of variation (CV) for Chl *a* and TI for each lake with multiple observations per year (>3). Since most lakes with multiple samples per season were sampled once a month, we had to utilize all the samples taken from May to September in order to make this analysis meaningful. CVs of Chl *a* were calculated based on ln-transformed Chl *a* values. Since CV is not meaningful for values close to zero, we standardized both TI and ln(Chl *a*) to a range between 1 and 2, prior to calculation of CVs. All statistical analyses were done using *R* (R Development Core Team, 2008).

**Fig. 1** Trophic index (TI) as a function of total phosphorus (TP; *upper panel*) and chlorophyll *a* (Chl *a*, *lower panel*). *Left*: *Black dots* represent samples from reference lakes, *grey dots* others. *Horizontal dashed line* gives the upper 95th percentile of the TI from reference lakes (= 2.11). *Right*: Same data, with quantile regression, showing the median (*bold line*) as well as the 5th and 95th percentiles (*dashed lines*). Note the low variability and steady median seen in TI on the left side of the gradient





**Fig. 2** Seasonal variability in trophic index (TI) and Chlorophyll *a* (Chl *a*) for lakes with multiple observations per year. *Left*: Each cross gives the mean value for Chl *a* and TI for a given lake-year. The horizontal and vertical bars represent the coefficient of variations (CV) of Chl *a* and TI, respectively.

## Results

### TI versus proxies of eutrophication

TI increases consistently with TP and Chl *a* (Fig. 1). The relationships seen in Fig. 1 are highly non-linear. TI increases only moderately among reference lakes, while there is a steep increase when a threshold is passed, which is found at approximately  $10 \mu\text{g TP l}^{-1}$ , and  $3 \mu\text{g Chl } a \text{ l}^{-1}$ , respectively. Reference lakes exhibit very similar TIs and are found entirely within the ‘flat’ area of the curve. Moreover, relative to the total variability seen in TI versus TP and Chl *a*, reference lakes group closely together (Fig. 1; see also seasonal variability, below).

We compared the seasonal variability of Chl *a* and TI for all lakes with multiple observations per year (Fig. 2), in order to assess the reliability of TI and Chl *a*. The analysis shows that TI is generally less variable with time than Chl *a*. The difference is most pronounced among oligotrophic lakes.

### TI in reference lakes and response to the environmental gradients

Norwegian lakes exhibit considerable environmental gradients not only in terms of climate (temperature, precipitation), but also in water colour (humic content), alkalinity and pH (Fig. 3). We assess the dependence of our index on these gradients in a multiple linear regression (Table 1). The TI shows significant response to alkalinity, temperature and

Colour code differentiates between lake-years where either Chl *a* (black) or TI (grey) exhibited higher CVs. *Right*: CVs of Chl *a* and TI for each lake-year plotted against each other. CVs are identical along the diagonal

season, the latter measured as day of year. TI does not change systematically with latitude, lake depth and lake humic content.

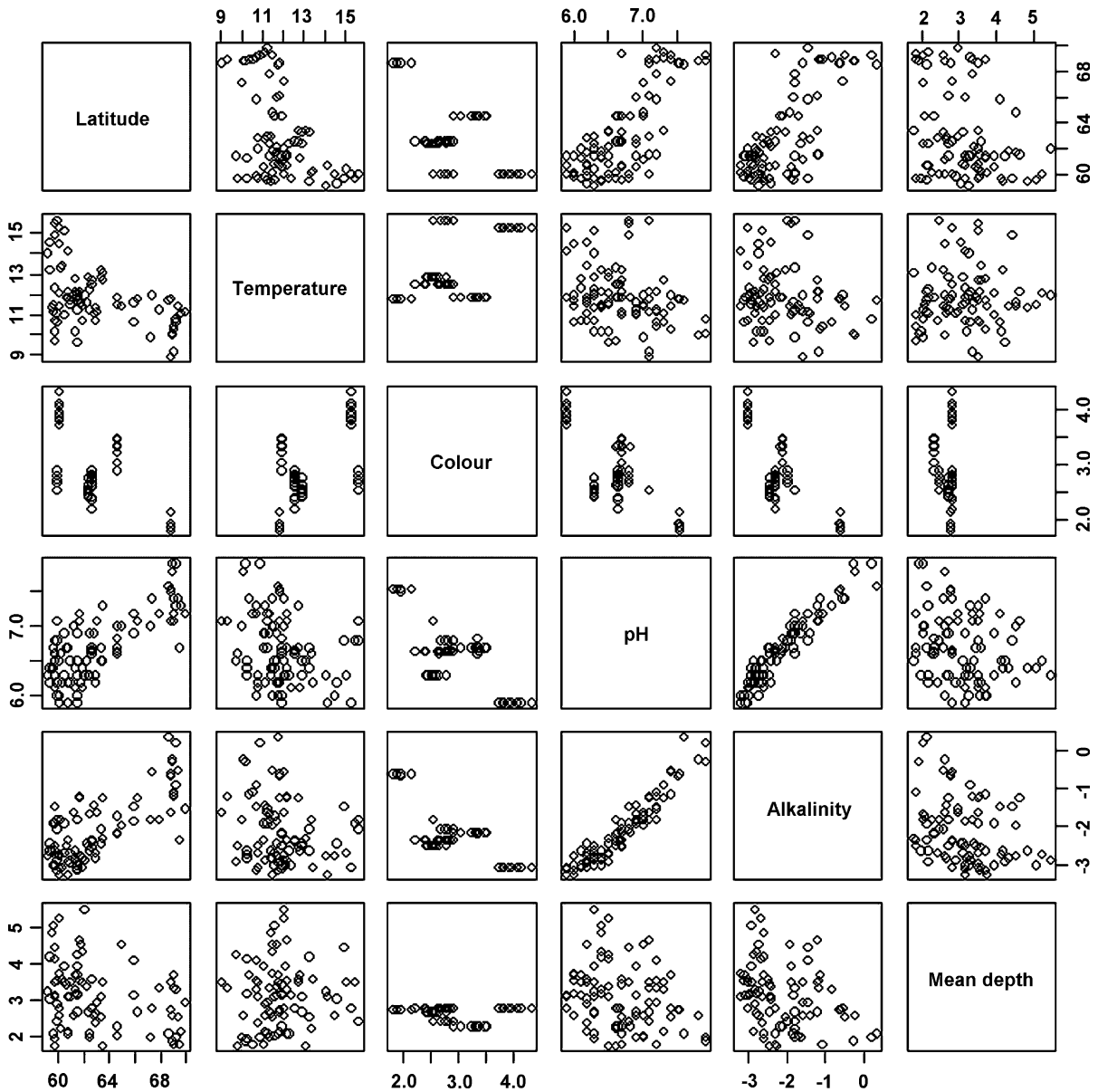
The relationship with season most likely reflects a relationship to temperature, since we included data from May to September. The significant effect of season disappears once the analysis is repeated with data from July to August only (not shown).

## Discussion

### TI and the eutrophication gradient

The TI as derived from community composition shows two important features which make it very suitable to monitor eutrophication in the WFD monitoring programs. First, it gives consistent information over time as evident from the fact that the seasonal variability seen in TI is smaller than that observed in Chl *a*. Second, TI exhibits a highly non-linear response, with very similar values among reference lakes, but responds sensitively to eutrophication. The stepped distribution seen in TI gives a ‘natural’ breakpoint for boundary settings in the WFD.

The TI appears to be very robust and similar among reference lakes, and may be adopted for particular lake types by correction for environmental gradients. However, the correlation with temperature may require adjusting the TI for long-term changes in climatic conditions. Since the data presented here are



**Fig. 3** Pair-wise illustration of important environmental parameters among reference lakes in the study area. Colour, alkalinity and Mean depth are ln-transformed

mainly from the period 1988–1995, a validation with more recent data would also be advantageous.

In contrast to alkalinity, colour had no measurable effect on TI in the combined model. However, since we only have a low number of humic reference lakes in our dataset, the power to detect an effect was low. Moreover, since colour was provided as a categorical variable only, a moderate effect may not be detected. A possible relationship between TI and colour remains to be tested using better data.

#### Chl *a* versus compositional indicators

The WFD requires indicators which reliably allow to assess whether, and if, how much a system deviates from a reference state. Concentrations of Chl *a* and total phosphorus are the often used proxies for eutrophication for the pelagial in lake ecosystems, and these parameters are often used to validate taxonomic biological indicators. Biological classification systems should reflect meaningful change in the

**Table 1** Multiple linear regression predicting trophic index (TI) in reference lakes as a function of environmental variables

	Estimate	Standard error	Student <i>t</i> -value	Significance <i>P</i>
Intercept)	1.81	0.04	50.1	<0.001
Ln (alkalinity)	0.03	0.004	6.7	<0.001
Temperature	0.01	0.002	5.37	<0.001
Day of year	0.0003	0.0001	2.66	0.008

Parameters were selected by two-way stepwise method using the Bayesian information criterion (BIC) for model selection. Deselected parameters were latitude, (categorical) humic content and lake mean depth.  $R^2 = 0.16$ ,  $n = 358$ . ‘Day of year’ becomes insignificant once samples from May, June and September are excluded. pH is highly correlated with alkalinity (Fig. 3), and therefore not taken into account

structure of communities and of the overall ecosystem functioning when affected by anthropogenic pressures (Solimini et al., 2009). We have shown here that the phytoplankton community composition, summarized with the TI, is a sensitive and a consistent indicator of eutrophication, and actually provides more reliable information compared to Chl *a*, especially at the lower end of the eutrophication gradient. Thus, the TI proposed here should be especially applicable for the discrimination of unimpacted lakes from lakes with low to moderate impact.

In contrast to earlier approaches (see ‘Introduction’ section), we used here the entire spectrum of taxa found in a geographic area for defining a lake TI. Ptacnik et al. (2008a) recently showed that species turnover is particularly high in productive, ‘eutrophic’ lakes. Eutrophic lakes may be characterized by a number of different taxa, and a given lake may exhibit blooms of different taxa from year to year. The high turnover may be one reason why indicators based on selected taxa often fall short in terms of reliability.

#### Non-linear response

While the data clearly show a non-linear response in community composition, indicating ecological breakpoints, we are currently unaware of the process underlying these patterns. An analysis of this question goes beyond the scope of this study, yet we want to mention some lines of reasoning worth further investigation. We may assume that the reference lakes in Norway are characterized by very low phosphorus levels and thus strong nutrient limitation, with dissolved SRP being below the analytical detection limit throughout the growing season (Henriksen et al., 1998). This is supported by a high share of mixotrophic chrysophytes

in oligotrophic Norwegian lakes (Ptacnik et al., 2008b). In addition to chrysophytes, small taxa (‘ $\mu$ -algae’) with very high surface to volume ratios, which usually cannot be identified under the microscope, are very common in these systems (see ‘Methods’ section). The ultimate change occurring in a lake subjected to eutrophication is an increase in limiting resources (nitrogen and/or phosphorus). With more nutrients in the system, a higher proportion of grazers can be sustained, and turnover of algal biomass increases (Andersen, 1997). At the same time, the availability of dissolved nutrients increases. The change from nutrient limitation towards increased turnover and the importance of top-down control may favour two strategies. Taxa which either may convert freely available nutrients rapidly into new biomass (= high growth rate), or taxa that have low susceptibility to grazing. The dominance of fast growing diatoms and grazing resistant cyanobacteria in productive Nordic lakes matches well with this reasoning (Ptacnik et al., 2008b). In other words, the breakpoint seen in community composition shown here may reflect the transition from phytoplankton adapted to strict nutrient limitation to those which either grows fast or are resistant to grazing. It should also be noted that the breakpoint in phytoplankton composition reported here has nothing to do with the regime-shift example reported by Scheffer (1990), which reflects the transition from a macrophyte dominated clear-water system to a turbid, phytoplankton dominated system, and occurs at higher TP levels in shallow lakes (50–100  $\mu\text{g TP}$ ).

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