Environmental variables and plankton communities in the pelagic of lakes: enclosure experiment and comparative lake survey

Stella A. Berger

Dissertation
zur Erlangung des Doktorgrades
der Fakultät für Biologie
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Summary

Most primary production of lakes and oceans occurs in the well-mixed surface layer that is subject to strong seasonal and geographical variation. With increasing mixed surface layer depth average light supply and specific nutrient supply decrease and so do light-dependent production rates and depth-dependent sinking loss rates of phytoplankton. Changes in mixing depth are expected to have important consequences for the dynamics of phytoplankton biomass, algal nutrient stoichiometry, light availability and nutrient retention in the mixed layer. Light absorption by enhanced concentrations of abiotic substances (humic substances, clay particles) furthermore negatively affects light availability and production.

I tested the predictions of a dynamical "closed system" model concerning the effects of mixing depth and background turbidity (K_{bg}) on phytoplankton biomass, light climate and nutrients in a field enclosure experiment. The natural phytoplankton community was exposed to high and low background turbidity along a gradient of mixing depth. For sinking algae, the model predicts that phytoplankton biomass should be most strongly limited by sedimentation losses in shallow mixed layers, by mineral nutrients at intermediate mixing depths and by a lack of light in deep mixed layers. As predicted, phytoplankton volumetric and areal biomasses showed a unimodal relationship to mixing depth and were negatively affected by background attenuation. With increasing K_{bg} the biomass peak shifted towards shallower mixing depth. The concentrations of dissolved and total nutrients were positively affected by increasing mixing depth but only marginally related to K_{bg} most likely due to a variable carbon to phosphorus cell quota.

For thermally stratified lakes I derived the following predictions from a dynamical "open system" model which includes variable algal cell quota: within a realistic mixing depth range (3-12m) light availability, phytoplankton density, and the carbon:phosphorus ratio of algal biomass should all be negatively related to mixing depth, while algal standing stock should be unimodally related, and total and dissolved nutrients be horizontally or positively related to mixing depth. All these prediction were in qualitatively good agreement with data from 65 central European lakes sampled during summer stratification. Notably, I observed the predicted negative relationship between phytoplankton density and mixing depth in spite of the rather limited range of mixing depths typical for medium sized temperate lakes.

Furthermore, I found a strong negative relationship among zooplankton biomass and mixing depth.

In a comprehensive comparative lake study of 40 northern German lakes, I sampled the surface mixed layers for a set of variables and focused on the taxonomic composition of phytoplankton and the relationships of taxonomic classes to environmental variables. I used high performance liquid chromatography to analyse the phytoplankton samples for 13 photosynthetic pigments and calculated the contributions of seven algal classes with distinct pigment signatures to total chlorophyll *a* using CHEMTAX, a matrix factorisation program. In multiple regression analyses, I examined the relationships of phytoplankton biomass and composition to total nitrogen (TN), total phosphorus (TP), total silica (TSi), mixing depth, water temperature, and zooplankton biomass. Total Chl-*a* was positively related to TN and TP and unimodally related to mixing depth. TN was the factor most strongly related to the biomasses of single taxa. I found positive relationships of chrysophytes, chlorophytes, cryptophytes, and euglenophytes to TN, and of diatoms and chrysophytes to TSi. Diatoms were negatively related to TN. Cryptophytes and chlorophytes were negatively and cyanobacteria positively related to zooplankton. Finally, the relative biomasses of chrysophytes and cryptophytes were negatively related to mixing depth. Most results were consistent with theoretical expectations. Some relationships may, however, have been masked by strong cross-correlations among several environmental variables.

Zusammenfassung

Der Großteil der Primärproduktion in Seen und Ozeanen findet in der gut durchmischten Oberflächendeckschicht statt, die starken saisonalen und geographischen Schwankungen unterliegt. Zunehmende vertikale Ausdehnung der durchmischten Oberflächendeckschicht (Durchmischungstiefe) führt zu verringertem mittleren Licht- und Nährstoffangebot und damit zu abnehmender lichtabhängiger Produktionsrate, sowie zu verringerter Sedimentationverlustsrate des Phytoplanktons. Veränderungen der Durchmischungstiefe beeinflussen somit die Dynamik der Phytoplanktonbiomasse, der Nährstoff-Stoichiometrie der Algen, der Lichtverfügbarkeit und der Nährstoffretention in der durchmischen Schicht. Zusätzlich verringert eine erhöhte Konzentration an Licht absorbierenden abiotischen Substanzen im Wasser (Huminstoffe, Tonpartikel) die Lichtverfügbarkeit und damit die Produktion des Phytoplanktons.

Ich überprüfte die Vorhersagen eines dynamischen Modells, das die Auswirkungen von Durchmischungstiefe und Hintergrundtrübung (K_{bg}) auf Phytoplanktonbiomasse, Lichtklima und Nährstoffverteilung in einem „geschossenen System“ beschreibt, mit einem Enclosure-Experiment. Entlang eines Gradienten zunehmender Durchmischungstiefe wurde die natürliche Phytoplanktongemeinschaft hoher und niedriger Hintergrundtrübung ausgesetzt. Für sinkendes Phytoplankton sagt das Modell vorher, dass die Phytoplanktonbiomasse stark durch Sedimentationsverluste in geringen Durchmischungstiefen limitiert ist, durch mineralische Nährstoffe in mittleren Durchmischungstiefen und durch Lichtmangel in einer tief durchmischen Wasserschicht. Wie vorhergesagt zeigten Algenkonzentration und Gesamtbiomasse eine unimodale Beziehung zur Durchmischungstiefe und waren beide negativ durch erhöhte Hintergrundattenuation beeinflusst. Mit erhöhter Hintergrundtrübung verschoben sich, wie vorausgesagt, die Maxima der Algenbiomassen in Richtung geringerer Durchmischungstiefe. Die Konzentrationen an gelöstem und Gesamtphosphor zeigten, wie erwartet, eine positive Beziehung zur Durchmischungstiefe und wurden nur marginal von der Hintergrundtrübung beeinflusst, höchstwahrscheinlich bedingt durch ein variables Kohlenstoff zu Phosphor Verhältnis in den Zellen.

Für thermisch geschichtete Seen („offenes System“) konnte ich aus einem dynamischen Modell mit integriertem variablen Kohlenstoff zu Phosphor Verhältnis der Algen folgende Vorhersagen ableiten: innerhalb realistischer Durchmischungs-

tiefenbereiche (3-12m) sollte mit zunehmender Durchmischungstiefe die mittlere Lichtverfügbarkeit, die Phytoplanktonkonzentration und das Kohlenstoff zu Phosphor Verhältnis der Algenbiomasse abnehmen. Die Gesamtalgenbiomasse sollte unimodal zur Durchmischungstiefe stehen und die gelösten und Gesamtnährstoffe sollten einen leicht ansteigenden Verlauf zeigen bzw. unbeeinflusst von der Durchmischungstiefe sein. Diese Modellvorhersagen konnten in einer vergleichenden Seenstudie, die eine Beprobung von 65 zentraleuropäischen Seen während der sommerlichen Schichtung umfasste, weitgehend bestätigt werden. Besonders hervorzuheben ist die negative Beziehung zwischen Phytoplanktonbiomasse und Durchmischungstiefe trotz der relativ geringen Durchmischungstiefenspanne, die typisch für Seen mittlerer Größe der gemäßigten Zone ist. Darüber hinaus konnte ich einen stark negativen Zusammenhang zwischen Zooplanktonbiomasse und Durchmischungstiefe aufdecken.

In einer sehr umfangreich beprobten Seenstudie 40 norddeutscher Seen untersuchte ich die taxonomische Zusammensetzung des Phytoplanktons und die Beziehungen einzelner Phytoplanktonklassen zu verschiedenen Umweltfaktoren. Ich verwendete eine moderne Methode zur Analyse der Phytoplanktonproben, die Hochdruckflüssigkeitschromatographie (HPLC) und konnte 13 photosynthetisch aktive Pigmente extrahieren. Ich ermittelte den jeweiligen Chorophyll-*a* Anteil von 7 Algen-klassen am Gesamtchlorophyll-*a* - Gehalt durch eindeutige Pigmentsignaturen mit Hilfe von CHEMTAX, einem Matrix-Faktorisierungs-Programm. Durch Regressionsanalysen untersuchte ich die Zusammenhänge zwischen Phytoplanktonbiomasse bzw. einzelner Taxa und den ´unabhängigen´ Variablen Gesamtstickstoff (TN), Gasamtphosphor (TP), Gasamtsilikat (TSi), Durchmischungstiefe, Wassertemperatur, und Zooplankton-biomasse. Der Gesamtchlorophyll *a*-Gehalt stand in positiver Beziehung zu TN und TP und zeigte eine unimodale Beziehung zur Durchmischungs-tiefe. TN schien der wichtigste Faktor zu sein und stand zu den meisten Algentaxa in Beziehung. Chrysophyten, Chlorophyten, Cryptophyten und Euglenophyten zeigten eine positive Beziehung zu TN, Diatomeen und Chrysophyceen eine positive zu TSi und Diatomeen eine negative zu TN. Cryptophyten und Chlorophyten zeigte eine negative, Cyanobakterien dagegen eine positive Beziehung zum Zooplankton. Die relativen Biomasseanteile von Chrysophyten und Cryptophyten hingen negativ mit der Durchmischungstiefe zusammen. Ein Großteil der Ergebnisse stimmte mit den theoretischen Erwartungen überein. Manche Beziehungen jedoch waren möglicherweise durch starke Korrelationen zwischen einigen Umweltfaktoren verdeckt.

Introduction

As all plants, phytoplankton requires light for photosynthesis and nutrients for growth, reproduction and metabolism. The supply of resources limiting algal production (light and nutrients) is highly variable depending on their environment. A major part of primary production in the pelagic of lakes and oceans occurs in the well-mixed surface layer. Light and nutrients fundamentally differ in their distribution within a well-mixed water column. While nutrients are relatively homogenously distributed, light decreases exponentially with depth due to absorption by water molecules. The vertical extension of the surface mixed layer (mixing depth) has strong negative effects on the mean light availability for passively entrained phytoplankton (Kirk 1994). The underwater light climate, particularly of lakes and estuaries is also affected by the concentration of light absorbing substances. Light attenuation by, e.g., humic substances or clay particles can be enormous and is summarised as background turbidity (K_{bg}) (Cuker 1987; Carpenter et al. 1998; Guildford et al. 1987). In many lakes, however, a major part of the available light may be absorbed by primary producers themselves (Tilzer 1983; Kirk et al. 1986). Thus, changes in phytoplankton biomass have direct feedback effects on the light climate in the mixed layer.

While increased background turbidity and mixing depth both negatively affect mean light intensity and, consequently, light-limited production of phytoplankton (Sverdrup 1953; Huisman 1999; Diehl et al. 2002) mixing depth also affects the sedimentation loss rates of phytoplankton. The sedimentation loss rate of negatively-buoyant algae is inversely related to mixing depth (Reynolds et al. 1984; Visser et al. 1996; Diehl et al. 2002; Ptacnik et al. 2003) and thus becomes most relevant to phytoplankton biomass in shallow mixed layers.

The vertical extension of the mixed surface layer can vary seasonally within lakes and geographically among lakes (Guildford et al. 1994; Soto 2002; Kunz and Diehl 2003). Furthermore, mixing depth depends on lake size and orientation to the dominating wind direction, water clarity and has been related to overall climate (Sterner 1990; Fee et al. 1996). It should therefore be regarded as an important environmental driver affecting phytoplankton biomass in a variety of ways.

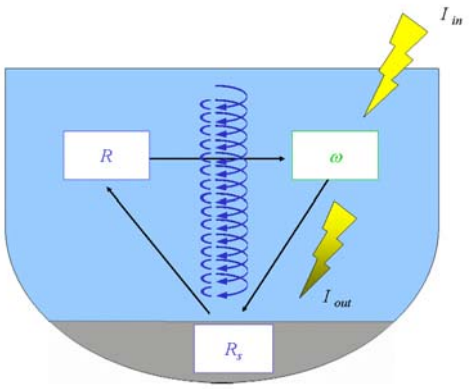
Since the onset of widespread anthropogenic eutrophication limnologists have focused mainly on the effects of nutrient enrichment on phytoplankton and later

became interested in effects of zooplankton grazing on food web structure in lakes (Sarnelle 1992; Persson et al. 1992; Mazumder et al. 1994). The strong positive relationship between chlorophyll a (Chl-*a*) and total phosphorus content (TP) became a dogma in freshwater ecosystems research (e.g. Dillon et al. 1974; Schindler 1978), while nitrogen (and silica) seemed to be more important as limiting nutrients in marine ecosystems and were rarely examined in freshwater ecosystems. The supply of light was examined to a lesser extent. Still, light and nutrients are interactively essential resources for phytoplankton growth sensu Tilman (1982); i.e., within limits an increase in either resource can overcome limitation by the other. As a consequence, increases in nutrient supply should ultimately increase the attenuation of light caused by phytoplankton biomass (Huisman et al. 1995; Scheffer 1998). Mechanistic understanding of the complex interrelationships and feedback mechanisms among mixing depth, nutrient supply, phytoplankton and light climate requires a dynamical modeling approach.

Theoretical Models

For a long time, dynamical models of resource competition in phytoplankton have focused on nutrients as limiting resources (Droop 1974; Tilman 1982). Huisman & Weissing (1995) were the first to mechanistically include light as a limiting resource in dynamical modeling of phytoplankton competition. Diehl (2002) extended Huisman and Weissing´s concept of enrichment with light by decreasing mixing depth, and included depth dependent sedimentation losses and nutrient supply into the model. This model assumes that phytoplankton production is limited by light and a single mineral nutrient and describes the dynamics of phytoplankton, light, and the nutrient in a well-mixed layer. Diehl (2002) distinguished between two modes of nutrient supply that represent endpoints of a continuum of natural conditions. In the closed-system model (Fig. 1) nutrient supply originates from remineralisation of sedimented algae representing shallow lakes or deep lakes during periods of mixing to the bottom. The open (stratified) system model (Fig. 2) describes the dynamics of the above state variables in the mixed surface layer of lakes which are stably stratified and nutrient supply originates from external sources, e.g. terrestrial run-off or deeper water layers (no recycling of sedimented algae is included). Physiological adaptation of algae to mixing depth-depended light and nutrient conditions i.e. a variable carbon:phosphorus ratio of algal biomass is not accounted for in the closed-system

model (on which Article 1 is based), but has been included to the open-system model (Article 2).



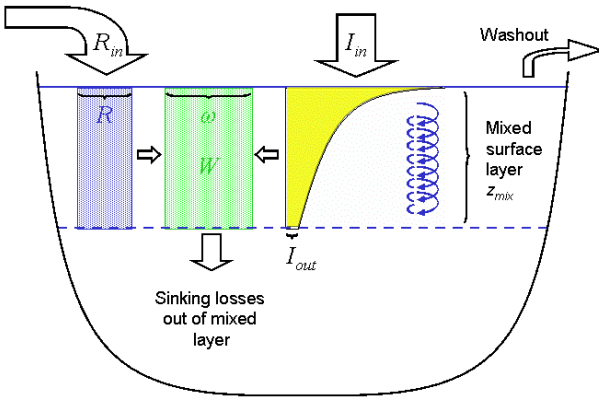


Figure 1 closed system

Figure 2 open "stratified" system

R = dissolved nutrients, R_s = sedimented nutrients, R_{in} = external nutrient supply, I_{in} = light supply, I_{out} = light at the bottom of the mixed layer, ω = algal biomass concentration, *W* = algal standing stock, z_{mix} = mixing depth

The Project

The main goals of this thesis were to investigate the influence of mixing depth on phytoplankton dynamics, and to explore the relationships of a set of environmental drivers to light, nutrients, seston stoichiometry, phytoplankton biomass and taxonomic composition in the mixed surface layer of lakes.

1. A field enclosure experiment was conducted to test the theoretical model of Diehl (2002) and focussed on effects of background attenuation and mixing depth on natural phytoplankton biomass, light and nutrients (Article 1). Experimental gradients of mixing depth were created by enclosing the natural phytoplankton community in cylindrical plastic bags of different depth (1-15 m) and subjecting it to a continuous mixing regime for 8 weeks (Fig. 3). High and low background attenuation was simulated by surrounding the enclosure walls with black and white silage foil. Sedimentation traps were installed at the bottom of each enclosure. In this field experiment, I dealt with the following model assumptions and predictions:

Assumptions:

- Average light intensity and specific phytoplankton production decrease with increasing mixing depth and are negatively affected by background turbidity
- Specific sedimentation loss rate decreases with increasing mixing depth
- The nutrient content per algal biomass is fixed and independent of mixing depth and background turbidity

Predictions:

- Concentration and standing stock of phytoplankton biomass show a unimodal relationship to mixing depth and are negatively affected by background turbidity
- The mixing depths at which the concentration and standing stock of biomass peak decrease with increasing background turbidity
- Light intensity at the bottom of the mixed layer decreases with mixing depth and is negatively affected by background turbidity
- The concentration of dissolved inorganic nutrients shows a u-shaped relationship to mixing depth and is positively affected by background turbidity
- The concentration of total nutrients in the mixed layer (i.e., dissolved nutrients plus nutrients stored in algal biomass) shows a u-shaped relationship to mixing depth and is positively affected by background turbidity
- The standing stock of sedimented nutrients shows a unimodal relationship to mixing depth and is negatively affected by background turbidity. Higher background turbidity leads to a shift of the peak towards shallower mixing depths.

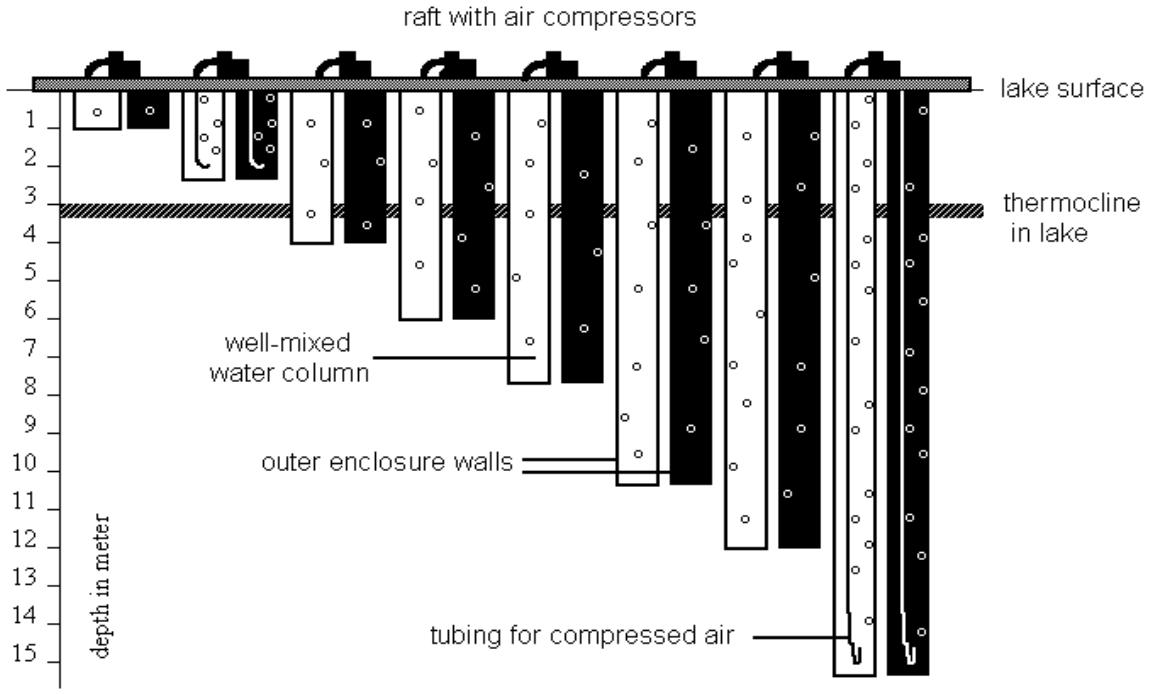


Figure 3 Design of the enclosure experiment. Mixing depth gradient (1-15 m) and low and high background attenuation treatment.

2. A comparative lake study of 65 central European lakes was conducted to test the open system model including variable seston stoichiometry in the field (Article 2). I sampled 40 dimictic, meso- to eutrophic lakes in northern Germany three times during summer 2001 for a whole set of parameters and included a data set of 25 oligo- to mesotrophic southern German lakes sampled twice during summer 1998 (Kunz and Diehl 2003) in a common analysis (Fig 4). In this extended data set I examined the influences of mixing depth, total phosphorus content (TP, a proxy for external nutrient supply) and water temperature on phytoplankton biomass, light and nutrients. For a realistic range of mixing depths, the model makes predictions as follows:

- Phytoplankton density and algal carbon:nutrient ratio are negatively related to mixing depth
- The areal standing stock of phytoplankton (summed over the mixed layer) is unimodally related to mixing depth
- Light intensity at the bottom of the mixed layer decreases with mixing depth

- Concentration of the limiting nutrient in dissolved mineral form and total nutrient concentration show a flat or increasing relationship to mixing depth
- Higher nutrient concentrations in the external supply lead to more phytoplankton biomass, lower light levels, higher concentrations of total and dissolved nutrients, and a lower algal carbon:nutrient ratio

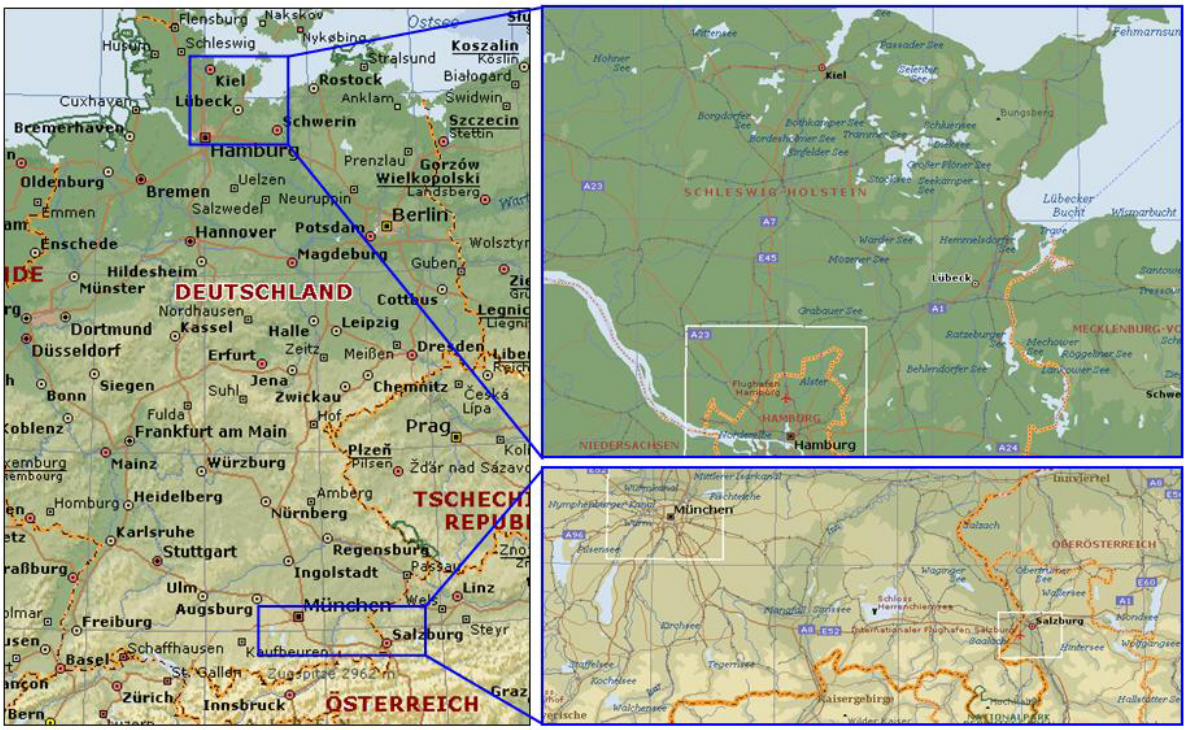


Figure 4 Geographical region of the lakes studied.

3. The comprehensive sampling program of the 40 northern German lakes allowed additional, detailed analyses of the relationships among environmental drivers and phytoplankton biomass and taxonomic composition (Article 3). Many ecologically relevant algal traits are shared by most or all members of higher taxonomic units (e.g., algal classes) that are also characterized by unique combinations of photosynthetically active and accessory pigments (Rowan 1989). To determine the contributions of different algal classes to the phytoplankton communities of the 40 lakes, I used one of the most modern methods for photosynthetic pigment analysis, high performance liquid chromatography (HPLC) (Barlow et al. 1997), and determined the content of chlorophyll *a* (Chl-*a*, the main pigment in all phytoplankton classes) and 12 accessory algal pigments (Fig 5). In one of the first application in freshwater, I used a matrix factorisation program, CHEMTAX (Mackey et al. 1996)

which uses class-specific ratios of Chl-*a* to marker pigments and Chl-*a* to diagnostic pigments and then calculates the contributions of single taxa to the total chlorophyll *a* concentration. CHEMTAX allowed distinguishing seven taxonomic phytoplankton classes: chrysophytes, chlorophytes, diatoms, cryptophytes, cyanobacteria, dinoflagellates, and euglenophytes. The program permits estimating the phytoplankton composition in the studied lakes. I then used multiple regressions to explore relationships among the environmental variables mixing depth, total phosphorus, total nitrogen, total silica, water temperature, zooplankton biomass and the absolute and relative biomasses of seven algal classes.

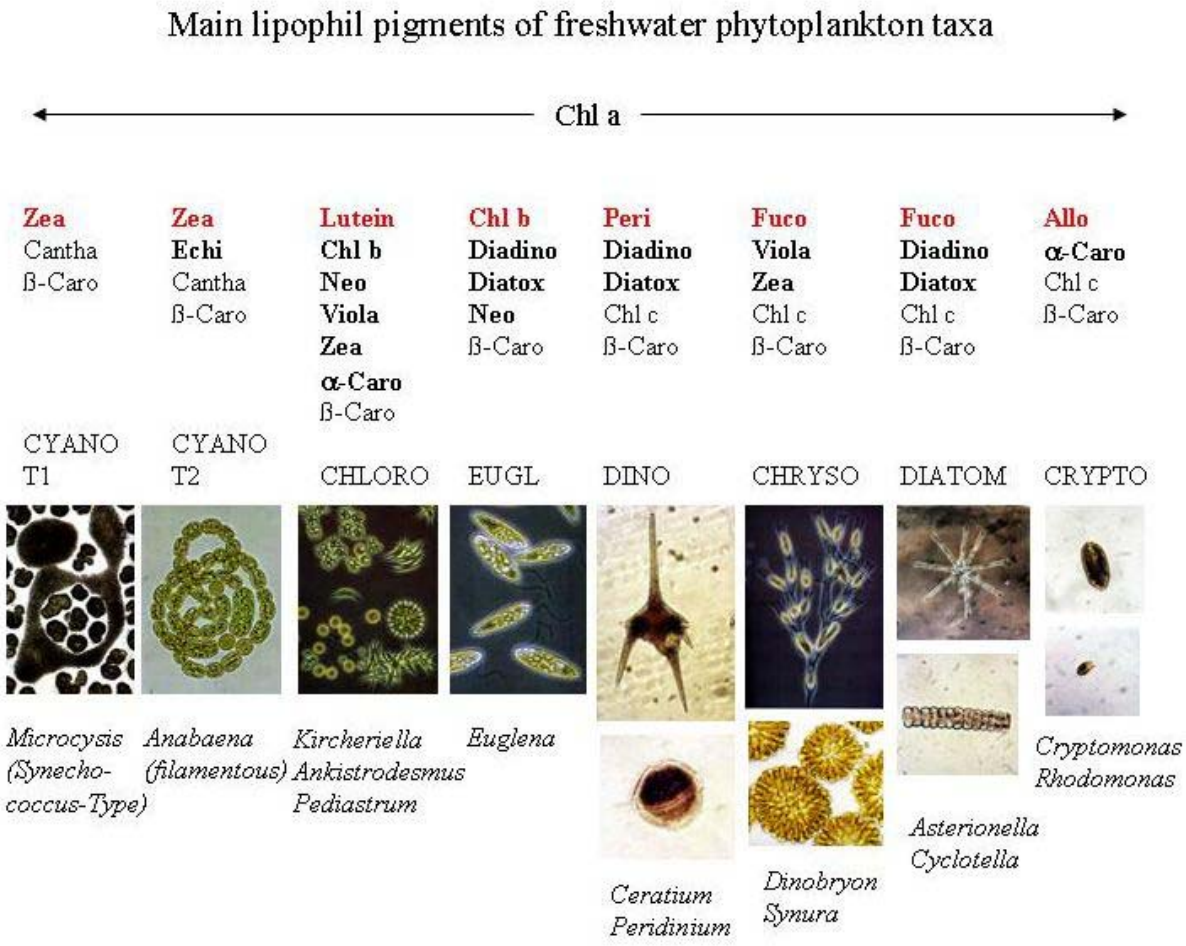


Figure 5 Chemotaxonomy of phytoplankton pigments

Chl a = chlorophyll *a*, Zea = zeaxanthin, Chl b = chlorophyll *b*, Peri = peridinin, Fuco = fucoxanthin, Allo = alloxanthin, Cantha = canthaxanthin, ß-Caro = ß-carotene, Echi = echinenone, Neo = neoxanthin, Viola = violaxanthin, α-Caro = α-carotene, diadino = diadinoxanthin, Diatox = diatoxanthin, Chl c = chlorophyll *c*). Algal classes: CYANO = cyanobacteria, CHLORO = chlorophyta, EUGL = euglenophyta, DINO = dinophyta, CHRYSO = chrysophyta, DIATOM = diatoms, CRYPTO = cryptophyta.

Summary of the articles

Article 1

Effects of mixing depth and background turbidity on phytoplankton biomass, light and nutrients

(Stella A. Berger, Sebastian Diehl, and Rainer Wöhrl)

As in all plants, the growth of pelagic primary producers living in the mixed surface layers of lakes and oceans depends on the supply of essential resources, namely light and nutrients. Environmental factors such as mixed layer depth and background turbidity (light absorption by abiotic substances) substantially influence the availability of production-limiting resources for phytoplankton. Light supply and thus the specific rate of (light-limited) phytoplankton production decrease with increasing mixing depth and background turbidity. For sinking phytoplankton, the specific sedimentation loss rate also decreases with increasing mixing depth, but is independent of background turbidity (Diehl et al. 2002, Ptacnik et al. 2003). A dynamic closed system model (Diehl 2002) predicts that algal biomass should be most strongly limited by sinking losses at shallow mixing depths, by mineral nutrients at intermediate mixing depths and by a lack of light at high mixing depths. Due to this mixing depth dependent pattern of resource limitation, the concentration and standing stock of phytoplankton biomass should show unimodal trends along the mixing depth gradient. Enhanced background turbidity should negatively influence the phytoplankton biomass. Along a gradient of mixing depth, the position of the biomass maxima should shift towards shallower mixing depths at higher background turbidity due to lower light availability at a given mixing depth but similar sedimentation losses. When nutrients are bound in biomass the dissolved nutrient concentration should be comparatively low and roughly show the opposite trend to algal biomass, leading to a u-shaped relationship to mixing depth and a positive relationship to increasing background attenuation. The standing stock of the sedimented nutrient fraction is expected to be unimodally related to mixing depth and negatively affected by background attenuation, as is the algal standing stock.

I tested the model predictions concerning the effects of water column mixing depth and background turbidity on phytoplankton biomass, light climate and nutrients

in a field enclosure experiment. The epilimnetic, 100 *µ*m-screened phytoplankton community of low productive Lake Brunnsee (Germany) was exposed to a gradient of mixing depths ranging from 1 to 15 m during 8 weeks. The enclosures had a diameter of 0.95 m and were open to the atmosphere. Well-mixed conditions within each enclosure were maintained by intermittently pumping air through tubing to the bottom of each enclosure. High and low background turbidity was simulated by surrounding the transparent enclosure walls with either black or white foliage. To calculate sedimentation losses I installed sedimentation traps just above the bottom of each enclosure. Throughout the experiment, the phytoplankton community was dominated by fast-sinking diatoms of the genera *Cyclotella* and *Fragilaria* at the beginning and at the end of the experiment, respectively.

Results were in qualitatively good accordance with most of the model predictions. As expected, phytoplankton concentration and standing stock were unimodally related to mixing depth and were lower in high background attenuation treatments. Because of the positive correlation of mean light intensity and specific phytoplankton gross production both decreased gradually with mixing depth and were lower at high background attenuation at all mixing depths. Sedimentation loss rate was inversely related to mixing depth and independent of background turbidity. Along the mixing depth gradient algal sinking loss rate decreased very sharply from relatively high values at shallow mixing depth to rather low values at intermediate and high mixing depth. At a given mixing depth algae experienced similar sedimentation rates but lower production rates at higher background turbidity. I observed the predicted shift of volumetric and areal biomass maxima towards shallower mixing depth at high background turbidity. Only partly in line with expectations, the concentrations of dissolved and total phosphorus in the water column were positively affected by mixing depth but showed only minor effects of background turbidity. Instead of the expected unimodal relationship with mixing depth, the standing stock of sedimented phosphorus increased only slightly with mixing depth and was unrelated to background attenuation. A possible explanation for these deviations from the model predictions is that the ratio of seston carbon to phosphorus was not constant as assumed in the model, but decreased with increasing mixing depth and background turbidity. This concept of flexible phytoplankton stoichiometry has been investigated in another paper (Diehl. et al. 2005).

Article 2

Light supply, plankton biomass and seston stoichiometry in a gradient of lake mixing depths

(S. A. Berger, T. J. Kunz, D. Albrecht, A. M. Oucible, S. Ritzer, S. Diehl)

Most aquatic primary production occurs in the well-mixed surface layer of lakes and oceans. With vertical expansion of the mixed surface layer average light intensity for passively entrained phytoplankton and, hence, specific phytoplankton production both decrease (Diehl et al. 2002). Furthermore, increasing mixing depth negatively affects external nutrient supply per volume of the mixed layer and the sinking loss rate of particulate matter into deeper layers (Diehl 2002). The relative supply of light and nutrients to the mixed surface layer does not only affect phytoplankton production and biomass, but also the elemental composition of algae which is known to be highly variable (Guildford et al. 1994; Sterner et al. 1997). Mixing depth is therefore expected to have important consequences for the dynamics of phytoplankton biomass, seston stoichiometry (C:P), light availability, nutrient concentration and nutrient retention in the mixed surface layer of lakes (Sterner et al. 1997).

To my knowledge, no theoretical model or empirical data set is yet available which comprehensively explores the impact of mixing depth on the dynamic interplay among the full set of these pelagic ecosystem components. We developed a dynamical open-system model that accounts for variability in algal nutrient stores and derive predictions on how the above state variables should be affected by mixing depth and external nutrient supply at equilibrium. I then compare the model predictions to the results of a comparative lake survey.

The model predicts that phytoplankton biomass concentration and algal carbon:nutrient ratio should be unimodally related to mixing depth due to high sinking losses of algal cells at the shallowest mixing depths and increasing light limitation towards deep mixed layers. However, for a realistic range of mixing depths typical for small to medium-sized lakes (2-15 m) phytoplankton density and algal C:P ratio are expected to be negatively related to mixing depth. The areal standing stock of phytoplankton should also be unimodally related to mixing depth with a maximum

occurring at considerably deeper mixed layers. In shallow mixed layers, depth-integrated biomass should be limited by the relatively low total amount of nutrients, whereas light should become limiting towards deep mixed layers. Light intensity at the bottom of the mixed layer should decrease with mixing depth. The concentration of dissolved and total nutrient concentration should show a flat-bottomed, u-shaped relationship to mixing depth. However, within a realistic range of mixing depths dissolved and total nutrient concentrations are expected to be nearly independent of mixing depth, because most nutrients are bound up in algal biomass leading to an increasing amount of nutrients stored in algae with increasing mixing depth. The model also predicts effects of enrichment from external sources on the state variables. Higher concentrations of the nutrient in the external supply should enhance phytoplankton biomass, decrease mean light levels and algal carbon:nutrient ratio, and lead to an increase in dissolved and total nutrient concentrations.

To test the model predictions I conducted a survey of 40 stratified northern German lakes in summer 2001 and included data from a lake survey comprising 25 lakes in southern Germany (north of the Alps) sampled in summer 1998 (Kunz and Diehl. 2003) in which all state variables included in the model were sampled. I used seasonal means of multiple mixed surface layer samplings from each lake to compare lake data with model predictions for equilibrium. The whole set of lakes (65) covered a moderate range of average mixing depths (3-10.3 m), and a broad range of mean total phosphorus (TP) contents (8-122 mg / m³) in the mixed surface layers. I performed multiple regression analyses to examine relationships among the dependent variables phytoplankton concentration and standing stock, light at the bottom of the mixed layer (I_{out}), and soluble reactive phosphorus (SRP) and the predictor variables mixing depth (z_{mix}) , squared mixing depth (z_{mix}^2) and total phosphorus (TP) and water temperature using a stepwise procedure. Qualitatively, the results agreed very well with model predictions for the range of examined mixing depths: Algal biomass concentration and the seston carbon:phosphorus ratio were negatively related to mixing depth. The standing stock of algal biomass was best fit by a unimodal relationship to mixing depth. Nutrient enrichment, as approximated by TP concentration, was positively related to the concentration and the standing stock of algal biomass as well as to the carbon:phosphorus ratio. The light intensity at the bottom of the mixed layer was negatively related to mixing depth. Likewise, TP concentration and I_{out} were negatively correlated because nutrient enrichment caused

a higher algal biomass that enhanced light attenuation. The concentration of soluble reactive phosphorus (SRP) was best fit by a u- shaped relationship to mixing depth and was positively related to TP concentration.

The robustness of this variable cell quota model is demonstrated by the fact that many assumptions of the comparatively simple model were not met in the natural lake systems investigated, as for example the absence of zooplankton grazing and identical background turbidity.

Depth of the mixed surface layer should be regarded as an important environmental factor determining algal biomass, seston stoichiometry and the distribution of nutrients among various pools in lakes. The effects of mixing depth should also propagate to higher trophic levels of lake food webs. Indeed, mesozooplankton biomass was strongly negatively related to mixing depth in the north-German subset of the lakes investigated. Mixed layer depth of lakes is determined by a complex combination of local climate conditions, lake orientation relative to the prevailing wind direction, lake size and water clarity (Fee et al. 1996). Seasonal and regional changes of mixing depth patterns due to large-scale climate variability such as the North Atlantic Oscillation (NAO) already lead to increased surface temperatures in central European lakes in years with positive NAO winter indices during the last decade. Also, recent climate changes already resulted in an advanced onset of thermal stratification and an alteration of mixis regimes in northern hemispheric lakes (Weyhenmeyer et al. 1999; Gerten et al. 2002).

Article 3

Phytoplankton taxonomic composition in relation to environmental variables: a comparative lake study using pigment analysis

(Stella A. Berger, Ilka Peeken, and Sebastian Diehl)

Chlorophyll *a*, the main photosynthetically active pigment of phytoplankton is usually used as a proxy for phytoplankton biomass in aquatic systems. Current research focuses on the modelling and discerning of empirical patterns relating phytoplankton biomass to environmental drivers. A well-researched topic is the positive relationship of chlorophyll *a* (Chl-*a*) to total phosphorus and its modifications by food web interactions (Dillon and Rigler 1974). The relationships of total nitrogen and total silica to phytoplankton biomass have, however, only recently began to be examined in freshwater systems (but see Huszar et al. 1998; Tilman et al. 1986; Jeppesen et al. 2000; Downing et al. 2001; Watson et al. 1997). It is well known that phytoplankton taxa differ in their resource requirements and in their responses to physical factors and grazing (Reynolds 1984). More detailed analyses of the functional and taxonomic composition of phytoplankton require time-consuming examination and enumeration of individual species under the microscope, so that such analyses are rarely included in routine monitoring or sampling programs of larger sets of lakes. Consequently, much empirical knowledge relating the abundances of single phytoplankton taxa to environmental factors is still largely based on descriptive case studies. Obviously, there is a need for more comprehensive, comparative studies of these relationships across larger sets of lakes. Phytoplankton taxa also differ in their light requirements and in sinking rates out of the mixed surface layer, both of which are mediated by depth of the mixed surface layer. With increasing mixing depth average mixed layer light intensity (affecting all taxa) and sinking losses (affecting negatively buoyant taxa) both decrease (Visser et al. 1996; Diehl et al. 2002; Soto 2002; Ptacnik et al. 2003). In spite of these apparent mechanisms, the influence of mixing depth on phytoplankton taxonomic composition has, to our knowledge, not yet been addressed with comparative lake data.

In the present study, I sampled phytoplankton, zooplankton, and a set of physical and chemical parameters in the mixed surface layers of 40 northern German lakes three times during summer stratification. I determined the taxonomic composition of the phytoplankton samples by analysing the samples for 13 algal pigments with high performance liquid chromatography (HPLC) and then calculated the quantitative contributions of seven algal classes differing in their pigment signatures to total chlorophyll-*a* using CHEMTAX program. CHEMTAX, a matrix factorisation program developed for marine phytoplankton (Mackey et al. 1996) combines information on a set of samples' contents of 'marker' pigments (which are unique to one or two algal groups) and 'diagnostic' pigments (shared by several algal groups) with taxon-specific pigment to Chl-*a* ratios. It calculates the contribution of each taxon to total Chl-*a* in an iterative process. So far, the use of CHEMTAX has been largely restricted to marine and brackish systems (Mackey et al. 1998; Wright et al. 1996; Wright et al. 2000; Higgins et al. 2000; Rodriguez et al. 2003; Schlüter et al. 2000; Schlüter et al. 2003). Very few studies have applied this technique in freshwater systems (Descy et al. 2000; Marinho et al. 2003; Fietz et al. 2004; Buchaca et al. 2005) with the consequence that class-specific pigment to Chl-*a* matrices for freshwater phytoplankton are still rare. Consequently, the output ratios of our lake study may help to establish this promising technique e.g. in large lake data sets.

Multiple regression analysis was used to examine the relationships of total phytoplankton biomass and taxonomic composition to a set of environmental variables including total nitrogen (TN), total phosphorus (TP), total silica (TSi), mixing depth, water temperature, and zooplankton biomass. The analyses showed that total Chl-*a* was positively related to TN and TP and unimodally related to mixing depth. Although molar TN:TP ratios suggested phosphorus to be relatively more limiting to phytoplankton growth than nitrogen, TN was the factor most strongly related to the biomass of the majority of individual taxa. I found positive relationships of chrysophytes and chlorophytes (the two most abundant taxa), cryptophytes, and euglenophytes to TN, and of diatoms and chrysophytes to TSi, whereas diatoms were negatively related to TN. Cryptophytes and chlorophytes were negatively, and cyanobacteria were positively related to zooplankton biomass. Finally, the relative biomasses of chrysophytes and cryptophytes were negatively related to mixing depth. Most, but not all, results are consistent with theoretical expectations or previously documented patterns. Some plausible relationships may, however, have

been masked by strong cross-correlations among several environmental variables. This study aims at contributing to the adaptation of the CHEMTAX procedure to freshwater systems and to the build-up of a comparative database relating the taxonomic composition of lake phytoplankton to a set of environmental drivers.

Synopsis

Interactions among environmental variables and plankton communities in lakes and oceans are in the focus of current aquatic ecological research in order to predict consequences of environmental changes for plankton communities. An important environmental factor is the depth of the mixed surface layer, where most aquatic primary production takes place. Mixing depth mediates both light availability (hence, light dependent production) and sedimentation losses of phytoplankton (Diehl 2002; Diehl et al. 2002). The field experiment and the comparative lake study along with a dynamical model that accounts for variable phytoplankton nutrient stoichiometry presented in this thesis provide an insight into proximate and ultimate effects of mixing depth, background turbidity, nutrient supply and their interrelationships with nutrient distribution, light, phytoplankton biomass and composition.

The results of the enclosure experiment produced a consistent picture of the effects of mixing depth and background attenuation (K_{bg}) on the light available for photosynthesis, phytoplankton production, phytoplankton biomass, and, partly, on the limiting nutrient (phosphorus) as predicted by the model of Diehl (2002). In the enclosure experiment where phytoplankton was exposed to a gradient of mixing depth under both high and low K_{bg} treatments I could demonstrate the negative effect of increased mixing depth and K_{bg} on mean light intensity and specific phytoplankton production rate. Furthermore, sedimentation loss rate was shown to be inversely related to mixing depth, leading to the predicted unimodal relationships of phytoplankton concentration and standing stock along a gradient of mixing depth (Diehl 2002). As expected, the maximum of the biomass peak shifted towards lower mixing depth at increased K_{bg}. With one exception, all assumptions of the model were corroborated in this experiment: Only the assumption of a constant algal cell quota proved to be incorrect: The seston carbon to phosphorus (C:P) ratio was negatively related to z_{mix} and K_{bg} and thus lead to the discrepancy between predicted and observed nutrient distributions among the different nutrient pools in the water column or in the sediment. Efforts to include a variable cell quota to a closed system model (Diehl 2002) are presented in (Diehl et al. 2005). The experimental phytoplankton communities were dominated by relatively fast sinking diatoms with mean sinking velocities of 0.3 m d^{-1} that most likely were favoured by the turbulent mixing regime and high silica concentrations in the enclosures. In lakes, the

phytoplankton composition generally is highly variable and consists of several morphologically different, positively or negatively buoyant algal taxa that I examined in article 3.

In a next step, we built on an open system model (Diehl 2002) which assumes the depth of the mixed surface layer to be lower than the whole water body, representing the situation in summer stratified lakes. We included variable seston carbon:phosphorus (C:P) ratio into the model, a feature which was detected in our previous field experiment and seems to be important in natural lake systems. In the comparative survey of 65 central European lakes that covered a broad range of total phosphorus (TP) concentrations and a moderate range of mixing depths (3-10 m) I observed most of the predicted relationships. Within the range of observed mixing depths, mean light availability, phytoplankton biomass, and seston C:P ratio were negatively related to mixing depth, whereas the standing stock of biomass showed a unimodal relationship, and total and dissolved nutrients showed a horizontal or increasing relationship to z_{mix}. Nutrient enrichment, here elevated total phosphorus (TP) concentrations, led to a decrease of light intensity at the bottom of the mixed layer because of the negative feed back of increased phytoplankton biomass on light. The seston C:P and dissolved and total phosphorus fractions were all positively related to TP. Water temperature was unrelated to any of the dependent variables, except for light climate. The results indicated that stratification depth is an important environmental driver of the dynamics of light, nutrients and phytoplankton biomass in the surface layer of lakes. The effects of mixing depth may propagate to higher trophic levels as indicated by a strong negative relationship of mixing depth and zooplankton biomass in a subset of the lakes studied.

In order to examine how the taxonomic composition of phytoplankton biomass in lakes relates to abiotic and biotic variables I extended the analysis of environmental variables in the north German subset of the comparative lake survey (40 lakes) to include total nitrogen (TN), total silica (TSi), water temperature and zooplankton biomass. The taxonomic composition of phytoplankton was estimated using pigment based HPLC analysis (Barlow et al. 1997) in combination with the CHEMTAX program (Mackey et al. 1996), a new and fast method for analysing phytoplankton samples of large data sets up to the class level. While CHEMTAX was originally developed for marine and brackish water systems, pigment to chlorophyll-*a* ratios for freshwater systems are still rare (Descy et al. 2000). This study aimed at contributing

to the adaptation of the CHEMTAX procedure to freshwater systems and to the build-up of a comparative database relating the taxonomic composition of lake phytoplankton to a set of environmental drivers. Most, but not all, results are consistent with theoretical expectations or previously documented patterns (Watson et al. 1997; Huszar et al. 1998). Some plausible relationships may, however, have been masked by strong cross-correlations among several environmental variables, especially the negative correlation between z_{mix} and TN. The analyses showed that total Chl-*a* was positively related to TN and TP and unimodally related to mixing depth. Although molar TN:TP ratios suggested phosphorus to be the nutrient limiting phytoplankton growth relatively more to TN was the factor most strongly related to the biomass of the majority of individual taxa. I found positive relationships of chrysophytes, chlorophytes, cryptophytes, and euglenophytes to TN, and of diatoms and chrysophytes to TSi, whereas diatoms were negatively related to TN. Cryptophytes and chlorophytes were negatively, and cyanobacteria were positively related to zooplankton biomass. Finally, the relative biomasses of chrysophytes and cryptophytes were negatively related to mixing depth. Although mixing depth was often retained in multiple regression analyses, suggesting that nutrient supply had a stronger effect, mixing depth was significantly negatively correlated to four of seven phytoplankton taxa.

With global climate change threatening to disrupt seasonal and regional stratification patterns (Weyhenmeyer et al. 1999; Gerten et al. 2002; Livingstone 2003) detailed knowledge of the role of mixed layer depth in plankton dynamics will help to forecast effects of climate change on the dynamics and essential compartments of lake ecosystems.

Outlook

The Intergovernmental Panel on Climate Change (IPCC) recently stated that the global increase in mean temperature is most likely due to the increase of greenhouse gases (e.g., CO_2). Global warming has already been related to changes in stratification patterns of lakes, e.g. earlier onset and delayed break-down of stratification in central European lakes, extended duration of spring turnover in Scandinavian lakes, and increase in summer stratification depth of small Canadian lakes (Weyhenmeyer et al. 1999; Sterner 1990; Livingstone 2003; Fee et al. 1996). Since mixed surface layer depth is determined by climatic conditions, (Fee et al. 1996) the effects of mixing depth on phytoplankton biomass, light and nutrients as described by our models and observed by empirical studies are of prime importance. Our comparative lake research indicates that the epilimnetic concentrations of algal and crustacean biomass during summer stratification are positively related to nutrient content oft the water, but negatively related to mixing depth and largely unrelated to temperature.

Phytoplankton biomass in lakes is influenced by various physical-chemical variables affecting primary production (mixing depth, nutrient supply) and grazing (temperature), but the complex interplay among these various climate related factors are not yet fully understood. Successful prediction of climate effects on lake ecosystems requires a detailed understanding of the consequences of climate driven stratification scenarios for the dynamic interplay of phytoplankton and zooplankton.

A possible summer scenario arises from an increase in air temperature, which will rise lake surface temperatures. Together with lower summer wind speeds, the stability of stratification should increase and mean mixing depth decrease. This should lead to an increase in light availability for phytoplankton that may be exploited by additional phytoplankton biomass. Depending on regional precipitation patterns, their possible change, and land use around lakes either nutrient enrichment (in case of increasing precipitation) or depletion (in case of decreasing precipitation) may occur in the surface mixed layer of lakes. As a result, phytoplankton growth then might either be less light- and/or nutrient limited and more pronounced algal blooms occur with possible implications for water quality, carbon sequestration and fish catch.

References

Barlow, R.G., D.G. Cummings, and S.W. Gibb, 1997. Improved resolution of mono- and divenyl chlorophylls a and b and zeaxanthin and lutein in phytoplankton extracts using reverse phase C-8 HPLC. *Mar. Ecol. - Proc. Ser.* **161:** 303-307.

Buchaca, T., M. Felip, and J. Catalan, 2005. A comparison of HPLC pigment analyses and biovolume estimates of phytoplankton groups in an oligotrophic lake. *J. Plankton Res.* **27:** 91-101.

Carpenter, S.R., J.J. Cole, J.F. Kitchell, and M.L. Pace, 1998. Impact of dissolved organic carbon, phosphorus, and grazing on phytoplankton biomass and production in experimental lakes. *Limnol. Oceanogr.* **43:** 73-80.

Cuker, B.E., 1987. Field experiment on the influences of suspended clay and P on the plankton of a small lake. *Limnol. Oceanogr.* **32:** 840-847.

Descy, J.P., H.W. Higgins, D.J. Mackey, J.P. Hurley, and T.M. Frost, 2000. Pigment ratios and phytoplankton assessment in northern Wisconsin lakes. *J. Phycol.* **36:** 274-286.

Diehl, S., 2002. Phytoplankton, light, and nutrients in a gradient of mixing depths: theory. *Ecology* **83:** 386-398.

Diehl, S., S.A. Berger, R. Ptacnik, and A. Wild, 2002. Phytoplankton, light, and nutrients in a gradient of mixing depths: field experiments. *Ecology* **83:** 399-411.

Diehl, S., S.A. Berger, and R. Wöhrl, 2005. Flexible algal nutrient stoichiometry mediates environmental influences on phytoplankton and its abiotic resources. *Ecology **86***: 2931-2945.

Dillon, P.J. and F.H. Rigler, 1974. Phosphorus-chlorophyll relationship in lakes. *Limnol. Oceanogr.* **19:** 767-773.

Downing, J.A., S.B. Watson, and E. McCauley, 2001. Predicting Cyanobacteria dominance in lakes. *Can. J. Fish. Aquat. Sci.* **58:** 1905-1908.

Droop, M.R., 1974. Nutrient status of algal cells in continuous culture. *J. Mar. Biol. Ass. U.K.* **54:** 825-855.

Fee, E.J., R.E. Hecky, S.E.M. Kasian, and D.R. Cruikshank, 1996. Effects of lake size, water clarity, and climatic variability on mixing depths in Canadian Shield lakes. *Limnol. Oceanogr.* **41:** 912-920.

Fietz, S. and A. Nicklisch, 2004. An HPLC analysis of the summer phytoplankton assemblage in Lake Baikal. *Freshw. Biol.* **49:** 332-345.

Gerten, D. and R. Adrian, 2002. Effects of climate warming, North Atlantic Oscillation, and El Niño-Southern Oscillation on thermal conditions and plankton dynamics in northern hemispheric lakes. *Sci. World J.* **2:** 586-606.

Guildford, S.J., F.P. Healey, and R.E. Hecky, 1987. Depression of primary production by humic matter and suspended sediment in limnocorral experiments at southern Indian lake, Northern Manitoba. *Can. J. Fish. Aquat. Sci.* **44:** 1408-1417.

Guildford, S.J., L.L. Hendzel, H.J. Kling, E.J. Fee, G.G.C. Robinson, R.E. Hecky, and S.E.M. Kasian, 1994. Effects of lake size on phytoplankton nutrients status. *Aqu. Sci.* **51:** 2769-2783.

Higgins, H.W. and D.J. Mackey, 2000. Algal class abundances, estimated from chlorophyll and carotenoid pigments, in the western Equatorial Pacific under El Niño and non-El Nino conditions. *Deep-Sea Res. Pt I – Oceanogr. Res. Papers* **47:** 1461-1483.

Huisman, J. and F.J. Weissing, 1995. Competition for nutrients and light in a mixed water column: a theoretical analysis. *Am. Nat.* **146:** 536-564.

Huisman, J., 1999. Population dynamics of light-limited phytoplankton: Microcosm experiments. *Ecology* **80:** 202-210.

Huszar, V.L.D. and N.F. Caraco, 1998. The relationship between phytoplankton composition and physical-chemical variables: a comparison of taxonomic and morphological-functional descriptors in six temperate lakes. *Freshw. Biol.* **40:** 679-696.

Jeppesen, E., T.L. Lauridsen, S.F. Mitchell, K. Christoffersen, and C.W. Burns, 2000. Trophic structure in the pelagial of 25 shallow New Zealand lakes: changes along nutrient and fish gradients. *J. Plankton Res.* **22:** 951-968.

Kirk, J.T.O. *Light and Photosynthesis in Aquatic Ecosystems.*, 2nd ed., Cambridge University Press (1994).

Kirk, J.T.O. and P.A. Tyler, 1986. The spectral absorption and scattering properties of dissolved and particulate components in relation to the underwater light yield of some Australian freshwaters. *Freshw. Biol.* **16:** 573-583.

Kunz, T.J. and S. Diehl, 2003. Phytoplankton, light and nutrients along a gradient of mixing depth: a field test of producer-resource theory. *Freshw. Biol.* **48:** 1050-1063.

Livingstone, D.M., 2003. Impact of secular climate change on the thermal structure of a large temperate central European lake. *Climatic Change* **57:** 205-225.

Mackey, D.J., H.W. Higgins, M.D. Mackey, and D. Holdsworth, 1998. Algal class abundances in the western equatorial Pacific: Estimation from HPLC measurements of chloroplast pigments using CHEMTAX. *Deep-Sea Res. Pt I – Oceanogr. Res. Papers* **45:** 1441-1468.

Mackey, M.D., D.J. Mackey, H.W. Higgins, and S.W. Wright, 1996. CHEMTAX - A program for estimating class abundances from chemical markers: Application to HPLC measurements of phytoplankton. *Mar.Ecol.-Proc.Ser.* **144:** 265-283.

Marinho, M.M. and S.V. Rodrigues, 2003. Phytoplankton of an eutrophic tropical reservoir: comparison of biomass estimated from counts with chlorophyll-*a* biomass from HPLC measurements. *Hydrobiologia* **505:** 77-88.

Mazumder, A. and W.D. Taylor, 1994. Thermal structure of lakes varying in size and water clarity. *Limnol. Oceanogr.* **39:** 968-976.

Persson, L., S. Diehl, L. Johansson, G. Andersson, and S.F. Hamrin, 1992. Trophic interactions in temperate lake ecosystems - a test of food-chain theory. *Am. Nat.* **140:** 59-84.

Ptacnik, R., S. Diehl, and S.A. Berger, 2003. Performance of sinking and non-sinking phytoplankton taxa in a gradient of mixing depths. *Limnol. Oceanogr.* **48:** 1903-1912.

Reynolds, C.S. *The ecology of freshwater phytoplankton.* 1984. Cambridge University Press.

Reynolds, C.S., S.W. Wiseman, and M.J.O. Clarke, 1984. Growth- and loss-rate responses of phytoplankton to intermittent artificial mixing and their potential application to the control of planktonic algal biomass. *J. Appl. Ecol.* **21:** 11-39.

Rodriguez, F., Y.Pazos, J.Maneiro, and M.Zapata, 2003. Temporal variation in phytoplankton assemblages and pigment composition at a fixed station of the Ria of Pontevedra (NW Spain). *Estuar. Coast. Shelf Sci.* **58:** 499-515.

Rowan, K.S. *Photosynthetic pigments of algae*. 1989. Cambridge University Press.

Sarnelle, O., 1992. Nutrient enrichment and grazer effects on phytoplankton in lakes. *Ecology* **73:** 551-560.

Scheffer, M. *The ecology of shallow lakes*. Chapman and Hall (1998).

Schindler, D.W., 1978. Factors regulating phytoplankton production and standing crop in the world´s freshwaters. *Limnol. Oceanogr.* **23:** 478-486.

Schlüter, L., F. Mohlenberg, H. Havskum, and S. Larsen, 2000. The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: testing the influence of light and nutrients on pigment/chlorophyll *a* ratios. *Mar. Ecol.- Progr. Ser.* **192:** 49-63.

Schlüter, L. and F. Mohlenberg, 2003. Detecting presence of phytoplankton groups with non-specific pigment signatures. *J. Appl. Phycol.* **15:** 465-476.

Soto, D., 2002. Oligotrophic patterns in southern Chilean lakes: the relevance of nutrients and mixing depth. *Rev. Chil. Hist. Nat.* **75:** 377-393.

Sterner, R.W., 1990. Lake morphometry and light in the surface-layer. *Can. J. Fish. Aquat. Sci.* **47:** 687-692.

Sterner, R.W., J.J. Elser, E.J. Fee, S.J. Guildford, and T.H. Chrzanowski, 1997. The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *Am. Nat.* **150:** 663-684.

Sverdrup, H.U., 1953. On conditions for the vernal blooming of phytoplankton. *J. Cons. Perm. Int. Explor. Mer* **18:** 287-295.

Tilman, D. *Resource competition and community structure*. 1982. Princeton University Press.

Tilman, D., R. Kiesling, R. Sterner, S.S. Kilham, and F.A. Johnson, 1986. Green, bluegreen and diatom algae - taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. *Arch. Hydrobiol.* **106:** 473-485.

Tilzer, M.M., 1983. The importance of fractional light absorption by photosynthetic pigments for phytoplankton productivity in Lake Constance. *Limnol. Oceanogr.* **28:** 833-846.

Visser, P.M., L. Massaut, J. Huisman, and L.R. Mur, 1996. Sedimentation losses of Scenedesmus in relation to mixing depth. *Arch. Hydrobiol.* **136:** 289-308.

Watson, S.B., E. McCauley, and J.A. Downing, 1997. Patterns in phytoplankton taxonomic composition across temperate lakes of different nutrient status. *Limnol. Oceanogr.* **42:** 487-495.

Weyhenmeyer, G.A., T. Blenckner, and K. Pettersson, 1999. Changes of the plankton spring outburst related to the North Atlantic Oscillation. *Limnol. Oceanogr.* **44:** 1788-1792.

Wright, S.W., D.P. Thomas, H.J. Marchant, H.W. Higgins, M.D. Mackey, and D.J. Mackey, 1996. Analysis of phytoplankton of the Australian sector of the Southern Ocean: Comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using the 'CHEMTAX' matrix factorization program. *Mar. Ecol.-Progr. Ser.* **144:** 285-298.

Wright, S.W. and R.L. van den Enden, 2000. Phytoplankton community structure and stocks in the East Antarctic marginal ice zone (BROKE survey, January-March 1996)

determined by CHEMTAX analysis of HPLC pigment signatures. *Deep-Sea Res. Pt.Ii –Top. Stud. Oceanogr.* **47:** 2363-2400.

Article 1

EFFECTS OF MIXING DEPTH AND BACKGROUND TURBIDITY ON PHYTOPLANKTON BIOMASS, LIGHT AND NUTRIENTS

(Stella A. Berger, Sebastian Diehl, and Rainer Wöhrl)

Effects of mixing depth and background turbidity on phytoplankton biomass, light and nutrients

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Abstract

Most phytoplankton production occurs in the turbulently mixed surface layer of lakes and oceans. Environmental factors such as mixed layer depth and background turbidity substantially influence the availability of essential, production-limiting resources, i.e., light and nutrients. We tested the predictions of a dynamic model concerning the effects of water column mixing depth and background turbidity on phytoplankton biomass, light climate and nutrients in a field enclosure experiment. The epilimnetic phytoplankton community of a low productive lake was exposed to high and low background turbidity along a gradient of mixing depths ranging from 1 to 15 m. The availability of light and thus the specific rate of light-limited phytoplankton production decreased with increasing mixing depth and background turbidity. Specific sedimentation loss rate decreased with increasing mixing depth, too, but was not influenced by background turbidity. Fast-sinking diatoms dominated the phytoplankton community. For this scenario, the model predicts that algal biomass should be most strongly limited by sinking losses at shallow mixing depths, by mineral nutrients at intermediate mixing depths and by a lack of light at high mixing depths. As predicted from this shifting pattern of limitation, phytoplankton volumetric and areal biomass showed unimodal trends along the mixing depth gradient and were negatively influenced by background turbidity. A predicted shift of the biomass maxima towards shallower mixing depths with increased background turbidity was also observed. Only partly in line with expectations the concentrations of dissolved and total phosphorus in the water column were positively affected by mixing depth but were only marginally affected by background turbidity. In addition, the standing stock of sedimented phosphorus did not conform to the expected unimodal relationship with mixing depth. A possible explanation for the latter deviations is that the ratio of seston carbon to phosphorus was not constant, but decreased with increasing mixing depth and background turbidity.

Introduction

Most pelagic primary production occurs in the light-transmitted, turbulently mixed surface layer of lakes and oceans (Sverdrup 1953; Kirk 1994; Huisman et al. 1999). The factors determining population growth rates of primary producers are gradually becoming understood (Mann and Lazier 1996; Falkowski and Raven 1997; Geider et al. 2001). Phytoplankton growth is primarily influenced by the availability of potentially limiting nutrients such as nitrogen, phosphorus, silica and iron, (Martin et al. 1990; Platt et al. 1992) by light availability (Mitchell et al. 1991; Huisman 1999), and by nutrient-light interactions (Mitchell et al. 1991; Sakshaug et al. 1991; Sterner et al. 1997; Sunda and Huntsman 1997; Lancelot et al. 2000; Fahnenstiel et al. 2000; Colijn and Cadee 2002). Light limitation seems to be relevant in upwelling regions and estuaries where the nutrient supply is high (Irigoien and Castel 1997; Bagheri et al. 2002; Bergmann et al. 2002; Gago et al. 2002), but also in nutrient-poor environments under conditions of deep mixing (Diehl et al. 2002; Soto 2002; Kunz and Diehl 2003; Ptacnik et al. 2003) and in turbid waters stained by humic substances or clay particles (Guildford et al. 1987; Cuker 1987; Carpenter et al. 1998). Environmental drivers such as mixing depth and background turbidity are subject to seasonal and geographical variation caused by regional climate, basin morphometry, and input of light-absorbing substances from the catchment (Wetzel 1983; Sterner 1990; Imboden and Wüest 1995). Mixing depth may range from a few meters to several tens of meters in lakes to more than 500 m in the open ocean (Kirk 1977; Wetzel 1983; Kirk 1994; Soto 2002; Farstey et al. 2002). Background turbidity, expressed as the vertical extinction coefficient of PAR, may vary from 0.03 m^{-1} in the ultraoligotrophic open ocean (Tyler 1975; Bienfang et al. 1984) to beyond 15 m^{-1} in turbid estuaries and humic lakes (Kirk 1994).

While background turbidity and depth of the mixed layer both affect production negatively, mixing depth also influences sedimentation losses of sinking phytoplankton species. Sedimentation loss rate is inversely related to mixing depth (Reynolds 1984; Visser et al. 1996; Diehl 2002; Ptacnik et al. 2003) and thus becomes most relevant in shallow mixed layers. Theoretical expectations and experimental studies (Boyd 2002; Diehl 2002; Diehl et al. 2002; Ptacnik et al. 2003) indicate that along a gradient of increasing mixing depth phytoplankton biomass becomes limited by different processes. When phytoplankton is dominated by sinking

species, algal biomass is most strongly limited by sedimentation losses in shallow mixed layers and by a lack of light in deep mixed layers. In between, where neither sinking losses nor light limitation are particularly intense, a biomass maximum occurs and nutrient limitation of algal growth becomes most important. Higher background turbidity does not only reduce light availability, but also affects the biomass - mixing depth relationship by shifting the biomass maximum towards shallower mixing depths (Diehl 2002). This is because higher background turbidity shifts the onset of strong light limitation towards shallower mixing depths.

In recent field enclosure experiments, we found evidence in support of the above expectations (Diehl et al. 2002). Light availability, algal production, and sedimentation losses decreased with increasing mixing depth, and biomass maxima occurred at intermediate mixing depths. In addition, our experimental results suggested that higher background turbidity reduced phytoplankton biomass at all mixing depths and shifted the biomass maximum towards shallower mixing depths. The experiments simulating moderate and high background turbidity were, however, carried out in different years. Initial conditions (e.g., phytoplankton biomass and composition) and the weather (e.g., air temperature and duration of daily sunlight) varied somewhat between years (Diehl et al. 2002). To verify that a significant portion of the observed differences between our former experiments could indeed be attributed to differences in background turbidity, we conducted a field enclosure experiment in which mixing depth and background turbidity were varied simultaneously. The experiment was furthermore designed to test a whole suite of steady state model predictions about the influence of mixing depth and background turbidity on natural phytoplankton, light climate, and nutrient distribution (Diehl 2002). The model of Diehl (2002) includes predictions about the distribution of the limiting nutrient among different compartments (dissolved, particulate suspended, and particulate sedimented fractions). In our previous experiments, only nutrients in the water column were investigated (Diehl et al. 2002). Here, we additionally test whether the (areal) standing stock of sedimented nutrients shows the predicted relationships to mixing depth and background turbidity.

Before we describe the experiment, we briefly review the qualitative predictions of the model (see Diehl 2002 for a detailed treatment). We illustrate these predictions with a numerical example that was parameterized to roughly mimic the environmental conditions in our experiment (see legend of Fig. 1 for parameter values). We

emphasize, however, that the illustrations are not meant to represent exact quantitative predictions.

Model assumptions and predictions

The model describes population growth and loss processes of phytoplankton and the dynamics of light and nutrients in a water column in contact with the sediment. The water column is assumed to be homogeneously mixed, i.e., algae and nutrients are uniformly distributed throughout the mixed layer. Photosynthetically active radiation (PAR) enters the mixed layer from above at constant incident intensity and declines exponentially with depth. Vertical light attenuation is caused by the water itself and by dissolved and particulate substances including the phytoplankton. Thus, the vertical light gradient is dynamic, i.e., changes in algal biomass feed back directly on the light climate. Following Huisman and Weissing (1995), specific phytoplankton production is assumed to be co-limited by light and a single mineral nutrient (e.g. phosphorus), i.e., specific production is a monotonously increasing function of light intensity and of the limiting mineral nutrient concentration. Consequently, specific algal production follows the vertical light gradient and is assumed to decrease with increasing mixing depth, background turbidity, and biomass density. Phytoplankton losses consist of a constant background rate and a specific sedimentation loss rate, which also is a decreasing function of mixing depth.

The nutrient content per unit algal biomass is assumed to be fixed and independent of mixing depth and background turbidity. The system is assumed to be closed and to contain a fixed amount of the limiting nutrient. Thus, the supply of nutrients to the mixed layer originates exclusively from remineralisation of sedimented biomass at a constant specific rate. This represents situations in which the mixed layer is in direct contact with the sediments, e.g. shallow lakes or deep systems during periods of destratification. If the external light supply and the total amount of limiting nutrient in the system are sufficiently high, the system has an interior equilibrium that is unique and locally stable (Diehl 2002). Equilibrium model predictions are derived formally and explained in Diehl (2002). Here, we summarize the predicted effects of mixing depth and background turbidity on phytoplankton biomass, light, and nutrients (in the following, symbols for state variables at equilibrium are marked by an asterisk): (1) along a gradient of increasing mixing depth algal biomass concentration, ω^*, shows a unimodal distribution and is

negatively affected by background turbidity, K_{bg} (Fig. 1a). (2) Algal standing stock of biomass per area, W^* ($= \omega^* \times z_{mix}$, where z_{mix} is the depth of the mixed layer), shows a unimodal relationship to mixing depth and is negatively affected by background turbidity (Fig. 1b). (3) The mixing depths at which the peaks in biomass concentration and standing stock occur decrease with increasing background turbidity (Fig. 1a, b). (4) Average light intensity in the mixed layer, I^*_{mix}, as well as the light intensity at the bottom of the mixed layer, I^*_{out}, decrease with mixing depth and are negatively affected by background turbidity (Fig. 1c). (5) The standing stock of nutrients in the sediment, R^*_s, shows the same unimodal relationship to mixing depth as does algal standing stock and is also negatively affected by background turbidity (Fig. 1f). At equilibrium, the remineralisation of nutrients must balance nutrient input to the sediments, because specific remineralisation rate is assumed to be constant and total (=areal) algal losses through sedimentation are proportional to algal standing stock. (6) Consequently, an increase in background turbidity pushes the mixing depth at which R^*_s peaks towards shallower depths (Fig. 1f). (7) Finally, and roughly inverse to the trends of ω^* and R_s^*, the water column concentrations of the dissolved mineral nutrient, R^*, and of the total (= dissolved plus particulate) nutrient, R^*_m, show a U-shaped relationship to mixing depth and are positively affected by background turbidity (Fig. 1 d, e). The decelerating limbs of the nutrient-mixing depth relationships occur, however, over a range of very shallow mixing depths (<1.5 m) unlikely to be observed in many natural systems over longer time scales (Fig. 1d, e; Diehl 2002).

Material and Methods

Study site and experimental design

We tested the dynamical model in an enclosure experiment carried out in Lake Brunnsee, which is situated close to the University of Munich's Limnological Research Station at Seeon, 90 km east of Munich, Germany. This small (area 5.8 ha), low productive hard water lake has a maximum depth of 19 m. Summer chlorophyll *a* concentration usually is around 2 µg L^{-1}, Secchi depth 7-15 m, and total phosphorus concentration 4-10 µg L^{-1}.

Experimental gradients of mixing depth were created by enclosing the natural, epilimnetic phytoplankton community in a series of 16 cylindrical plastic bags of 8

different depths (1; 2.5; 4; 6; 8; 10; 12; 15 m). The enclosures had a diameter of 0.95 m, extended 0.2 m above the water level, and were open to the atmosphere. The inner enclosure walls were of clear plastic (Tricoron R, BP Chemicals Wasserburg, Germany). To manipulate background attenuation we covered the outer enclosure walls with white or black silage film simulating two different background turbidities at each depth. The resulting vertical light (PAR) extinction coefficients on day 1 of the experiment were 0.93 m^{-1} and 1.31 m^{-1}, respectively, which we call 'low' and 'high' background turbidity treatments. The corresponding extinction coefficient in the lake was 0.43 m^{-1} (all extinction coefficients were calculated for the upper 5 m by linear regression of log-transformed PAR against depth).

When filling the enclosures we filtered the epilimnetic water through 100-µm mesh gauze that should retain mesozooplankton to ensure that sedimentation and not grazing was the principal loss process of phytoplankton. To monitor the abundance of zooplankton we took vertical hauls with a 35-µm mesh net in each enclosure every 2 weeks, fixed the samples with Lugol's solution and counted them under a dissecting scope. Mesozooplankton density was low during the experiment. When necessary, we removed mesozooplankton by vertical hauls with a 200-µm mesh net of a diameter slightly smaller than the enclosures. The abundances of copepods and cladocerans (mostly *Bosmina* and *Scapholeberis*) never exceeded 1.5 individuals L^{-1} and 3 individuals L^{-1}, respectively, in any enclosure and thus mesozooplankton grazing should not have strongly influenced phytoplankton biomass (Sommer et al. 2001). Periphyton started to visibly grow on the upper 2-3 m of the inner enclosure walls after 5-6 weeks and was thereafter removed weekly by brushing.

Concentrations of dissolved inorganic nutrients in the enclosures at the beginning of the experiment were 5.2 mg nitrate-N L^{-1} and 5.9 mg Si L^{-1}. N and Si remained far in excess of phytoplankton demands for phosphorus throughout the experiment (atomic N:P > 800 and Si:P > 300). We initially enriched all enclosures with 8 µg L^{-1} phosphorus to a total phosphorus content of 14 µg L^{-1} to stimulate primary production moderately in the phosphorus-poor water. Well-mixed conditions within the enclosures were maintained by intermittently pumping air through tubings to the bottom of each enclosure (for one minute every 5 minutes). The mixing was highly effective and water temperatures within the enclosures showed no vertical differences. There were, however, temperature differences among enclosures.

Initially, shallow enclosures were warmer than the deeper ones, which extended deeper into the colder hypolimnion of the lake and therefore forced a heat exchange. At the beginning of the experiment water temperatures ranged from 11.6 °C in the deepest to 17.4 °C in the shallowest enclosures. Temperature differences among enclosures diminished as the autumn cooling of the epilimnion progressed. In week 4, temperatures ranged from 10.1 °C to 14.4 °C and at the end of week 8 only from 9.1 °C to 9.5 °C. Background turbidity treatment did never affect water temperatures (paired T-tests, $p > 0.5$). To calculate sedimentation losses two sets of triplicate sedimentation traps (height 90 mm, opening diameter 34 mm) were installed just above the bottom of each enclosure. One set was sampled and replaced weekly. Another set was recovered at the end of the experiment to assess the final standing stock of sedimented phosphorus. The field experiment lasted for 8 weeks from 8 September to 2 November 1999.

Sampling program and laboratory analysis

Once per week all enclosures were sampled for physical, chemical and biological parameters. Starting just below the water surface we measured water temperature and electrical conductivity with a conductivity meter (Lf 191 with probe LT1/T, WTW, Weilheim, Germany) in steps of 1 m. Photosynthetically active radiation (PAR) was measured from just below the water surface in 1 m steps with a spherical quantum sensor (LI-139SA) connected to the data logger LI-1000 (LICOR, Lincoln, Nebraska, USA). Parallel to each underwater reading we took a reading of incident PAR with a flat quantum sensor (LI-190SA) just above the water surface. Thus, variations in cloud cover during the measurements could be corrected for in the subsequent calculations for relative light levels. Water column concentrations of dissolved silica, dissolved inorganic phosphorus (SRP), particulate phosphorus (PP) and total phosphorus (TP) as well as TP in the sediment traps were measured using standard methods (Wetzel and Likens 1991). SRP measurements were done during the whole experimental period. Concentrations of SRP at the end of the experiment (week 8) were below the detection limit. To estimate the concentration of dissolved (organic plus inorganic) phosphorus we calculated the difference of TP minus PP. Nitrate was analysed on 0.45 µm filtered samples using a Dionex DX 100 ion chromatograph (Dionex, Idstein, Germany). Particulate (seston) organic carbon (POC) in the water column as well as POC in the sediment traps was filtered on pre-combusted 0.45 µm

glass fiber filters and determined by infrared-spectrophotometry (C-Mat 500, Ströhlein, Germany).

For phytoplankton counts, we took water samples of 200 ml and immediately preserved them with Lugol's solution. Subsamples of 50 ml were transferred to sedimentation chambers and the phytoplankton therein was identified and counted in an inverted microscope according to Utermöhl (1958) after 20 hours of sedimentation. A digital camera (model VCB-3512 P, Sanyo) was connected to the microscope and an appropriately sized area was scanned to count at least 100 individual cells from each of the abundant taxa. Small taxa (< 50 µm) were counted at 400x magnification and larger taxa at 100x magnification. To estimate the mean biovolume of each of these taxa 20 to 50 individual cells were measured using an image analysis program (Analysis Pro 3.00, Soft Imaging Software GmbH) and calculated using geometrical forms (Tümpling and Friedrich 1990). Total phytoplankton biovolume resulted as the sum of all taxa counted. Biovolume was converted to fresh biomass assuming a conversion factor of 1 cm³ = 1 g.

Calculation of light parameters, production and loss rates

Average light intensity, I_{mix}, characterizes the mean availability of PAR to algae in the mixed layer. We estimated I_{mix} as the percentage of incident PAR, I_0, immediately below the water surface as:

$$I_{mix} = \frac{1}{z}\sum_{i=1}^{i=z}\left[\frac{I_{i-1} + I_i}{2}\right] \qquad (1)$$

where i is depth in meters, z is enclosure depth and I_i is PAR at depth i in percent of I_0.

For each enclosure we calculated seston net and gross growth rates and the sedimentation rates of particulate organic carbon over weekly intervals. Specific daily net production, p_n, was calculated as:

$$p_n = \frac{1}{t}\ln\left(\frac{W_t}{W_o}\right) \qquad (2)$$

where t is the sampling interval in days, and W_0 and W_t are the standing stocks of seston organic carbon at the beginning and at the end of the sampling interval, respectively.

Assuming that sedimentation was the only relevant loss process, specific sedimentation loss rate, l_s, was calculated as:

$$l_s = p_n\left(\frac{F}{W_t - W_0}\right) \quad (3)$$

where *F* is the amount of particulate organic carbon per unit area lost through sedimentation in the time interval 0 to *t*. Sedimentation loss rate was plotted against mixing depth and power functions with exponents close to –1 gave an excellent fit to the data. Thus, we estimated sinking velocity, *v*, according to the theoretically expected form (Reynolds 1984):

$$v = l_s z_{mix} \quad (4)$$

Specific gross production rate, p_g, was calculated for each enclosure as the sum of specific net production and sedimentation losses.

$$p_g = p_n + l_s \quad (5)$$

Statistical analyses

For statistical analyses of monotonic relationships between dependent variables and mixing depth we first transformed variables to linearize relationships, if necessary, and then performed ANCOVAs with background turbidity as a categorical variable and mixing depth as the covariate. Interaction terms were never significant ($P>0.5$) and therefore dropped from the models. For statistical analyses of unimodal relationships among variables we used the approaches described in the section 'Test of model predictions'. When effects of background turbidity in ANCOVAs were marginally non-significant ($0.1>P>0.05$) and in the case of unimodal relationships to mixing depth we tested for background turbidity effects with pairwise t-tests after pairing turbidity treatments by mixing depth.

Results

We first test whether the results of our enclosure experiment are in agreement with four key assumptions of the dynamical model. We then compare the results with the model predictions illustrated in Fig. 1. Because predictions are for equilibrium conditions, we use data from the last sampling date (week 8).

Verification of model assumptions

Average light intensity decreases with increasing mixing depth and is negatively affected by background turbidity. These assumptions were corroborated. Mean intensity of PAR, I_{mix} on day 1 decreased with increasing mixing depth and was

negatively affected by background turbidity at all enclosure depths (Fig. 2a, ANCOVA of effects of log-transformed mixing depth and background turbidity on log-transformed I_{mix} ; r^2 = 0.97; $P(z_{mix})$ < 0.001; $P(K_{bg})$ = 0.001). Average intensity of PAR in the upper 5m, the actual mixing depth of Lake Brunnsee was 41 % of incident PAR in the lake (Fig. 2a) compared to 21 % and 15 % of incident PAR at low and high background turbidity, respectively.

Specific production decreases with increasing mixing depth and is negatively affected by background turbidity. As assumed, specific gross production rate of seston POC, p_g, decreased with increasing mixing depth during week 1 (Fig. 2b) and throughout the entire experiment (data not shown) and was negatively affected by background turbidity (Fig. 2b, ANCOVA of effects of mixing depth and background turbidity on log-transformed specific gross production rate; r^2 = 0.87; P (z_{mix}) < 0.001; P (K_{bg}) = 0.07; paired t-test, P (K_{bg}) = 0.029). There was a very tight correlation between I_{mix} and specific gross production rate in the enclosures on day 1 (Pearson's R = 0.95; n = 16; P < 0.001).

Specific sedimentation loss rate decreases with increasing mixing depth. Throughout the experiment, relatively fast sinking diatoms dominated the phytoplankton community, *Cyclotella* at the beginning, *Synedra* and *Fragilaria* at the end of the experiment. As assumed, specific sedimentation loss rate of seston POC decreased with mixing depth during week 1 (Fig. 2c) and throughout the entire experiment (data not shown) and was independent of background attenuation (ANCOVA of effects of $1/z_{mix}$ and background turbidity on sedimentation loss rate; r^2 = 0.97; P (z_{mix}) < 0.001; P (K_{bg}) = 0.85). We fitted the log-transformed data to a linear function of the form log l_s = log (v/z_{mix}), where algal sinking velocity, v, was 0.25 m d^{-1} (Fig. 2c).

The Nutrient content per algal biomass is fixed and independent of mixing depth and background turbidity. This simplifying model assumption was violated. The atomic seston C:P ratio at the end of week 8 declined with increasing mixing depth and background turbidity (Fig. 2d, ANCOVA of log-transformed mixing depth and background turbidity on log-transformed C:P ratio; r^2 = 0.83; P (z_{mix}) < 0.001; P (K_{bg}) = 0.007).

Test of model predictions

The concentration, ω^*, and standing stock, W, of phytoplankton biomass show a unimodal relationship to mixing depth and are negatively affected by background turbidity (Fig. 1a,b)*. In accordance with expectations, phytoplankton biomass concentration and areal standing stock were negatively affected by background turbidity (Fig. 3a,b; paired t-tests, P (K_{bg}) = 0.022 and 0.042 for ω and W, respectively). Similar to the numerical example, this effect was clearly visible only for mixing depths > 2.5 m.

Also in accordance with expectations, visual inspection of the data suggested that the concentration and standing stock of phytoplankton biomass peaked at intermediate mixing depths (Fig. 3a,b). Because treatments were unreplicated, we used the following approach to test statistically for the existence of biomass maxima at intermediate mixing depths. We grouped the mixing depths into the three categories 'low' (1-4m), 'intermediate' (6-10m), and 'high' (12-15m). We then ran two-way ANOVAs with the treatment categories mixing depth (low, intermediate, high) and background turbidity (low, high) on log-transformed biomass data. Effects of mixing depth on phytoplankton biomass concentration and on algal standing stock were significant (P (z_{mix}) ≤ 0.003). Biomass concentration and areal standing stock were indeed higher at intermediate mixing depth than at both low and high mixing depths (Bonferroni corrected post hoc test, P (z_{mix}) ≤ 0.033).

The mixing depths at which the concentration and standing stock of biomass peak decrease with increasing background turbidity (Fig. 1a,b). To test this prediction, we fitted standard unimodal functions separately to the data from each background turbidity treatment. We used several functions, because all of them are purely phenomenological descriptions of unimodal relationships and none of them would be expected to give a best fit to all data sets. The fits of these functions were modest to excellent (r^2 = 0.40 – 0.94, Table 1). We compared the positions of the peaks between high and low background turbidity treatments separately for each standard function. In accordance with model predictions, the peaks of these functions always shifted towards shallower mixing depths with increased background turbidity (Table 1).

Average light intensity, I^*_{mix}, in the mixed layer and light intensity at the bottom of the mixed layer, I^*_{out}, decrease with mixing depth and are negatively affected by

background turbidity (Fig. 1c). As expected, average intensity of PAR, I_{mix} , decreased with increasing mixing depth and background turbidity. (Fig. 3c, ANCOVA of effects of log-transformed mixing depth and background turbidity on log-transformed I_{mix}; r^2 = 0.99; P (z_{mix}) < 0.001; P (K_{bg}) = 0.004). Light at the bottom of the mixed layer, I_{out}, at that time was strongly correlated with the average light intensity I_{mix} (Pearson's R = 0.94; n =16; P < 0.001). Consequently, I_{out} also decreased with mixing depth but the negative effect of background turbidity on I_{out} was not statistically significant (data not shown, ANCOVA of effects of log-transformed mixing depth and background turbidity on log-transformed I_{out}; r^2 = 0.94; P (z_{mix}) < 0.001; P (K_{bg}) = 0.152). In comparison to day 1 (Fig. 2a) differences in I_{mix} and I_{out} values between low and high background turbidity were less pronounced at the end of week 8. Because all enclosures had similar algal starting densities, this is in accordance with expected and observed compensatory biomass responses to the higher light availability at low background turbidity.

The concentration of dissolved inorganic nutrients, R, shows a U-shaped relationship to mixing depth and is positively affected by background turbidity (Fig. 1d).* Because SRP was below the detection level from week 5 onwards, we used total dissolved phosphorus as a measure of the limiting nutrient not bound in biomass. The concentration of dissolved phosphorous increased with mixing depth levelling off at intermediate depths (Fig. 3d, ANCOVA of effects of log-transformed mixing depth and background turbidity on dissolved phosphorus; r^2 = 0.89; P (z_{mix}) < 0.001). The predicted positive effects of decreasing mixing depth at very shallow depths and of increased background turbidity on dissolved phosphorus (Fig. 1d) could not be observed. (Fig. 3d, ANCOVA, P(K_{bg}) = 0.45)

The total concentration of nutrients in the mixed layer, R^*_m , (i.e., dissolved nutrients plus nutrients stored in biomass) shows a U-shaped relationship to mixing depth and is positively affected by background turbidity (Fig. 1e). Largely following the pattern of dissolved phosphorus, total phosphorus concentration (TP) increased with increasing mixing depth (Fig. 3e). Background turbidity tended to have a weak positive effect on TP, which, however, was not statistically significant and occurred at intermediate rather than high mixing depth (Fig. 3e, ANCOVA of effects of log-transformed mixing depth and background turbidity on TP; r^2 = 0.94; P(z_{mix}) < 0.001; P(K_{bg}) = 0.08; paired t-test, P(K_{bg}) = 0.095).

The standing stock of sedimented nutrients, R^*_S, shows a unimodal relationship to mixing depth and is negatively affected by background turbidity. Higher background turbidity leads to a shift of the peaks towards shallower mixing depths (Fig. 1f). In contrast to predictions, the standing stock of sedimented phosphorus showed no clear indications of a background turbidity effect, but weakly increased with mixing depth. (Fig. 3f, ANCOVA of effects of log-transformed mixing depth and background turbidity on standing stock of sedimented P; $r^2 = 0.37$; P (z_{mix}) = 0.031; P (K_{bg}) = 0.21).

Discussion

The main results of our enclosure experiment are summarized and compared to the model predictions in Table 2. The qualitatively good agreement of most of our results with model predictions indicates that mixing depth and background turbidity can have substantial influence on phytoplankton biomass, underwater light climate, and nutrient distribution (dissolved, bound in biomass or sedimented) in the pelagic. In accordance with model predictions and supporting the results of previous experiments (Diehl et al. 2002) we observed a unimodal distribution of algal biomass concentration and standing stock along a gradient of increasing mixing depth. Likewise, increased background turbidity negatively affected algal biomass at almost all mixing depths and shifted the biomass maxima towards shallower mixing depths. The underlying mechanisms leading to these patterns are discussed below.

Sedimentation losses, production and mixing depth

Phytoplankton in the pelagic of rivers, lakes and oceans suffers from several loss processes such as consumption by herbivores, cell mortality and sedimentation (Reynolds 1984). Phytoplankton-zooplankton interactions are not considered in the model we have tested with our experiment. Consequently, we largely excluded mesozooplankton from our enclosures at the beginning of the experiment in order that sedimentation of algae was the most important loss process for negatively buoyant algae. In accordance with theoretical expectations and other experimental data (Reynolds 1984; Visser et al. 1996; Diehl et al. 2002; Ptacnik et al. 2003) algal sedimentation loss rate in our study was inversely related to mixing depth at the beginning (Fig. 2c) and throughout the entire experiment (data not shown). Along the mixing depth gradient sinking loss rate decreased very sharply from relatively high

values at the lowest mixing depth to rather low values at intermediate mixing depths, but did not decrease much further towards higher mixing depths (Fig. 2c). In contrast, specific production decreased more gradually with increasing mixing depth down to very low (and in some weeks negative) values at the highest mixing depths (Fig. 2b). Consequently, the difference between production and loss rates in most weeks was greatest at intermediate depths, leading to the observed unimodal biomass-mixing depth relationships (Fig. 3a, b). This suggests that net production was most strongly limited by sedimentation losses in the shallowest enclosures and by light limitation in the deepest enclosures. The increased biomass concentration at a mixing depth of 1 m compared to the 2.5 m value was possibly caused by resuspension of algae into the mixed layer through intense mixing (it is likely that sedimented algae remained viable for considerable time at the well-lit bottom of the 1-m enclosures).

The phytoplankton communities in all enclosures were dominated by relatively fast sinking diatoms with mean sinking velocities of 0.25 m d^{-1} in week 1 (Fig. 2c) and 0.3 m d^{-1} averaged over the entire experiment. At the end of week 8, diatoms accounted for 86 - 99% of total biomass in most treatments and were dominated by the genera *Fragilaria* and *Synedra.* Only in the 12-m enclosures and the 15-m high background turbidity treatment did the proportion of diatoms constitute a smaller fraction of the phytoplankton community, being 65%, 30%, and 53% of the total biomass, respectively. The remaining proportion was primarily made up of *Mougeotia*, a filamentous, non-pelagic green alga which had most likely be detached from wall periphyton of the upper 2-3 m. This species occurred mainly in the deeper enclosures characterized by low light availability for pelagic phytoplankton species and therefore probably lower competition for nutrients among pelagic taxa.
One could argue that fast-sinking phytoplankton taxa such as diatoms should be poor competitors in shallow mixed systems due to high sedimentation losses and that these conditions should be more beneficial to positively buoyant or motile phytoplankton species. It is conceivable that our experimental mixing procedure may have benefited diatoms, because the rather intense turbulence created by blowing air into the bottom part of the enclosures should have created a rapidly changing light environment that is thought to favour diatoms (Litchman 1998). For example, PAR levels at the bottom of the water column were only 44% (43%) of incident PAR in the 1-m enclosures and as low as 4% (7%) in the 2.5-m enclosures at high (low) background turbidity, resulting in a highly fluctuating light field for passively entrained

algal cells. Fluctuations in light fields are known to affect species specific growth rates and thus competitive abilities (Mallin and Pearl 1992; Reynolds 1994; Litchman 2000; Litchman and Klausmeier 2001).Hence, the turbulent mixing regime and high silica concentrations (see below) in our experiment may have favoured diatoms to become the dominant algal group (Kiørboe 1993). For example, in situ growth under fluctuating light increased the growth rate of the diatom *Skeletonema* and decreased the growth rate of a chlorophyte and some cyanobacteria (Mitrovic et al. 2003).

Effects of background turbidity

While algal sedimentation losses depend directly and inversely on physical mixing depth, algal specific production is related to the underwater light climate (Huisman 1999; Geider et al. 2001; Diehl et al. 2002). We observed a tight correlation between mean light intensity, I_{mix}, and specific production rate of seston POC in our enclosure experiment, both being decreasing functions of increasing mixing depth and background turbidity. Because increased background turbidity reduced light availability at any given mixing depth, volumetric and areal biomass was generally lower in the high versus low background turbidity treatments. In a field study of shaded versus unshaded enclosures, similar results were obtained for algal biomass concentration (Elser et al. 2003). Interestingly, we also detected effects of simultaneous variation in background turbidity and mixing depth on the biomass-mixing depth relationship. Following the concept of an optically defined depth [the product of physical depth and background turbidity (Kirk 1994)], enclosures of the same physical depth were optically deeper at increased background turbidity. Integrating this concept with the turbidity-independent sedimentation losses means that at a given mixing depth algae experienced similar loss rates but lower production rates at higher background turbidity because of increased light limitation. Therefore, the combined processes of light-limited production and depth-dependent sedimentation losses resulted in a shift of the biomass maximum towards a shallower mixing depth with increased background turbidity.

Nutrient responses to mixing depth and background turbidity

Phytoplankton may become limited by the availability of nutrients when light supply is sufficient and loss rates are not excessive. In most cases of phytoplankton nutrient deficiency in freshwater systems, phosphorus is the limiting macronutrient

(Guildford and Hecky 2000), whereas open ocean environments are frequently more nitrogen or iron deficient (Hecky and Kilham 1988). In our enclosures we generally kept TP at maximal concentrations of 12 µg L^{-1} to comply with the model assumption that phytoplankton is limited by light and a single mineral nutrient, i.e. phosphorus. The concentration of SRP was close to or below the detection limit in all enclosures during and at the end of the experiment, indicating that phosphorus was the main limiting nutrient. Dissolved silica concentration ranged from 1.5 to 9.1 mg L^{-1} during the experimental period, with the exception of the 1-m enclosures on the final day of the experiment when silica concentrations had dropped to 0.11 mg L^{-1} at low and 0.03 mg L^{-1} at high background turbidity. Concentrations of dissolved nitrate-N varied between 3.8 mg L^{-1} and 4.6 mg L^{-1} during the experimental period. Hence, the nutrients silica and nitrogen were usually far beyond levels limiting algal growth. Atomic ratios of dissolved silica and nitrate to total phosphorus were above 300:1 (with the one exception above) and 800:1, respectively, indicating that phosphorus was the main growth-limiting nutrient throughout the experiment. While all model predictions concerning the response variables biomass concentration, standing stock, and light climate were supported by our data, nutrient data were only in partial agreement with model expectations (Table 2). This partial lack of agreement may in part be a consequence of the limited experimental mixing depth gradient, but also of transient dynamics and of some simplifying model assumptions. To address the former argument first: instead of a U-shaped relationship, an increasing relationship of the concentrations of dissolved and total phosphorus with mixing depth was found across the entire gradient of mixing depths. The decelerating limb of the U-shaped relationship is, however, predicted to occur over a narrow range of very shallow mixing depths (usually <1.5 m or less, Fig. 1 d,e; Diehl 2002), which is largely outside the range covered by our experiment. Because mixing depths below 1 m are not frequently observed over extended periods in most natural systems (Fee et al. 1996), we did not include such depths in our experimental design.

Second, instead of the predicted unimodal relationship between the standing stock of sedimented nutrients and mixing depth, we observed a weakly positive one (Table 2). It is quite likely that the contents of those traps that accumulated sediments over the whole experimental period did not yet reflect equilibrium conditions. At the beginning of the experiment algal biomass concentration (and, consequently, the amount of sedimented biomass per area) did not differ among

treatments. The build-up of the unimodal relationship across the mixing depth gradient was slow, becoming first clearly apparent by week 6. Thus, it is likely that the standing stock of sedimented biomass in the traps largely reflected conditions during the transient phase of the experiment before differences caused by treatment had become established. The relatively high amount of sedimented PP in both 1 m-enclosures was possibly related to more than proportional influence of airborne particulate phosphorus. A mass balance calculation of the total phosphorus content within each enclosure ($R_{tot} = R_m + R_s/z_{mix} \approx 12$ µg L^{-1}) indicates that TP contents in both 1 m enclosures were overestimated by more than double (27 µg L^{-1}) and nearly double (20 µg L^{-1}) in the high and low K_{bg} treatments. TP contents in the other treatments deviated from the expected value of 12 µg L^{-1} by maximally $\pm$ 2 µg L^{-1}.

Finally, while the model predicts a positive effect of background turbidity on dissolved and total water column nutrients, and a negative effect on the standing stock of sedimented nutrients, only the prediction for total water column nutrients was weakly supported by our data (Table 2). While the model assumes that the nutrient content per unit biomass of phytoplankton is constant, the nutrient:carbon stoichiometry of phytoplankton can be highly variable (Sterner et al. 1997). In our experiment the seston C:P ratio decreased with increasing mixing depth and with increasing background turbidity (Fig. 2d). Consequently, for each sedimented unit of biomass, a higher amount of phosphorus was lost from the water column at higher mixing depths and higher background turbidity. This increased nutrient loss may have resulted in a higher than expected amount of sedimented nutrients at higher background turbidity and at high mixing depths, and also a lower than expected concentration of dissolved nutrients in the water columns (Table 2). Several other studies have documented negative influences of mixing depth and background turbidity on the seston C:P ratio (Guildford et al. 1994; Diehl et al. 2002; Kunz and Diehl 2003). This is in line with studies that related the variability of seston C:P ratios to the light supply per unit nutrient and found that the ratio of I_{mix}:TP was positively related to the seston C:P ratio (Sterner et al. 1997, Elser et al. 2003). Because the variability of the C:P ratio is known to influence a large number of ecosystem processes such as secondary production and nutrient cycling (Sterner et al. 1998) an important step would be to include variable stoichiometric cell ratios in dynamic phytoplankton models.

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References

Bagheri, S., M. Rijkeboer, and H.J. Gons 2002. Inherent and apparent optical measurements in the Hudson/Raritan Estuary. *Aqu. Ecol.* **36:** 559-562.

Bergmann, T., T.L. Richardson, H.W. Paerl, J.L. Pinckney, and O. Schofield 2002. Synergy of light and nutrients on the photosynthetic efficiency of phytoplankton populations from the Neuse River Estuary, North Carolina. J. *Plankton Res.* **24:** 923-933.

Bienfang, P.K., J.P. Szyper, M.Y. Okamoto, and E.K. Noda 1984. Temporal and spatial variability of phytoplankton in a subtropical ecosystem. *Limnol. Oceanogr*. **29:** 527-539.

Boyd, P.W. 2002. Environmental factors controlling phytoplankton processes in the Southern Ocean. *J. Phycol.* **38:** 844-861.

Carpenter, S.R., J.J. Cole, J.F. Kitchell, and M. L. Pace 1998. Impact of dissolved organic carbon, phosphorus, and grazing on phytoplankton biomass and production in experimental lakes. *Limnol. Oceanogr.* **43:** 73-80.

Colijn, F. and G.C. Cadee 2002. Is phytoplankton growth in the Wadden Sea light or nitrogen limited? *J. Sea Res.* **49:** 83-93.

Cuker, B.E. 1987. Field experiment on the influences of suspended clay and P on the plankton of a small lake. *Limnol. Oceanogr*. **32:** 840-847.

Diehl, S. 2002. Phytoplankton, light, and nutrients in a gradient of mixing depths: theory. *Ecology* **83:** 386-398.

Diehl, S., S.A. Berger, R. Ptacnik, and A. Wild 2002. Phytoplankton, light, and nutrients in a gradient of mixing depths: field experiments. *Ecology* **83:** 399-411.

Elser,J.J., M.Kyle, W. Marinko, T. Yoshida, J. Urabe 2003. Ecological stoichiometry in the microbial food web: a test of the light : nutrient hypothesis. *Aquat. Microbiol. Ecol.* **31**: 49-65.

Fahnenstiel, G.L., R.A. Stone, M.J. McCormick, C.L. Schelske, and S.E. Lohrenz 2000. Spring isothermal mixing in the Great Lakes: evidence of nutrient limitation and nutrient-light interactions in a suboptimal light environment. *Can. J. Fish. Aquat. Sci*. **57:** 1901-1910.

Falkowski, P.G. and Raven, J.A. *Aquatic Photosynthesis*. Blackwell Science, 1997.

Farstey, V., B. Lazar, and A. Genin 2002. Expansion and homogeneity of the vertical distribution of zooplankton in a very deep mixed layer. *Mar. Ecol. Prog. Ser*. **238:** 91-100.

Gago, J., X.A. Alvarez-Salgado, M. Gilcoto, and F.F. Perez 2002. Assessing the contrasting fate of dissolved and suspended organic carbon in a costal upwelling system (Ria de Vigo, NW Iberian Peninsula). *Estuar. Coast. Shelf S* **56:** 271-279.

Geider, R.J. and others. 2001. Primary productivity of planet earth: biological determinants and physical constraints in terrestrial and aquatic habitats. *Global Change Biol*. **7:** 849-882.

Guildford, S.J., F.P. Healey, and R.E. Hecky 1987. Depression of primary production by humic matter and suspended sediment in limnocorral experiments at southern Indian lake, Northern Manitoba. *Can. J. Fish. Aquat. Sci.* **44:** 1408-1417.

Guildford, S.J. and R.E. Hecky 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnol. Oceanogr*. **45:** 1213-1223.

Guildford, S.J., L.L. Hendzel, H.J. Kling, E.J. Fee, G.G.C. Robinson, R.E. Hecky, and S.E.M. Kasian 1994. Effects of lake size on phytoplankton nutrients status. *Aqu. Sci*. **51:** 2769-2783.

Hecky, R.E. and P. Kilham 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent-evidence on the effects of enrichment. *Limnol. Oceanogr*. **33:** 796-822.

Huisman, J. 1999. Population dynamics of light-limited phytoplankton: Microcosm experiments. *Ecology* **80:** 202-210.

Huisman, J., P. van Oostveen, and F. J. Weissing 1999. Critical depth and critical turbulence: Two different mechanisms for the development of phytoplankton blooms. *Limnol. Oceanogr*. **44:** 1781-1787.

Imboden, D.M. and Wüest, A. Mixing mechanisms in lakes, p.83-138. *In:* Lerman, A., Imboden, D. M., and Gat, J. R. (eds*.), Physics and chemistry of lakes.* Springer Verlag, 1995.

Irigoien, X. and J. Castel, 1997. Light limitation and distribution of chlorophyll pigments in a highly turbid estuary: the Gironde**.** *Estuar. Coast. Shelf S* **44:** 507-517.

Kiørboe, T. 1993. Turbulence, phytoplankton cell size, and the structure of pelagic food webs. *Adv. Mar. Biol*. **29:** 1-72.

Kirk, J.T.O. 1977. Use of a quanta meter to measure attenuation and underwater reflectance of photosynthetically active radiation in some inland and coastal southeastern Australian waters. *Austal*. *J. Mar. Freshw. Res.* **28:** 9-21.

Kirk, J.T.O., *Light and Photosynthesis in aquatic ecosystems*. 2nd ed. Cambridge University Press, 1994.

Kunz, T.J. and S. Diehl 2003. Phytoplankton, light and nutrients along a gradient of mixing depth: a field test of the producer-resource theory. *Freshw. Biol*. **48:** 1050-1063.

Lancelot, C., E. Hannon, S. Bequefort, C. Veth, and H.J.W. DeBaar 2000. Modelling phytoplankton blooms and carbon export production in the Southern Ocean: dominant controls by light and iron in the Atlantic sector in Austral spring 1992. *Deep-Sea Res. Pt I – Oceanogr. Res. Papers* **47:** 1621-1662.

Litchman, E. 1998. Population and community responses of phytoplankton to fluctuating light. *Oecologia* **117:** 247-257.

Litchman, E. 2000. Growth rates of phytoplankton under fluctuating light. *Freshw. Bio*l. **44:** 223-235.

Litchman, E. and C. A. Klausmeier 2001. Competition of phytoplankton under fluctuating light. *Am. Nat*. **157:** 170-187.

Mallin, M.A. and H. W. Pearl 1992. Effects of variable irradiance on phytoplankton productivity in shallow estuaries. *Limnol. Oceanogr*. **37:** 54-62.

Mann, K. H. and Lazier, J.R.N. *Dynamics of Marine Ecosystems: Biological-physical Interactions in the Oceans*., 2nd ed. Blackwell, 1996.

Martin, J.H., R.M. Gordon, and S.E. Fritzwater 1990. Iron in Antarctic waters. *Nature* **345:** 156-158.

Mitchell, B.G., E.A. Brody, O. Holm-Hansen, C. McClain, and J. Bishop 1991. Light limitation of phytoplankton biomass and macronutrient utilization in the southern-ocean. *Limnol. Oceanogr*. **36:** 1662-1677.

Mitrovic, S.M., C.G. Howden, L.C. Bowling, and R.T. Buckney 2003. Unusual allometry between in situ growth of freshwater phytoplankton under static and fluctuating light environments: possible implications for dominance. *J. Plankton Res*. **25:** 517-526.

Platt, T., S. Sathyendranath, O. Ulloa, W.G. Harrison, N. Hoepffner, and J. Goes 1992. Nutrient control of phytoplankton photosynthesis in the western North-Atlantic. *Nature* **356:** 229-231.

Ptacnik, R., S. Diehl, and S.A. Berger 2003. Performance of sinking and non-sinking phytoplankton taxa in a gradient of mixing depths. *Limnol. Oceanogr*. **48:** 1903-1912.

Reynolds, C.S., *The ecology of freshwater phytoplankton*. Cambridge University Press, 1984.

Reynolds, C.S. 1994. The long, the short and the stalled - on the attributes of phytoplankton selected by physical mixing in lakes and rivers. *Hydrobiologia* **289:** 9-21.

Sakshaug, E., D. Slagstad, and O. Holm-Hansen 1991. Factors controlling the development of phytoplankton blooms in the Antarctic ocean - a mathematical model. *Mar. Chem*. **35:** 259-271.

Sommer, U., F. Sommer, B. Santer, C. Jamieson, M. Boersma, C. Becker, and T. Hansen 2001. Complementary impact of copepods and cladocerans on phytoplankton *Ecol. Lett*. **4:** 545-550.

Soto, D. 2002. Oligotrophic patterns in southern Chilean lakes: the relevance of nutrients and mixing depth. *Rev. Chil. Hist. Nat*. **75:** 377-393.

Sterner, R.W. 1990. Lake morphometry and light in the surface-layer. *Can. J. Fish. Aquat. Sci*. **47:** 687-692.

Sterner, R.W., J.J. Elser, E.J. Fee, S.J. Guildford, and T.H. Chrzanowski 1997. The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *Am. Nat*. **150:** 663-684.

Sterner, R.W., J. Clasen, W. Lampert, and T. Weisse 1998. Carbon: phosphorus stoichiometry and food chain production. *Ecol. Lett*. **1:** 146-150.

Sunda, W.G. and S. Huntsman 1997. Interrelated influence of iron, light and cell size on marine phytoplankton growth. *Nature* **390:** 389-392.

Sverdrup, H.U. 1953. On conditions for the vernal blooming of phytoplankton. *J. Cons. Perm. Int. Explor. Mer* **18:** 287-295.

Tümpling, W.,. and Friedrich, G., *Biologische Gewässeruntersuchung*. Gustav Fischer Verlag, 1990.

Tyler, J.E. 1975. The *in situ* quantum efficiency of natural phytoplankton populations. *Limnol. Oceanogr*. **20:** 976-980.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplanktonmethodik. *Int. Ver. Theor. Ang. Limnol. Mitteilungen* **9:** 1-38.

Visser, P.M., L. Massaut, J. Huisman, and L.R. Mur 1996. Sedimentation losses of Scenedesmus in relation to mixing depth. *Arch. Hydrobiol*. **136:** 289-308.

Wetzel, R. G., *Limnology*, 2nd ed. Sounders College Publishing, 1983.

Table 1 Depth of maximal biomass concentration, ω, and maximal standing stock of biomass, *W*, at low and high background turbidity at the end of week 8 estimated with several different standard functions. Also given are the r^2 values of the fit, where statistical significance ($P < 0.005$) is given at $r^2 > 0.7$ ($N = 8$).

State variables affected	Low background turbidity		High background turbidity		Function
	Depth of maximum	r^2	Depth of maximum	r^2	
Log ω	9.30	0.67	6.69	0.40	Polynomial quadratic
ω	8.72	0.50	7.31	0.58	Polynomial quadratic
ω	8.24	0.64	7.57	0.70	Gaussian 3 parameter [1]
ω	8.14	0.77	7.75	0.74	Lorentzian 3 parameter [2]
Log *W*	10.97	0.86	9.18	0.90	Polynomial quadratic
W	11.70	0.70	8.79	0.72	Polynomial quadratic
W	10.93	0.67	8.90	0.93	Gaussian 3 parameter [1]
W	9.48	0.60	9.17	0.94	Lorentzian 3 parameter [2]

[1] $f = a \times e^{-0.5 \times \left(\frac{x - x_0}{b}\right)^2}$

[2] $f = \frac{a}{1 + \left(\frac{x - x_0}{b}\right)^2}$

Table 2 Predicted (Diehl 2002) and observed changes in equilibrium values of state variables along a gradient of increasing mixing depth and increasing background turbidity, respectively. Also shown are the effects of increasing background turbidity on the locations of maxima (ω^*, W^*, R^*_s) and minima (R^*, R^*_m) along an axis of increasing mixing depth. State variables are described in the legend of Fig. 1. Symbols are: +, state variable or position of maximum/minimum increases; -, state variable or position of maximum/minimum decreases; + -, state variable first increases then decreases; - +, state variable first decreases then increases; 0, no explicit effect on state variable observed; n. m., no minimum/maximum predicted and/or observed; symbols in brackets indicate marginally but not statistically significant effect on state variable. [1)] The predicted decrease in state variable at very low mixing depths is not expected to be included in the range of mixing depths examined in the experiment.

Parameters varied	ω^*	W^*	I^*_{mix}	I^*_{out}	R^*	R^*_m	R^*_s
Mixing depth (z_{mix})							
Predicted	+ –	+ –	–	–	– +	– +	+ –
Observed	+ –	+ –	–	–	+[1)]	+[1)]	+
Background turbidity (K_{bg})							
Predicted	–	–	–	–	+	+	–
Observed	–	–	–	(–)	0	(+)	0
Effects of K_{bg} on max./min.							
Predicted	–	–	n.m.	n.m.	–	–	–
Observed	–	–	n.m.	n.m.	n.m.	n.m.	n.m.

Fig. 1

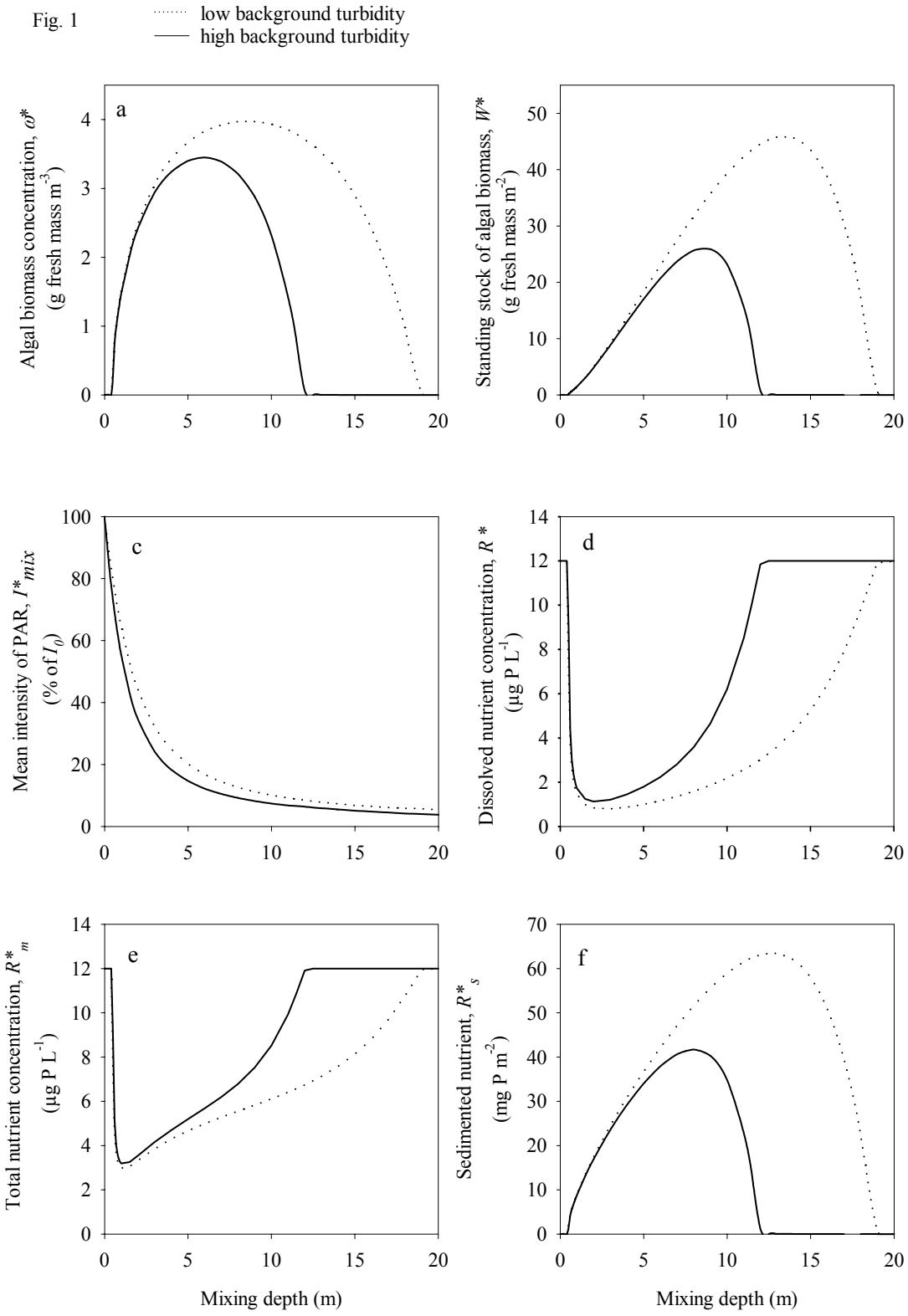
low background turbidity
high background turbidity
a
Algal biomass concentration, a*
(g fresh mass m-3)
Standing stock of algal biomass, W*
(g fresh mass m-2)
c
Mean intensity of PAR, I*mix
(% of I0)
d
Dissolved nutrient concentration, R*
(µg P L-1)
e
Total nutrient concentration, R*m
(µg P L-1)
f
Sedimented nutrient, R*s
(mg P m-2)
Mixing depth (m)
Mixing depth (m)

Figure 1 Theoretical expectations according to the closed system model in Diehl (2002). Effects of mixing depth and background turbidity on equilibrium values of (a) algal biomass concentration in the mixed layer, ω^*; (b) standing stock of algal biomass in the mixed layer, W^*; (c) mean intensity of PAR in the mixed layer, I^*_{mix}; (d) concentration of dissolved nutrient in the mixed layer, R^*; (e) concentration of total nutrient in the mixed layer R^*_m; (f) standing stock of nutrient lost from the mixed layer through sedimentation, R^*_s. Values of parameters and state variables are as in Table 1, Diehl (2002), with the following exceptions: background light attenuation coefficient K_{bg} = 0.93 m^{-1} and 1.31 m^{-1} for low and high background turbidity, respectively; total amount of limiting nutrient in the system R_{tot} = 12 µg phosphorus L^{-1}; incoming light intensity at the water surface I_{in}, (averaged over 24 h) = 300 µmol $m^{-2}s^{-1}$. Predictions for biomass in Figs. a and b are scaled as fresh mass but were originally generated from calculations in which biomass was scaled in units of carbon (as in the original model of Diehl 2002) assuming a carbon content corresponding to 5 % of fresh mass.

Fig. 2

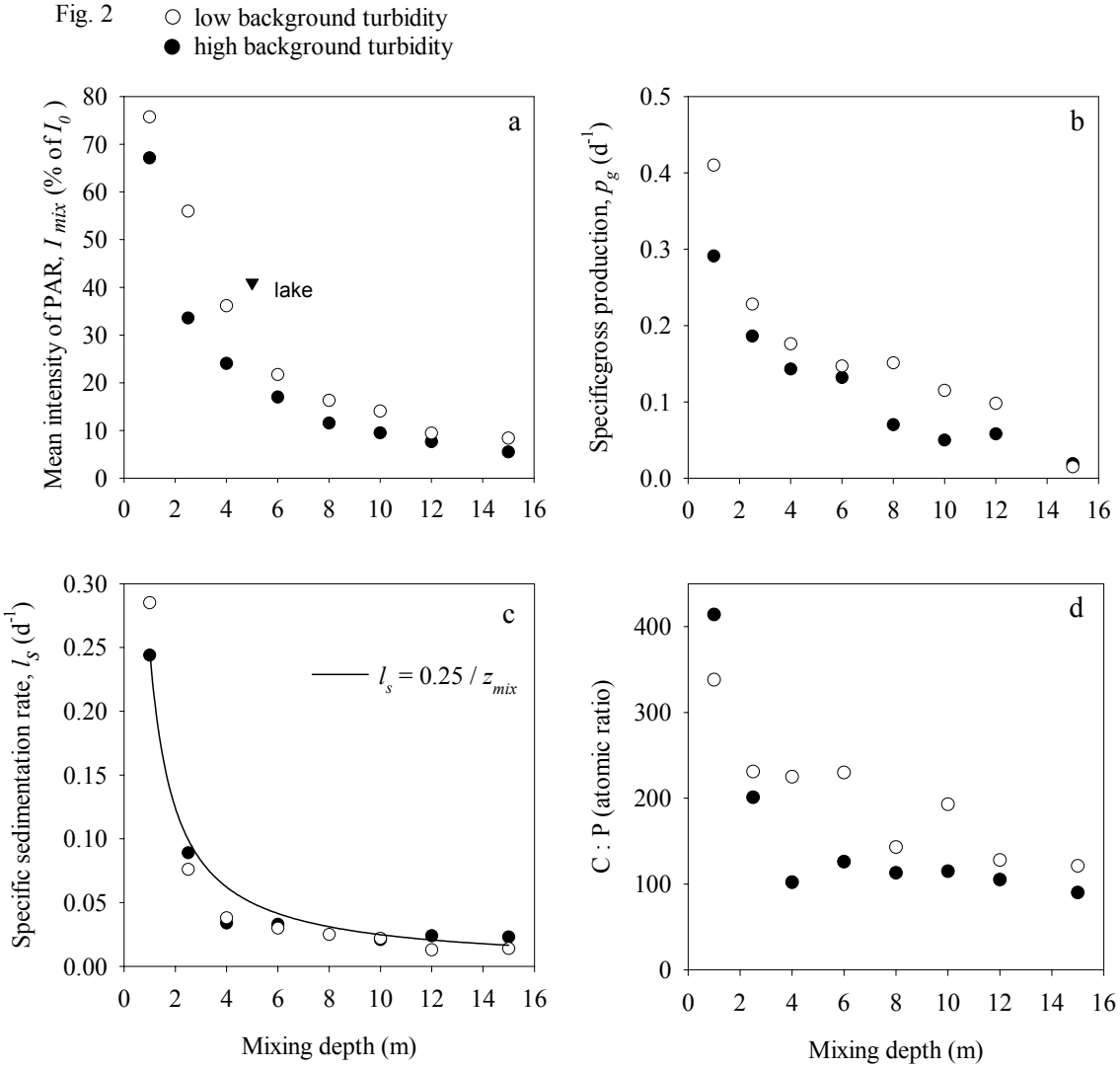

Figure 2 Field experiment. (a) Mean intensity of PAR in the mixed layer, I_{mix} , as percentage of incident PAR (I_0) in relation to mixing depth on day 1. Also shown is I_{mix} in the lake at the actual mixing depth of 5 m (solid triangle). (b) Specific gross production rate, p_g, of seston organic carbon in relation to mixing depth during week 1. (c) Specific sedimentation rate, I_s of particulate organic carbon in relation to mixing depth during week 1. Also shown is the back-transformed regression line according to the equation log I_s = log (v /z_{mix}), yielding an estimate of average sinking velocity v = 0.25 m d^{-1}. Regressions statistics are r^2 = 0.96; p < 0.001. (d) Atomic seston C:P ratio in relation to mixing depth at the end of week 8.

Fig. 3

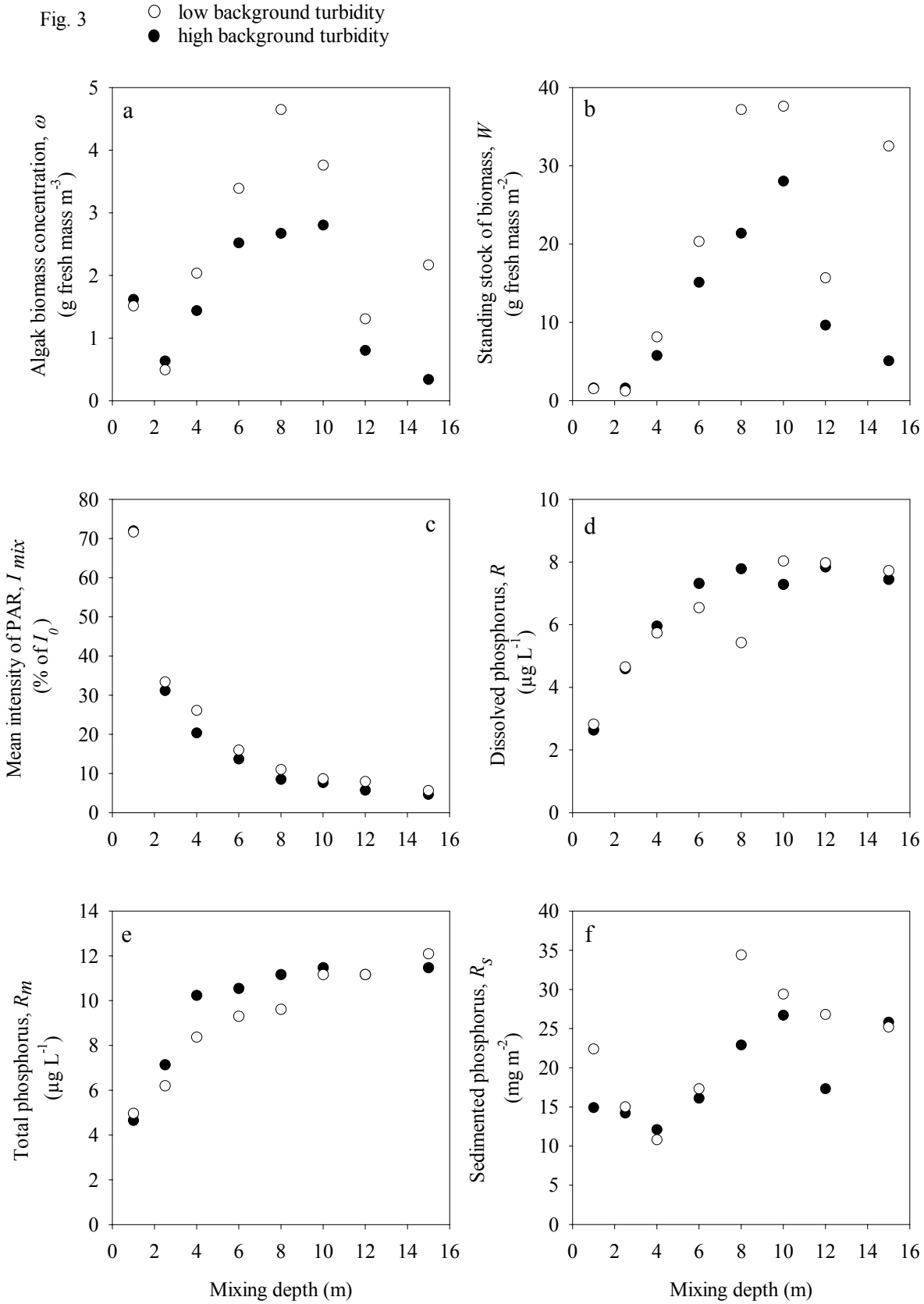
low background turbidity
high background turbidity
a
Algak biomass concentration, ω
(g fresh mass m-3)
b
Standing stock of biomass, W
(g fresh mass m-2)
c
Mean intensity of PAR, I mix
(% of I0)
d
Dissolved phosphorus, R
(µg L-1)
e
Total phosphorus, Rm
(µg L-1)
f
Sedimented phosphorus, Rs
(mg m-2)
Mixing depth (m)
Mixing depth (m)

Figure 3 Effects of mixing depth and background turbidity on (a) algal biomass concentration, ω; (b) standing stock of algal biomass, W; (c) mean intensity of PAR in the mixed layer, I_{mix}; (d) concentration of dissolved phosphorus, R; (e) concentration of total phosphorus, R_m, in the mixed layer; and (f) standing stock of sedimented phosphorus, R_s, at the end of week 8.

Article 2

LIGHT SUPPLY, PLANKTON BIOMASS, AND SESTON STOICHIOMETRY IN A GRADIENT OF LAKE MIXING DEPTHS

(Stella A. Berger, Thomas J. Kunz, Dieter Albrecht, Amine M. Oucible, Sylvie Ritzer & Sebastian Diehl)

Light supply, plankton biomass and seston stoichiometry in a gradient of lake mixing depths

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Abstract

In the mixed surface layer of thermally stratified lakes average light supply and specific nutrient supply decrease with increasing mixed layer depth and so do depth-averaged specific phytoplankton production and sedimentation losses. Mixing depth is therefore expected to have important consequences for the dynamics of phytoplankton biomass, algal nutrient stoichiometry, light availability, nutrient supply, and nutrient retention in the mixed surface layer. Here, we derive from a dynamical model that light availability, phytoplankton density, and the carbon:nutrient ratio of phytoplankton biomass should all be negatively related to mixed surface layer depth, whereas the areal standing stock of phytoplankton should show a unimodal, and total and dissolved nutrients a horizontal or increasing relationship to mixing depth. These predictions agree closely with data from 65 Central European lakes during summer stratification. In addition, zooplankton biomass was strongly negatively related to mixing depth in a subset of the lakes. Stratification depth is thus an important driver of the dynamics of light, nutrients and phytoplankton in the surface layer, the effects of which propagate to higher trophic levels.

Introduction

The elemental composition of plants varies in response to the relative supplies with energy and materials (Sterner et al. 1997; Rastetter et al. 1997) and affects ecosystem processes such as the sequestration and storage of atmospheric carbon dioxide by living and dead plant biomass, the transfer of energy and matter along the food chain, and the transport, storage, and recycling of nutrients within and among ecosystems (Andersen 1997; Cebrian 1999; Hessen et al. 2004). Aquatic plants 'compete' for light with abiotic absorbents such as water molecules. Light supply to passively entrained planktonic producers decreases with water depth. Consequently, specific production and algal carbon to nutrient ratio are negatively affected by the vertical extension of the mixed water column (Reynolds 1984; Huisman et al. 1995; Sterner et al. 1997; Diehl et al. 2002), which can vary seasonally within lakes and geographically among lakes over more than an order of magnitude (Guildford et al. 1994; Soto 2002; Kunz et al. 2003). Mixed surface layer depth furthermore negatively affects volumetric nutrient supply to the surface layer from external sources and the sinking loss rate of particulate nutrients to strata below the thermocline (Reynolds 1984; Visser et al. 1996; Ptacnik et al. 2003). Mixing depth is therefore expected to have important consequences for the dynamics of phytoplankton biomass, algal nutrient stoichiometry, light availability, nutrient supply, and nutrient retention in the mixed surface layer (Huisman et al. 1995; Diehl 2002).

To our knowledge, the dynamic interplay among the full set of these pelagic ecosystem components and the impact of mixing depth on it have not yet been comprehensively explored – neither in theory nor with empirical data. Here, we present a simple dynamical model that accounts for the mixing-depth dependence of most pelagic processes and for variability in algal nutrient stores. We derive predictions on how the above state variables should be affected by mixing depth and external nutrient supply and draw comparisons to results of a lake survey in which we measured all relevant state variables.

Model structure

The model considers the well-mixed surface layer of a stratified water column and describes the dynamics of the concentrations of algal biomass (A) and dissolved mineral nutrients (R), the nutrient content (quota) per algal biomass (Q), and the light intensity ($I(s)$) at depth s of the mixed layer:

$$\frac{dA}{dt} = \frac{A}{z}\int_0^{z_{mix}} p(I(s),Q)ds - l_m A - \frac{v+D}{z_{mix}}A \quad (1)$$

$$\frac{dR}{dt} = \frac{D}{z_{mix}}(R_{in} - R) - \rho(Q,R)A \quad (2)$$

$$\frac{dQ}{dt} = \rho(Q,R) + l_m Q - \frac{Q}{z}\int_0^{z_{mix}} p(I(s),Q)ds \quad (3)$$

$$I(s) = I_{in}e^{-(kA+K_{bg})s} \quad (4)$$

Specific algal growth rate (p) is assumed to be a multiplicative (Huisman et al. 1995), saturating function of light intensity and nutrient quota (Droop 1974) $p(I,Q) = p_{\max} I/(I+H)(1-Q_{\min}/Q)$, which averaged over a mixed water column of depths z_{mix} yields

$$\frac{1}{z_{mix}}\int_0^{z_{mix}} p[I(s),Q]ds = \frac{p_{\max}}{kA+K_{bg}}\ln\left(\frac{H+I_{in}}{H+I_{out}}\right)\left(1-\frac{Q_{\min}}{Q}\right) \quad (5)$$

Here, p_{max} is maximum specific production rate, Q_{min} the minimum nutrient quota, k the algal biomass-specific light attenuation coefficient, K_{bg} the background light attenuation coefficient (describing attenuation by non-algal components), H the half-saturation constant of light-dependent production, and I_{in} the (constant) light intensity at the water surface. Light intensity decreases exponentially with depth. Light intensity at the bottom of the mixed layer (Huisman et al. 1994), I_{out}, is defined by eq. 4 for $s = z_{mix}$. Algae respire carbon at rate l_m and leave the mixed layer at rates proportional to their sinking velocity v and the water exchange rate D. Nutrients enter and leave the mixed layer at rate D and at concentrations R_{in} and R, respectively. Algal loss and nutrient exchange rates are inversely proportional to mixing depth z_{mix}. Finally, nutrient uptake is a decreasing function of algal nutrient quota (Morel 1987) and a saturating function of external nutrient concentration

$$\rho(Q,R) = \rho_{\max}\left(\frac{Q_{\max}-Q}{Q_{\max}-Q_{\min}}\right)\frac{R}{M+R} \quad (6)$$

Here, ρ_{max} is maximum specific nutrient uptake rate, M the half-saturation constant of nutrient uptake, and Q_{max} the maximum nutrient quota. Algal nutrient quota increases through nutrient uptake and carbon respiration and decreases through growth.

Model predictions

Below we explore how the model system responds to two environmental drivers: depth of the mixed water column and external nutrient supply. We focus on equilibrium conditions. A formal stability analysis is not feasible, but in numerical runs the system always settled to a unique, globally stable equilibrium. We performed simulations in MATLAB covering a range of plausible parameter values. The qualitative results described below have been observed in all of these simulations and will be illustrated with a few parameterized examples. Baseline parameter values are listed in the caption of Fig. 1 and were chosen to reflect realistic algal traits (see, e.g., chapter 3.5 in Andersen 1997) and to roughly match environmental conditions in our study lakes.

At equilibrium, the model predicts that phytoplankton density and algal carbon:nutrient ratio are unimodally related to mixing depth (Fig. 1a,d), because algal carbon sequestration is limited by high sinking losses at the shallowest mixing depths and by low average light availability in deeply mixed layers. For realistic parameter values, both maxima occur, however, at very shallow mixing depths (< 2 m, Fig. 1a,d) which are not normally observed over extended periods of time (see Supplementary Table 1). Thus, in the mixing depth range usually observed we expect negative relationships of both phytoplankton density and the algal carbon:nutrient ratio to mixing depth (unshaded areas in Fig. 1). The areal standing stock of phytoplankton (summed over the mixed layer) is also unimodally related to mixing depth, but the maximum occurs in considerably deeper mixed layers (Fig. 1b), because in shallow mixed layers depth-integrated biomass is limited by the total amount of nutrients in the mixed water column (Huisman et al. 1995). Light intensity at the bottom of the mixed layer decreases with mixing depth (Fig. 1c). Finally, the concentration of the limiting nutrient in dissolved mineral form as well as total (dissolved plus particulate) nutrient concentration show flat-bottomed U-shaped relationships to mixing depth, the decreasing limbs of which occur below realistic mixing depths (< 0.2 m, Fig. 1e,f). Thus, although nutrient supply rate is inversely related to mixing depth in the model, we expect total and dissolved nutrients to be nearly independent of mixing depth across a mixing depth range typical for small to medium-sized lakes (unshaded areas in Fig. 1). Within that range, essentially all nutrients are sequestered by algae, keeping dissolved nutrients at very low levels and leading to the predicted decrease in algal carbon:nutrient ratio with increasing mixing depth. Note that sinking losses

depress total nutrient concentration considerably below the nutrient concentration in the external source (Fig. 1f). Once a threshold mixing depth is reached, algae become saturated with nutrients and cannot sequester all available nutrients. Beyond that threshold algal biomass depends solely on light availability and dissolved and total nutrients increase with mixing depth (Fig. 1b,e,f).

The model also predicts straightforward effects of nutrient supply: higher nutrient concentrations in the external supply lead to more phytoplankton biomass (except where algae are nutrient saturated), lower light levels, higher concentrations of total and dissolved nutrients, and a lower algal carbon:nutrient ratio (Fig. 1).

Lake survey - Material and methods

Study lakes and sampling

To confront the model predictions with data, we sampled the surface layers of 65 central European lakes (area 0.03-80 km^2) several times during summer stratification and measured all state variables included in the model. For nutrients, we focussed on phosphorus as the nutrient limiting production in the majority of freshwater lakes (Reynolds 1984; Hessen et al. 2004). Twenty-five lakes in southern Germany and northern Austria were sampled twice in 1998 (06 - 17 July, 16 - 28 September) and 40 lakes in northern Germany were sampled three times in 2001 (05 - 29 June, 12 July - 3 August, 14 August - 06 September). Lake characteristics are listed in Supplementary Table 1. The lakes covered a moderate range of mixing depths (3.0 - 10.3 m; seasonal average) and a broad range of nutrient supply rates as inferred from total phosphorus (TP) concentrations (8 -122 mg TP m^{-3}) (see Supplementary Table1).

On each sampling occasion we first recorded a vertical profile of temperature, conductivity, oxygen concentration, and pH. Mixing depth (z_{mix}) was then defined as the depth at which the temperature difference to the lake surface (or a depth of 1m if other parameters indicated short-term heating of the surface) did not exceed 1 °C. We then recorded a vertical profile of photosynthetically active radiation (PAR) with a spherical underwater quantum sensor and collected an integral water sample of the mixed surface layer. In 2001, we also collected a mixed-layer zooplankton sample with a 50-µm mesh net.

Sample analyses

TP concentration was determined from unfiltered water samples. All other analyses were carried out on 200 µm-filtered samples to exclude mesozooplankton. We determined soluble reactive phosphorus (SRP) and total dissolved phosphorus (TDP) after filtration through membrane filters (0.45 µm), and TP after oxidation of all organic phosphorus according to the phosphorus-molybdate method. Particulate phosphorus was calculated as the difference between TP and TDP. After filtration of seston onto glass-fiber filters (GF/C, Whatman) we determined chlorophyll-*a* spectrophotometrically in 1998 and by HPLC analysis in 2001, and particulate organic carbon via infrared spectrometry. Microzooplankton was counted in preserved water samples using an inverted microscope. Mesozooplankton was counted and measured under a dissecting microscope. Total dry biomass of zooplankton (crustaceans+ rotifers +ciliates) was calculated using length-mass regressions (Botrell et al. 1976).

Comparison with model expectations – statistical analyses

To compare lake data with equilibrium model expectations we used seasonal means averaged over the multiple samplings from each lake. We adopted this procedure to include each lake as a single, independent data point and to reduce the influence of sampling error and transient temporal variability. Although fluctuations in plankton biomass are often minor during summer stratification (Sommer et al. 1986), seasonal averages should more closely approach equilibrium conditions than would single measurements.

Because many model parameters (e.g., water exchange rate, background turbidity, algal sinking velocity and physiological rates) were expected to vary among lakes but could not be assessed, we did not attempt a quantitative comparison between model expectations and lake data. Rather, we used stepwise, multiple regression to ask whether the lake data matched the qualitative expectations described above (Fig. 1). As predictor variables we used mixing depth (z_{mix}), squared mixing depth (z_{mix}^2), and TP. Since data on external nutrient supply are very difficult to obtain TP is conventionally used as a proxy of phosphorus supply from external sources (Vollenweider 1976; Sterner et al. 1997). The strong positive relationship between external nutrient supply and total nutrient concentration predicted by our model (Fig. 1f) actually gives a theoretical justification for this approach. As predicted for the range of observed mixing depths, TP and z_{mix} were indeed uncorrelated in the

data (Pearson r = - 0.11, P > 0.1). Mixing depth and water temperature are likely not independent from one another, the latter possibly affecting phytoplankton production. In our data set water temperature and mixing depth were indeed negatively correlated (Pearson r = - 0.54, P < 0.001). We therefore separated their contributions by including water temperature as an additional variable to the regression analyses.

We performed stepwise multiple regressions with the backward elimination method. Predictor variables were included in the regression at P < 0.05 and excluded at P > 0.1. Response variables included the volumetric density and areal standing stock of phytoplankton biomass (as Chl-*a*), the density of zooplankton (as dry mass), the SRP concentration, the seston carbon:phosphorus ratio, and the intensity of PAR at the bottom of the mixed layer [I_{out} in per cent of I_{in} = 100 exp (-K z_{mix}), with K being the slope of a linear regression of ln-transformed PAR vs. depth]. As predictor variables we used z_{mix}, z_{mix}^2, TP, and water temperature. Because northern and southern German lakes differ in basin geology and were sampled in different years, we included 'region' (southern vs. northern) as a dummy variable. 'Region' was related to I_{out} (P < 0.001) but excluded from all other regressions (P ≥ 0.44). Inclusion of 'region' did not affect the qualitative relationships of z_{mix} and TP to I_{out} and is therefore not mentioned in Table 1. Prior to analysis, zooplankton biomass, Chl-*a*, I_{out}, SRP, and TP were log - or log (x+1) - transformed and averaged across multiple samplings. Studentized residuals of all dependent variables were normally distributed (Kolmogorov-Smirnov test P > 0.05) and there was only one outlier (Cook's d > 1.0), the SRP value of Lake Wittensee (Cook's d = 1.99). Omitting this data point leads to the exclusion of z_{mix} from the predictor variables but does not change the qualitative relationships of z_{mix}^2 and TP to SRP.

Results and discussion

Generally, we found very good qualitative agreement between observed and predicted relationships among variables (compare Fig. 2 and Table 1 to unshaded areas in Fig. 1). Both, algal biomass concentration (as chlorophyll-*a*) and seston carbon:phosphorus ratio were negatively related to mixing depth and positively related to TP concentration (Fig. 2a,d, Table 1). The areal standing stock of algal biomass was best described by a unimodal relationship with mixing depth (Table 1). Similar to the numerical example, the peak of the relationship occurred close to the highest observed mixing depths and was rather flat (Fig. 2b). Light intensity at the bottom of the mixed layer was negatively related to both mixing depth and TP. The

latter suggests that nutrient enrichment enhanced light attenuation by increasing phytoplankton density (Fig. 2a,c, Table 1). Finally, the concentration of soluble reactive phosphorus was best fit by a positive relationship to TP and a U-shaped relationship to mixing depth (Table 1). Similar to the numerical example, the relationship was almost flat at low to intermediate mixing depths (Fig. 2e). Mean seasonal water temperature ranged from 13.5-21.2 °C and was retained in most regression models (Table 1), suggesting that effects of temperature were independent of mixing depth effects.

To our knowledge, no other strategic ecosystem model describes the relationships among phytoplankton biomass, light, nutrients, and mixing depth as comprehensively as ours and our lake survey is the only available data set that reports all state variables described by the model. Given that unaccounted factors (e.g., algal taxonomic composition, zooplankton grazing) should have produced considerable noise in the lake data, the qualitative agreement between theoretical expectations and data is striking. Notably, we observed the predicted negative relationships of phytoplankton density and algal C:P ratio to mixing depth in spite of the rather limited range of mixing depths covered by our study. Nutrient supply explained, however, a relatively larger part of the variation in chlorophyll-*a* concentrations than did mixing depth (simple regressions of log Chl-*a* vs. TP, vs. z_{mix}, and vs. z_{mix}^2 yield R^2-values of 0.69, 0.22, and 0.20, respectively). This is not surprising given that the TP-Chl-*a* correlation is one of the most pervasive empirical patterns in limnology (Peters 1986). Our data suggest, however, that a substantial portion of the residuals from this relationship is explained by variation in mixing depth. Clearly, the relative importance of mixing depth should increase towards more deeply mixed systems. Very strong negative effects of mixing depth on algal chlorophyll-*a* levels have indeed been reported from very deeply mixed lakes (Soto 2002) and from the ocean (Mitchell et al. 1991; Boyd 2002).

Mixed surface layer depth is determined by climatic conditions, lake size and orientation, and water clarity (Sterner 1990; Fee et al. 1996). Not surprisingly, global warming has already been related to changes in stratification patterns, e.g. earlier onset and delayed break-down of stratification in Central European lakes, extended duration of spring turnover in Scandinavian lakes, and increase in summer stratification depth of small Canadian lakes (Weyhenmeyer et al. 1999; Livingstone 2003; Fee et al. 1996). We expect that the effects of mixing depth predicted by our

model should propagate to higher trophic levels. Zooplankton biomass was indeed strongly negatively related to mixing depth in a subset of the surveyed lakes (Fig. 2f). Clearly, understanding the consequences of global climate change for carbon and nutrient dynamics of pelagic food chains will require detailed knowledge of climate impacts on stratification patterns.

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References

Andersen, T. *Pelagic nutrient cycles: herbivores as sources and sinks*. Springer Verlag, Berlin, 1997.

Botrell, H.H., A. Duncan, Z.M. Gliwicz, E. Grygiereck, A. Herzig, A. Hillbricht-Ilkowska, H. Kurasawa, P. Larrson, and T. Weglenska, 1976. A review of some problems in zooplankton production studies. *Nor. J. Zool.* **24:** 419-456.

Boyd, P.W., 2002. Environmental factors controlling phytoplankton processes in the Southern Ocean. *J. Phycol.* **38:** 844-861.

Cebrian, J., 1999. Patterns in the fate of production in plant communities. *Am. Nat.* **154:** 449-468.

Diehl, S., 2002. Phytoplankton, light, and nutrients in a gradient of mixing depths: theory. *Ecology* **83:** 386-398.

Diehl, S., S.A. Berger, R. Ptacnik, and A. Wild, 2002. Phytoplankton, light, and nutrients in a gradient of mixing depths: field experiments. *Ecology* **83:** 399-411.

Droop, M.R., 1974. Nutrient status of algal cells in continuous culture. *J. Mar. Biol. Ass. U.K.* **54:** 825-855.

Fee, E.J., R.E. Hecky, S.E.M. Kasian, and D.R. Cruikshank, 1996. Effects of lake size, water clarity, and climatic variability on mixing depths in Canadian Shield lakes. *Limnol. Oceanogr.* **41:** 912-920.

Gerten, D. and R. Adrian, 2002. Effects of climate warming, North Atlantic Oscillation, and El Niño-Southern Oscillation on thermal conditions and plankton dynamics in northern hemispheric lakes. *Sci. World J.* **2:** 586-606.

Guildford, S.J., L.L. Hendzel, H.J. Kling, E.J. Fee, G.G.C. Robinson, R.E. Hecky, and S.E.M. Kasian, 1994. Effects of lake size on phytoplankton nutrients status. *Can. J. Fish. Aqu. Sci.* **51:** 2769-2783.

Hessen, D.O., G.I. Agren, T.R. Anderson, J.J. Elser, and P.C. De Ruiter, 2004. Carbon, sequestration in ecosystems: The role of stoichiometry. *Ecology* **85:** 1179-1192.

Huisman, J. and F.J. Weissing, 1994. Light-limited growth and competition for light in well-mixed aquatic environments - an elementary model. *Ecology* **75:** 507-520.

Huisman, J. and F.J. Weissing, 1995. Competition for nutrients and light in a mixed water column: a theoretical analysis. *Am. Nat.* **146:** 536-564.

Kunz, T.J. and S. Diehl, 2003. Phytoplankton, light and nutrients along a gradient of mixing depth: a field test of producer-resource theory. *Freshw. Biol.* **48:** 1050-1063.

Kunz,T.J. and S. Diehl, 2005. Effects of mixing depth and nutrient enrichment on phytoplankton, zooplankton, light and nutrients: a marine enclosure experiment. *(in preparation).*

Livingstone, D.M., 2003. Impact of secular climate change on the thermal structure of a large temperate central European lake. *Climatic Change* **57:** 205-225.

Mitchell, B.G. and O. Holm-Hansen, 1991. Observations and modelling of the Antarctic phytoplankton crop in relation to mixing depth. *Deep-Sea Res. Pt. A* **38:** 981-1007.

Morel, F.M.M., 1987. Kinetics of nutrient-uptake and growth in phytoplankton. *J. Phycol.* **23:** 137-150.

Peters, R.H., 1986. The role of prediction in limnology. *Limnol. Oceanogr.* **31:** 1143-1159.

Ptacnik, R., S. Diehl, and S.A. Berger, 2003. Performance of sinking and non-sinking phytoplankton taxa in a gradient of mixing depths. *Limnol. Oceanogr.* **48:** 1903-1912.

Rastetter, E.B., G.I. Agren, and G.R. Shaver, 1997. Responses of N-limited ecosystems to increased CO_2: A balanced-nutrition, coupled-element-cycles model. *Ecol. Applic.* **7:** 444-460.

Reynolds, C.S. *The ecology of freshwater phytoplankton*. Cambridge University Press, 1984.

Sommer, U., Z.M. Gliwicz, W. Lampert, and A. Duncan, 1986. The PEG-model of seasonal succession of planktonic events in fresh waters. *Arch. Hydrobiol.* **106:** 433-471.

Soto, D., 2002. Oligotrophic patterns in southern Chilean lakes: the relevance of nutrients and mixing depth. *Rev. Chil. Hist. Nat.* **75:** 377-393.

Sterner, R.W., 1990. Lake morphometry and light in the surface-layer. *Can. J. Fish. Aquat. Sci.* **47:** 687-692.

Sterner, R.W., J.J. Elser, E.J. Fee, S.J. Guildford, and T.H. Chrzanowski, 1997. The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *Am. Nat.* **150:** 663-684.

Visser, P.M., L. Massaut, J. Huisman, and L.R. Mur, 1996. Sedimentation losses of Scenedesmus in relation to mixing depth. *Arch. Hydrobiol.* **136:** 289-308.

Vollenweider, R.A., 1976. Advances in defining the critical loading levels for phosphorus in lake eutrophication. *Mem. Ist. Ital. Idrobiol.* **33:** 53-83.

Weyhenmeyer, G.A., T. Blenckner, and K. Pettersson, 1999. Changes of the plankton spring outburst related to the North Atlantic Oscillation. *Limnol. Oceanogr.* **44:** 1788-1792.

Table 1 Relationships among response and predictor variables in the study lakes

Response variables	Regression equations with partial *P*-values					R^2	Variables excluded
Algal biomass concentration							
log Chl-*a*	-1.03	+ 0.95 logTP	- 0.003 z_{mix}^2	+ 0.049 Temp		0.79	z_{mix}
		P < 0.001	*P* = 0.005	*P* = 0.007			
Standing stock of algal biomass							
log Chl-*a*	- 1.16	+ 0.93 logTP	+ 0.17 z_{mix}	- 0.010 z_{mix}^2	+ 0.049 Temp	0.73	
		P < 0.001	*P* = 0.016	*P* = 0.054	*P* = 0.002		
Light at the bottom of the mixed layer							
log I_{out}	2.16	- 1.79 log TP	- 0.11 z_{mix}	+ 0.072 Temp		0.65	z_{mix}^2
		P < 0.001	*P* < 0.001	*P* = 0.017			
Seston C:P ratio							
C:P	201	- 141 log TP	- 13.07 z_{mix}	+ 12.50 Temp		0.39	z_{mix}^2
		P < 0.001	*P* = 0.002	*P* = 0.012			
Dissolved nutrient concentration							
log (SRP+1)	-0.23	+ 0.62 logTP	- 0.16 z_{mix}	+ 0.016 z_{mix}^2		0.52	Temp
		P < 0.001	*P* = 0.056	*P* = 0.012			
Zooplankton biomass							
log Zoo	3.61	+ 0.31 log TP	- 0.075 z_{mix}	- 0.058 Temp		0.51	z_{mix}^2
		P = 0.002	*P* < 0.001	*P* = 0.036			

Response variables are algal biomass concentration (mg Chl-*a* m^{-3}); standing stock of algal biomass (mg Chl-*a* m^{-2}); light at the bottom of the mixed layer, I_{out} (% of incident PAR); seston C:P ratio (mol:mol); soluble reactive phosphorus concentration, SRP (mg m^{-3}), and zooplankton density, Zoo (g dry mass m^{-3}). Predictor variables are total phosphorus concentration, TP (mg m^{-3}); mixing depth, z_{mix} (m), squared mixing depth, z_{mix}^2 (m^2); and water temperature, Temp (°C). N = 40 for zooplankton; N = 65 for all other variables.

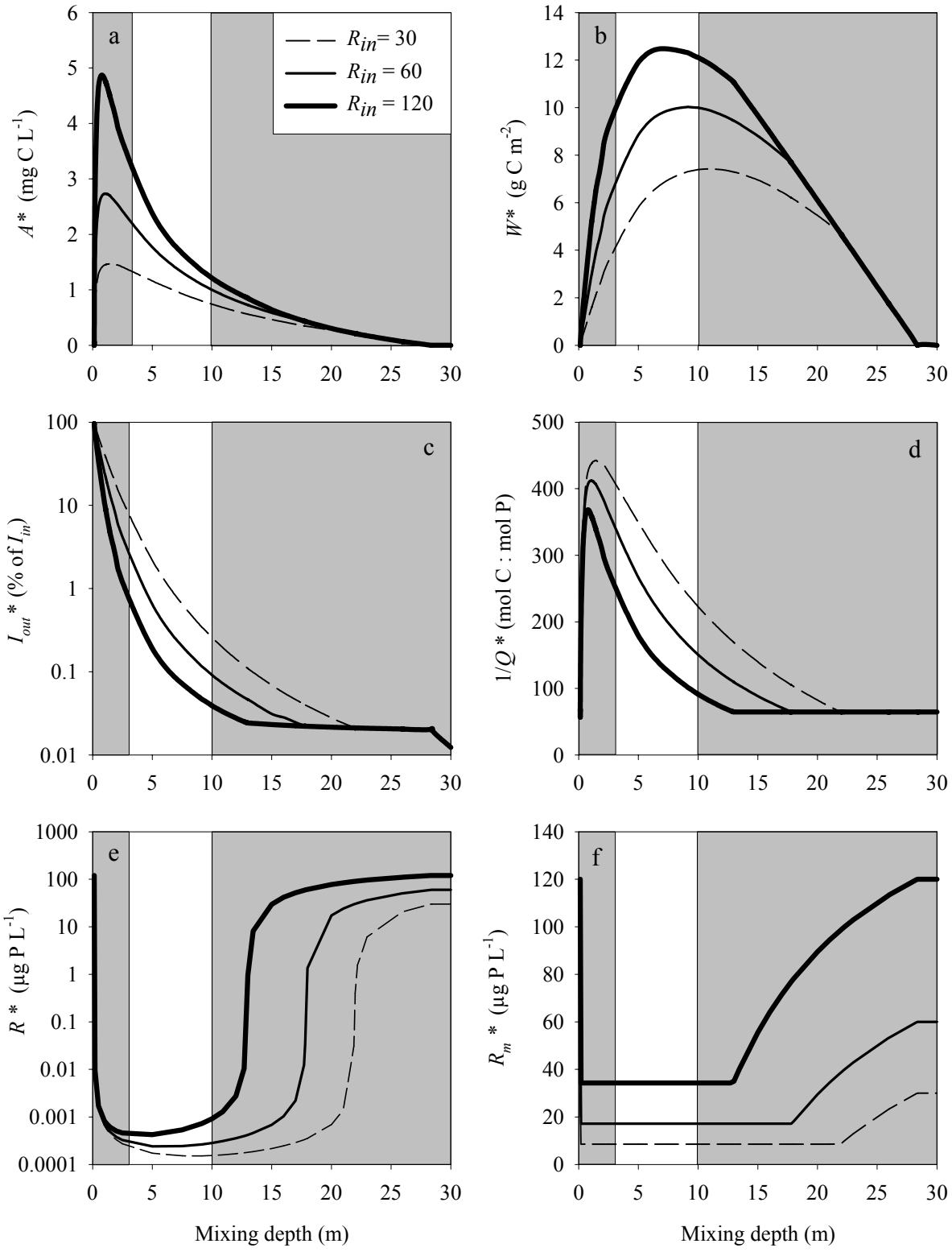
a
b
c
d
e
f
R_{in}= 30
R_{in} = 60
R_{in} = 120
A^* (mg C L^{-1})
W^* (g C m^{-2})
I_{out} * (% of I_{in})
1/Q^* (mol C : mol P)
R^* (µg P L^{-1})
R_m * (µg P L^{-1})
Mixing depth (m)
Mixing depth (m)

Figure 1 Equilibrium relationships among mixing depth, nutrient supply, and six state variables predicted by the model. a, algal biomass concentration, A^*; b, standing stock of algal biomass, W^*; c, light at the bottom of the mixed layer, I_{out}^*; d, carbon:nutrient ratio of algal biomass, $1/Q^*$; e, dissolved mineral nutrient concentration, R^*; f, total (dissolved+particulate) nutrient concentration, R_m^*. The illustrated examples are for realistic parameter values and three dissolved mineral phosphorus concentrations in the external source, R_{in}: 30 mg P m^{-3} (dashed line), 60 mg P m^{-3} (fine solid line), 120 mg P m^{-3} (bold solid line). The remaining parameter values are: D=0.02 day^{-1}, H=120 µE $m^{-2}s^{-1}$, I_{in}=300 µE $m^{-2}s^{-1}$, k=0.0004 m^2 mg^{-1} C, K_{bg}=0.3 m^{-1}, l_m=0.13 day^{-1}, M=1.5 mgP m^{-3}, p_{max}=1.0 day^{-1}, Q_{max}=0.04 gP gC^{-1}, Q_{min}=0.004 gP gC^{-1}, ρ_{max}=1 gP $gCday^{-1}$, v=0.05 m day^{-1}. Unshaded areas indicate the mixing depth range of the lake survey.

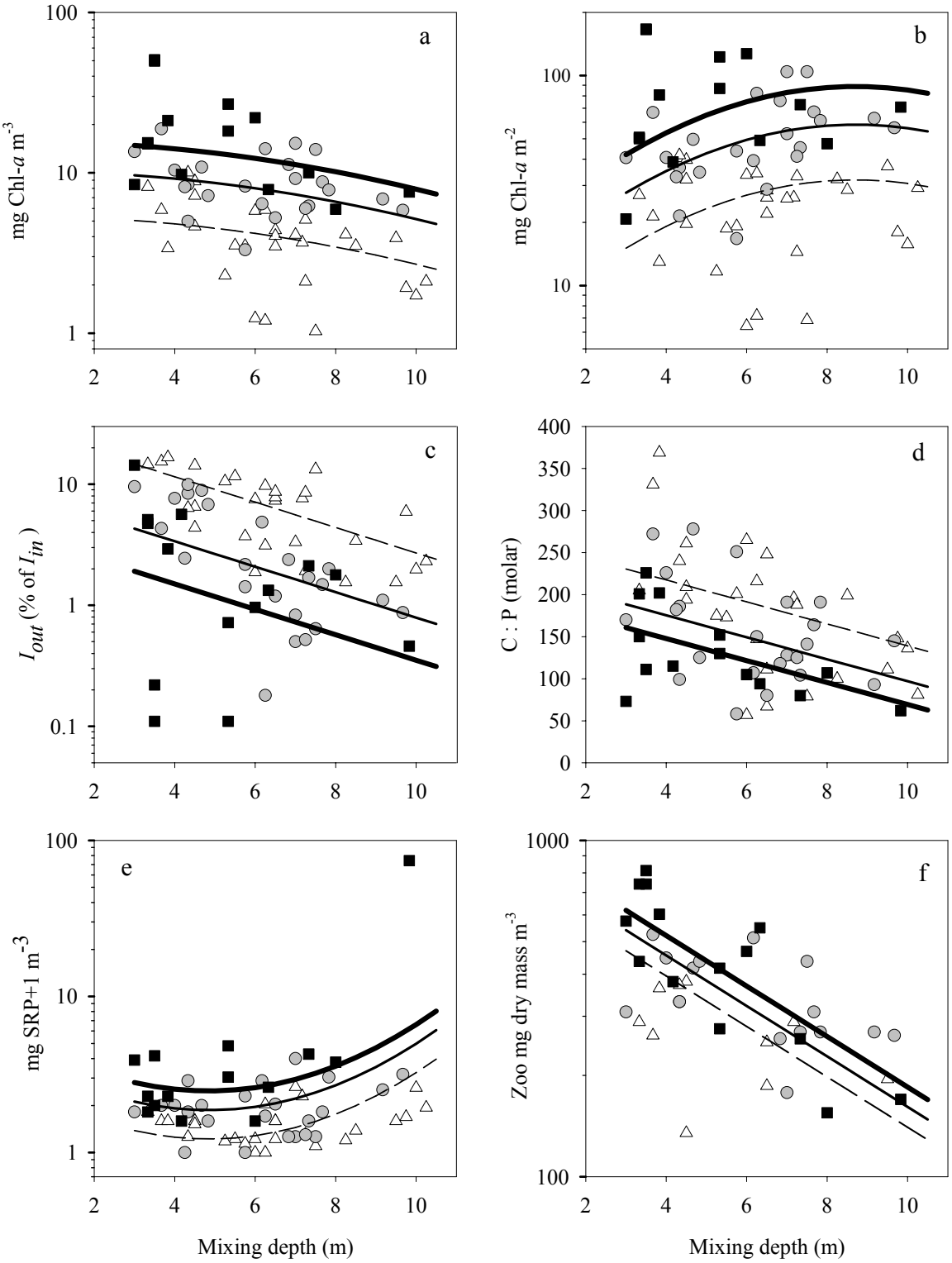
a
b
c
d
e
f
mg Chl-a m-3
mg Chl-a m-2
Iout (% of Iin)
C : P (molar)
mg SRP+1 m-3
Zoo mg dry mass m-3
Mixing depth (m)
Mixing depth (m)

Figure 2 Relationships among mixing depth, nutrient loading, and six variables in the surveyed lakes. a, algal biomass concentration (mg chlorophyll-*a* m^{-3}). b, standing stock of algal biomass (mg chlorophyll-*a* m^{-2}). c, intensity of photosynthetically active radiation at the bottom of the mixed layer (% of surface irradiation). d, molar seston carbon:phosphorus (C:P) ratio. e, dissolved mineral phosphorus concentration (mg (SRP+1)m^{-3}). f, Zooplankton density (g dry mass m^{-3}). Nutrient loading is approximated by total phosphorus (TP) concentration and lines illustrate the empirical regression models (Table 1) for the median TP values in three nutrient categories: TP<20 mg m^{-3} (white triangles, dashed line), TP=20-30 mg m^{-3} (grey circles, fine solid line), TP>30 mg m^{-3} (black squares, black solid line). N=65 in a-e, N=40 in f.

Supplementary Table 1 Lake characteristics. Lake area (A), mixing depth (z_{mix}), total phosphorus (TP), water temperature (Temp), volumetric chlorophyll-*a* (Chl-*a*), zooplankton dry mass (Zoo); light (PAR) at the bottom of the mixed layer (I_{out}) in percent of incoming light, soluble reactive phosphorus (SRP), and molar seston carbon to phosphorus (C:P) ratio. Data are seasonal averages of integral mixed surface layer samples collected on three occasions during summer 2001 (Region 1) and two occasions during summer 1998 (Region 2).

Nr	Lake	A	z_{mix}	TP	Temp	Chl-*a*	Zoo	I_{out}	SRP	C:P
		km^2	m	$mg\ m^{-3}$	°C	$mg\ m^{-3}$	$mg\ m^{-3}$	% of I_{in}	$mg\ m^{-3}$	mol:mol
	Region 1: Northern Germany									
1	Behler See	3.10	7.7	25	17.9	9.2	319	1.6	1.0	164
2	Blunker See	0.20	3.7	25	19.7	20.5	631	4.6	1.3	272
3	Bornhöveder See	0.73	3.5	94	19.8	57.2	853	4.6	6.3	111
4	Dieksee	3.86	9.2	27	17.7	7.1	278	1.5	2.7	93
5	Edebergsee	0.08	4.7	27	18.7	13.7	487	10.5	1.3	278
6	Garrensee	0.19	4.5	16	20.0	8.4	157	15.2	0.7	209
7	Grebiner See	0.29	4.5	19	18.8	11.4	409	9.3	0.7	261
8	Großensee	0.75	4.3	16	20.6	10.8	392	9.4	0.3	240
9	Großer Eutiner See	2.18	5.3	98	20.6	27.3	276	4.0	4.7	152
10	Großer Madebröckensee	0.07	3.7	16	18.8	6.5	272	15.8	0.7	331
11	Großer Plöner See (Ascheberg)	17.40	8.0	33	18.8	7.3	164	2.5	5.0	107
12	Großer Plöner See (Plön)	12.39	9.7	25	18.2	6.7	279	1.4	2.3	145
13	Großer Pönitzer See	1.08	4.0	25	20.8	11.2	465	10.0	1.0	226
14	Großer Segeberger See	1.70	4.2	35	20.5	10.0	398	5.9	0.7	115
15	Hemmelsdorfer See	4.10	3.5	90	21.2	52.9	773	0.4	1.0	226
16	Höftsee	0.19	7.8	24	17.3	9.4	304	2.1	2.7	191
17	Ihlsee	0.28	3.8	9	20.8	3.4	502	17.3	0.7	369
18	Kellersee	5.52	7.5	29	18.8	14.6	472	1.1	0.3	141
19	Kleiner Plöner See	2.39	7.3	50	17.9	11.2	271	2.5	5.0	80
20	Kolksee	0.03	3.0	21	19.0	13.9	308	9.9	1.0	170
21	Lankauer See	0.14	3.3	18	20.9	9.0	306	15.3	1.0	205
22	Lüttauer See	0.41	3.3	32	19.8	22.2	549	9.7	1.0	150
23	Muggesfelder See	0.27	4.3	22	19.3	9.0	339	8.6	2.0	186
24	Plußsee	0.14	3.0	38	19.0	6.9	626	14.7	4.0	73

Nr	Lake	A	z_{mix}	TP	Temp	Chl-*a*	Zoo	I_{out}	SRP	C:P
		km^2	m	mg m^{-3}	°C	mg m^{-3}	mg m^{-3}	% of I_{in}	mg m^{-3}	mol:mol
25	Ratzeburger See	16.20	7.0	30	18.8	15.4	224	1.4	0.3	128
26	Schaalsee	6.22	6.8	27	19.4	11.6	256	3.6	0.3	118
27	Schierensee (Grebin)	0.15	4.8	22	18.3	8.7	530	8.0	0.7	125
28	Schluensee	1.27	6.2	26	18.7	6.6	633	5.1	2.3	107
29	Schmarksee	0.07	3.3	35	18.3	15.8	792	6.6	1.3	158
30	Schöhsee	0.78	6.5	12	17.5	4.7	218	8.0	0.7	201
31	Selenter See	22.39	9.5	19	17.9	4.4	211	3.5	0.7	67
32	Stocksee	2.11	6.5	16	17.8	4.1	317	7.8	0.7	111
33	Stolper See	1.40	6.0	40	18.3	28.0	558	1.0	0.7	105
34	Suhrer See	1.37	7.2	11	17.9	3.8	376	8.4	1.3	196
35	Trammer See	1.63	7.3	22	17.8	7.0	291	2.0	0.7	104
36	Ukleisee	0.32	4.3	21	19.9	6.5	339	11.4	1.0	99
37	Vierer See	1.32	6.3	39	17.3	10.8	576	1.9	3.0	94
38	Westensee	7.70	5.3	58	19.8	18.6	430	2.4	9.7	130
39	Wielener See	0.25	3.8	35	18.6	24.6	630	4.0	1.3	202
40	Wittensee	10.30	9.8	122	18.8	9.1	170	1.9	74.0	62
	Region 2: Southern Germany									
41	Ammersee	46.60	10.0	9	14.9	1.7		4.5	1.6	136
42	Brunnsee	0.06	6.5	10	16.9	3.7		10.2	0.3	248
43	Chiemsee	79.90	8.3	12	15.9	4.1		3.9	0.2	100
44	Fohnsee	0.21	5.3	10	15.6	2.3		13.9	0.2	175
45	Griessee	0.09	6.0	13	16.4	5.8		3.7	0.3	265
46	Hartsee	0.87	5.8	9	16.3	3.6		4.6	0.2	201
47	Klostersee	0.47	8.5	9	16.8	3.6		5.9	0.5	199
48	Langbürgener See	1.04	7.3	8	17.8	2.1		14.8	0.4	188
49	Obinger See	0.31	7.3	20	15.5	6.2		0.6	0.4	125
50	Ostersee	1.18	6.3	9	15.6	1.2		11.8	0.0	147
51	Pelhamer See	0.71	6.3	24	15.2	15.2		0.5	0.8	150
52	Riegsee	1.89	7.0	19	16.0	4.2		10.6	1.7	126
53	Schliersee	2.22	5.8	20	14.2	8.4		5.0	0.0	251
54	Schloßsee	0.27	5.5	11	17.9	3.6		14.0	0.3	173
55	Simssee	6.49	7.3	15	17.2	5.2		7.3	0.4	126
56	Staffelsee	7.66	5.8	20	15.4	3.6		6.7	1.3	58
57	Starnberger See	56.36	10.3	15	14.9	2.6		2.5	1.0	81
58	Tachinger See	2.36	6.3	15	17.4	6.2		7.6	1.3	216
59	Tegernsee	8.93	6.0	17	13.8	1.3		14.0	0.0	57
60	Thalersee	0.04	4.3	24	17.1	8.4		14.5	0.0	182

Nr	Lake	A	z_{mix}	TP	Temp	Chl-*a*	Zoo	I_{out}	SRP	C:P
		km^2	m	mg m^{-3}	°C	mg m^{-3}	mg m^{-3}	% of I_{in}	mg m^{-3}	mol:mol
61	Tüttensee	0.11	4.5	14	17.7	4.8		10.3	0.7	194
62	Waginger See	6.61	6.5	20	16.9	5.4		8.0	1.3	80
63	Walchensee	16.20	7.5	11	13.5	1.1		21.1	0.1	79
64	Wesslinger See	0.17	7.0	28	16.0	9.3		4.7	3.1	191
65	Wörthsee	4.34	9.8	9	15.3	2.0		7.3	0.9	148

Article 3

PHYTOPLANKTON TAXONOMIC COMPOSITION IN RELATION TO ENVIRONMENTAL VARIABLES: A COMPARATIVE LAKE STUDY USING PIGMENT ANALYSIS

(Stella A. Berger, Ilka Peeken, Sebastian Diehl)

Phytoplankton taxonomic composition in relation to environmental variables: a comparative lake study using pigment analysis

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Abstract

Comparative studies of the taxonomic composition of lake phytoplankton are rare and have been impeded by the time-consuming examination and enumeration of individual species under the microscope. We sampled the mixed surface layers of 40 Northern German lakes repeatedly during summer stratification, analysed the samples for 13 algal pigments with high pressure liquid chromatography, and calculated the contributions of seven algal classes with distinct pigment signatures to total chlorophyll-*a* using CHEMTAX. We then performed multiple regressions to relate phytoplankton biomass and composition to total nitrogen (TN), total phosphorus (TP), total silica (TSi), mixing depth, temperature, and zooplankton biomass. Total Chl-*a* was positively related to TN and TP and unimodally related to mixing depth. Although molar TN:TP ratios suggested that phosphorus was the more limiting nutrient, TN was the factor most strongly related to the biomasses of single taxa. We found positive relationships of chrysophytes, chlorophytes (the two most abundant taxa), cryptophytes, and euglenophytes to TN, and of diatoms and chrysophytes to TSi. Diatoms were negatively related to TN. Cryptophytes and chlorophytes were negatively, and cyanobacteria were positively related to zooplankton. Finally, the relative biomasses of chrysophytes and cryptophytes were negatively related to mixing depth. Most, but not all, results are consistent with theoretical expectations or previously documented patterns. Some plausible relationships may, however, have been masked by strong cross-correlations among several environmental variables.

Introduction

The content of chlorophyll *a* (Chl-*a*), the major photosynthetically active pigment in most phytoplankton, has for decades been routinely used as an inexpensive, highly reproducible, and easily measurable proxy of phytoplankton biomass in lake water. Consequently, a wealth of comparative data on Chl-*a* concentrations from lakes all over the world has accumulated. This rich data base has, in turn, stimulated the search for empirical patterns relating phytoplankton biomass to environmental factors, the positive relationship of Chl-*a* to total phosphorus (TP) and its modifications by food web interactions being the perhaps most prominent example (Dillon and Rigler 1974; Schindler 1978; Persson et al. 1992; Sarnelle 1992; Mazumder 1994). Because more detailed analyses of the functional and taxonomic composition of phytoplankton require time consuming microscopic counts by highly skilled personal, such analyses are rarely included in routine monitoring or sampling programs of larger sets of lakes. Consequently, much empirical knowledge relating the abundances of single phytoplankton taxa to environmental factors is still largely based on descriptive case studies. Clearly, there is a need for more comprehensive, comparative studies of these relationships across larger sets of lakes.

It is well known that phytoplankton taxa differ in their resource requirements and in their responses to physical conditions and grazing. For example, phytoplankton taxonomic composition usually changes with increasing nutrient levels due to differences among taxa in nutrient uptake, storage, and growth rates (Reynolds 1984). While previous comparative lake studies have primarily focused on TP as the nutrient of interest (Sandgren 1988; Jensen et al. 1994; Watson et al. 1997), growth of phytoplankton may be limited by other elements such as nitrogen, silica or carbon (Hecky et al. 1988). The relationships of total nitrogen (TN) and total silica (TSi) to phytoplankton biomass and taxonomic composition have, however, rarely been examined in freshwater systems (but see Huszar et al. 1998; Tilman et al. 1986; Jeppesen et al. 2000; Downing et al. 2001). Phytoplankton taxa also differ in their requirements for light and in the rates at which they sink out of the mixed surface layer. Both of these aspects are mediated by depth of the mixed surface layer: increasing mixing depth decreases both average light supply (affecting all taxa) and sinking losses (affecting negatively buoyant taxa), (Visser et al. 1996; Diehl et al. 2002; Soto 2002; Ptacnik et al. 2003). In spite of these obvious mechanisms, the influence of mixing depth on phytoplankton taxonomic composition has, to our

knowledge, not yet been addressed with comparative lake data. Finally, phytoplankton taxa differ vastly in their susceptibilities to different types of grazers. Although grazing rates are often highly taxon- and size-specific, studies of grazing impacts on the taxonomic composition of natural phytoplankton assemblages are so far largely restricted to experimental systems (Sarnelle 1992, 1993; 2003; Sommer et al. 2001).

Many ecologically relevant algal traits are shared by most or all members of higher taxonomic units (e.g., algal classes) that are also characterized by unique combinations of photosynthetically active and accessory pigments (Rowan 1989). Pigment analysis by high-performance liquid chromatography (HPLC) in combination with chemotaxonomical classification therefore promises to be a highly useful method for the rapid functional and taxonomical characterization of phytoplankton assemblages in large data sets (Jeffrey et al. 1997; Peeken 1997). Until recently, the use of pigment analysis for the functional characterization of plankton samples has been limited, because chemotaxonomical classifications were based on marker pigments that are not always unique to single algal classes. Consequently, pigment analysis could not distinguish among functionally distinct taxa sharing the same marker pigments, e.g., chrysophytes and diatoms sharing fucoxanthin (Wilhelm et al. 1991). CHEMTAX, a matrix factorization program developed for marine phytoplankton (Mackey et al. 1996), greatly alleviates this shortcoming. CHEMTAX combines information on a set of samples' contents of 'marker' pigments (unique to one or two algal groups) and 'diagnostic' pigments (shared by several algal groups) with taxon-specific pigment to Chl-*a* ratios and calculates in an iterative process the contribution of each taxon to total Chl-*a*. So far, the use of CHEMTAX has been largely restricted to marine and brackish systems (Mackey et al. 1998; Wright et al. 1996, 2000; Higgins et al. 2000; Rodriguez et al. 2003; Schlüter et al. 2000, 2003). Very few studies have applied this promising technique in freshwater (Descy et al. 2000; Marinho et al. 2003; Fietz et al. 2004; Buchaca et al. 2005) with the consequence that class-specific pigment to Chl-*a* matrices for freshwater phytoplankton are still rare in the literature.

In the present study, we sampled phytoplankton, zooplankton, and a host of physical and chemical parameters in the mixed surface layers of 40 Central European lakes several times during summer stratification. We determined the taxonomic composition of the phytoplankton samples at the level of algal classes

after extraction of 13 lipophilic pigments with high-performance liquid chromatography (HPLC) followed by CHEMTAX calculations of the quantitative contributions of the different algal classes to total Chl-*a*. We then used multiple regressions to examine the relationships of total phytoplankton biomass and taxonomic composition to a set of environmental variables including nutrients (total nitrogen, phosphorus, and silica), mixing depth, water temperature, and zooplankton density. Our study aims at contributing to the adaptation of the CHEMTAX procedure to freshwater systems and to the build-up of a comparative database relating the taxonomic composition of lake phytoplankton to environmental drivers.

Materials and methods

Study lakes and sampling

We selected 40 dimictic study lakes (size range 0.03 - 22.39 km^2, Table 1) in an area southwest of the Baltic Sea (northern Germany) located around the Max-Planck-Institute of Limnology in Plön (Schleswig-Holstein). The lakes are meso- to eutrophic, have similar geochemistry and experience similar weather regime. Each lake was sampled three times in 2001, shortly after the beginning (05-29 June), in the middle (12 July - 03 August), and towards the end (14 August - 06 September) of summer stratification.

On each sampling occasion, we first determined the depth of the mixed surface layer by recording a vertical profile of temperature, conductivity, oxygen concentration, and pH at 1 m steps, reducing step size to 0.5 m close to the suspected lower limit of the mixed layer. Mixing depth was defined as the depth at which the temperature difference to the lake surface (or to a depth of 1m, if other parameters indicated short-term heating of the surface) did not exceed more than 1 °C. We next collected an integral mixed layer water sample by combining three to five water samples taken with a 2-L-Ruttner sampler at regularly spaced depth intervals in the mixed surface layer. All water samples were transported to the laboratory in cooled, opaque boxes within a few hours. Total phosphorus (TP), total nitrogen (TN), and total silica (TSi) contents were determined from unfiltered water samples. TP was analysed after persulfate oxidation of all organic phosphorus according to the phosphorus-molybdate method (Wetzel et al. 1991). The oxidation of organic nitrogen and silica was carried out following Wetzel & Likens (1991). TN was then determined by anion-chromatography as NO_3-N (DIONEX, Sunnyvale, CA,

U.S.A.) and TSi by spectrophotometry at a wavelength of 700 nm (Wetzel et al. 1991). We finally took a zooplankton sample of the mixed surface layer by pulling a 50-µm net (8.5 cm effective opening diameter) vertically from the bottom of the mixed layer to the lake surface. The samples were fixed with 40 % sugar-formalin to a final concentration of 4 %. We identified and counted all mesozooplankton under a binocular and measured the body length of up to 30 individuals of each taxon using an image analysis program (Analysis, Soft Imaging System, Münster, Germany). We then calculated dry biomass of mesozooplankton (cladocerans, adult copepods, and copepodites) using length-weight-relationships (Botrell et al. 1976).

Phytoplankton biomass and pigment measurements

To estimates seston biomass we measured particulate organic carbon (POC) and suspended particle volume. For POC, 100-800 ml aliquots of 200 µm-screened water samples were filtered on pre-combusted 0.45 µm Whatman GF/C glass-fibre filters and POC was determined by infrared-spectrophotometry (C-Mat 500, Ströhlein, Korschenbroich, Germany). Suspended particle volume (particle size range 3-30 µm equivalent spherical diameter) was measured on 1200 µl aliquots of unfiltered samples in a cell counter and analysis system (CASY-1, model TTC, Schärfe, Reutlingen, Germany).

Lipophilic photosynthetic marker pigments were determined by high-performance liquid chromatography (HPLC) using a modified method of Barlow et al. (1997). The HPLC system (Waters, Millford, U.S.A) was equipped with an auto sampler (717 plus), a pump (series 600), a fluorescence detector (474), a photo diode array (PDA 996), and Millenium software. 100-800 ml of 200 µm-filtered water samples were filtered through Whatman GF/C glass-fibre filters, wrapped in aluminium foil and immediately stored at –70 °C. Pigment extraction was carried out by adding 2.5 ml of cold acetone (100 %), 100 µl cooled internal standard (canthaxanthin) and glass beads (2 and 4 mm) to each filter. After homogenizing for 5 minutes in a cell mill the suspensions were centrifuged for 10 minutes at 5500 rpm in a cooled (4 °C) centrifuge (Sigma 3 K12). The extracts were filtered through 0.2 µm cellulose filters, placed in Eppendorf cups and stored at –30 °C until analysis within the next 12 hours. Aliquots of 120 µl were injected into the HPLC system. The pigments were analysed by reverse-phase HPLC using a C_8 Hypersil MOS-2 3 µm (4.6 x 100 mm) Altech column and HPLC-grade solvents (solvent A = 70 % methanol + 30 % 0.5 M ammonium acetate, solvent B = 100 % methanol). Starting with 65 % A and 35 % B

the gradient and was run to 100 % B within 25 minutes and then run back to start conditions, all at a continuous flow rate of 1 ml min^{-1}. Eluted pigments were detected by absorbance (440 nm) and fluorescence (extinction: 410 nm; emission: > 600 nm). The resulting chromatogram shows a peak for each pigment at its characteristic retention time. Additionally, pigments were identified by their absorption curves and checked against library pigment spectra of standards and algal extracts using Millenium software. Pigment concentrations were quantified based on peak areas of each pigment and normalized to the internal standard canthaxanthin. Published specific extinction coefficients were used to determine the amounts of carotenoids (Bidigare 1991).

The HPLC method effectively separated 12 distinct lipophilic photosynthetic pigments (Table 2) in addition to chlorophyll *a* (Chl-*a*). Pigment degradation products were negligible. Chl-*a* (including its allomeric and epimeric forms), the major pigment of algal light absorption which is routinely used as an estimate of photosynthetically active phytoplankton biomass (Sakshaug 1997), was highly correlated with seston POC (Pearson Correlation 0.92, $p < 0.0001$) and total particle volume (Pearson Correlation 0.96, $p < 0.0001$).

CHEMTAX procedure

We first calculated pigment to Chl-*a* mass ratios from our samples. Then CHEMTAX (Mackey et al. 1996), a matrix factorisation program, was applied to our pigment data set. CHEMTAX estimates Chl-*a* biomass for various algal classes by using several pigments per algal class and allows thus the distinction between algal classes that share the same marker pigments (i.e., diatoms vs. chrysophytes and chlorophytes vs. euglenophytes), which is not possible using more conventional multiple regression approaches. The program uses a factor analysis and a steepest descent algorithm to find the best fit to the data based on an initial pigment ratio matrix for the classes to be determined. As input ratio matrix we used a modified pigment matrix that originates from a pigment analysis of mixed surface layer samples of several northern Wisconsin lakes during summer stratification (Descy et al. 2000), (Table 2a). Because carotenoid to Chl-*a* ratios within lake phytoplankton assemblages may vary depending on light levels (Millie et al. 2002), the data set comprising 120 (3 x 40 lakes) mixed surface layer samples was divided into three subgroups differing in mixing depth (shallow 2–4 m, intermediate 4.5-6.5 m, deep 7-13.5 m). CHEMTAX processing was run separately on each depth group in order to

minimize potential errors in the estimation of pigment ratios caused by differences in light climate (Goericke et al. 1998; Henriksen et al. 2002).

The pigments used for fitting the algal class abundances were peridinin (dinoflagellates), fucoxanthin (diatoms, chrysophytes), violaxanthin (chlorophytes, chrysophytes), neoxanthin (chlorophytes, euglenophytes), diadinoxanthin (diatoms, dinoflagellates, euglenophytes), alloxanthin (cryptophytes), zeaxanthin (chlorophytes, chrysophytes, cyanobacteria type 1, type 2), lutein (chlorophytes), diatoxanthin (diatoms, dinoflagellates, euglenophytes), chlorophyll *b* (chlorophytes, euglenophytes), echinenone (cyanobacteria type 2), and α-carotene (most chlorophyta, cryptophyta) (Table 2). Descy et al. (2000) differentiated two types of cyanobacteria (type 1, type 2) which we combined into a single group, because there does not seem to be a clear functional difference between the two types. We thus distinguished seven phytoplankton taxa, chlorophytes, chrysophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates, and euglenophytes and expressed their biomasses as absolute and relative contributions to total Chl-*a*.

Generally, the pigment to Chl-*a* output ratios calculated by CHEMTAX program were lower in our data set than the input pigment ratios (Table 2). The output ratios of most algal classes were however well within the ranges documented in the freshwater literature, with the exception of the ratios of fucoxanthin to Chl-*a* in chrysophytes, which were about 2-fold lower than previously documented values (Wilhelm et al. 1991; Soma et al. 1993; Woitke et al. 1996; Yacobi et al. 1996; Descy et al. 2000; Schagerl et al. 2003 a,b; Marinho et al. 2003; Deydier-Stephan et al. 2003; Buchaca et al. 2005). After CHEMTAX processing most pigment to Chl-*a* ratios showed only minor deviations among the three subgroups differing in mixing depth (Table 2b).

Multiple regression analyses

We investigated relationships of phytoplankton biomass and taxonomic composition to the environmental variables nutrient content (TN, TP, TSi), mixing depth, water temperature, and zooplankton biomass using multiple, linear regression with stepwise variable selection (backward procedure, p_{out} = 0.1, p_{in} = 0.05) in SPSS 12.0.1. Since we were interested in average relationships during summer stratification, we performed the analyses on seasonal means averaged across the three sampling periods. Where data transformations were necessary to conform with the assumptions of multiple regression, data were first transformed and then

averaged. Total nutrient concentrations, zooplankton biomass, as well as total and class-specific phytoplankton biomass (Chl-*a*) were log-transformed. Relative biomasses of the algal classes were arc sine square root transformed. To allow for the possibility of hump-shaped relationships of algal biomasses to mixing depth (Diehl 2002; Diehl et al. 2002; Ptacnik et al. 2003) we included squared mixing depth as an environmental variable. In all analyses, Cook´s distance was < 1 and studentized residuals were normally distributed for all but a single data point in a single analysis.

Results

Averaged across the three samplings, the mixed surface layers of the 40 studied lakes covered a wide range of nutrient concentrations (TP: 9 - 122 µg L^{-1}; TN: 0.49 - 1.65 mg L^{-1}; TSi: 0.21 – 10.33 mg L^{-1}) and a moderate range of mixing depths (3.0 – 9.8 m) and water temperatures (17.3 – 21.2 °C), (Table 1). Average mesozooplankton density ranged from 28 to 655 µg dry weight L^{-1}, with cladocerans and copepods each contributing on average 50 % to total mesozooplankton. The mean Chl-*a* concentration ranged from 3.4 µg L^{-1} in low productive Lake Ihlsee to a maximum of 57.2 µg L^{-1} in nutrient-rich Bornhöveder See (Table 1). Over the sampling season, average total Chl-*a* content of the 40 lakes increased from 9.2 µg L^{-1} in June to 18.9 µg L^{-1} in August/September (Fig. 1). With the exception of chlorophytes all algal taxa showed a similar seasonal increase, so the relative contributions of different taxa to total chlorophyll did not change dramatically over time (Fig. 1). Overall, chrysophytes were the most abundant algal taxon (contributing 27 % to total Chl-*a*) followed by chlorophytes (23 %), cyanobacteria (18 %), cryptophytes (12 %), dinoflagellates (9 %), diatoms (8 %), and euglenophytes (4 %).

Relationships among phytoplankton and environmental factors

Between-lake differences in total nutrient concentrations, mixing depth, and water temperature are largely driven by physical properties of the lakes and by geology and land use in their catchments and can therefore be regarded as abiotic drivers of phytoplankton biomass and community composition. In contrast, zooplankton biomass is likely influenced by some of these abiotic variables and by feedback processes linking zooplankton to their algal food. In addition, several of these variables were cross-correlated, which makes it difficult to separate all of their independent contributions to the between-lake variance in phytoplankton biomass

and community composition. We therefore briefly present the correlation structure among all variables characterizing the algal environment (Table 3). We found significant positive correlations of TN with TP, zooplankton and temperature, and of TP with TSi. Mixing depth was negatively correlated with TN, temperature and zooplankton.

Stepwise multiple regression revealed that total Chl-*a* concentration was strongly positively related to total nitrogen and total phosphorus concentrations and showed a weak unimodal relationship to mixing depth (indicated by a positive linear and a negative quadratic term in z_{mix}), (Table 4a, Fig. 2). According to the regression equation a maximum in Chl-*a* concentration occurred around a mixing depth of 5.4 m (Fig. 2b). Because TN was highly correlated with TP (Table 3) we only show the relationship of Chl-*a* to TN (Fig. 2a). Excluded from the regression model as non-significant were temperature, total silica, and zooplankton biomass.

The absolute and relative biomasses (expressed as Chl-*a*) of the seven phytoplankton taxa showed diverse patterns in relation to the set of environmental variables (Table 4). The concentration of TN was the factor most frequently related to single taxa. TN was positively related to the absolute biomasses of chrysophytes, chlorophytes, cryptophytes, and euglenophytes and negatively related to the biomass of diatoms (Table 4a, Fig. 3a-d). In addition, the proportional contribution of diatoms to total phytoplankton biomass was negatively related to TN, as was that of dinoflagellates (Table 4b, Fig. 3e,f). Both the absolute and the relative biomasses of chrysophytes and diatoms were positively related to TSi (Table 4, Fig. 4). TP was positively related to the absolute biomass of cryptophytes and the relative biomass of dinoflagellates and negatively related to the relative biomass of cyanobacteria. Both the relative and absolute biomasses of cryptophytes were negatively related to zooplankton biomass, whereas the relative and absolute biomass of cyanobacteria showed a strongly positive relationship to zooplankton biomass (Table 4, Fig. 5). Although the absolute biomasses of four phytoplankton taxa (chrysophytes, chlorophytes, cyanobacteria, euglenophytes) were negatively correlated with mixing depth in bivariate correlations (correlation coefficients range from - 0.32 to - 0.41, $p < 0.05$), most taxa showed no relationship to mixing depth in multiple regression, probably because the explanatory variables TN and zooplankton were highly correlated with mixing depth (Table 3). Exceptions were a unimodal relationship of absolute dinoflagellates biomass to mixing depth and negative relationships of the

relative biomasses of chrysophytes and dinoflagellates to mixing depth, all of which were rather weak (Table 4). Temperature was in almost all cases excluded from multiple regressions; only the absolute biomass dinoflagellates showed a weak positive relationship to temperature (Table 4).

Discussion

Possibilities and limitations of CHEMTAX

CHEMTAX program offers several advantages compared to more conventional approaches to determine phytoplankton taxonomic composition. Compared to regression approaches based on exclusive marker pigments, CHEMTAX uses additional, diagnostic pigments. CHEMTAX therefore has the potential to separate algal taxa that cannot be distinguished based on exclusive marker pigments alone (e.g., diatoms and chrysophytes, chlorophytes and euglenophytes), (Mackey et al. 1996; Descy et al. 2000). Compared to microscopic counts, which are very time-consuming and require considerable training, pigment analysis is not only much easier to learn and faster to execute, but may also more accurately assess autotrophic picoplankton. Picoplankton, which occurs in several algal classes (chlorophytes, chrysophytes, cyanobacteria), does not settle quantitatively in sedimentation chambers and is therefore usually underestimated in microscopic counts (Schlüter et al. 2000; Fietz et al. 2004). Of course, pigment analysis can currently only distinguish phytoplankton on the relatively coarse taxonomic level of classes. Pigment analysis may therefore be most usefully applied in research addressing patterns and processes related to class-specific algal physiological and ecological traits.

The CHEMTAX approach gives reproducible and accurate results on the taxonomic composition of phytoplankton only if the ratios of marker and diagnostic pigments to Chl-*a* differ sufficiently among taxa and are largely unaffected by environmental conditions. The pigment content of various algal taxa has been found to be sensitive to light intensity, with photo protective pigments usually increasing with light intensity (Schlüter et al. 2000; Henriksen et al. 2002). We therefore calculated separate pigment ratio matrices for shallow, intermediate, and deep mixed layers. Variation among the three output matrices was, however, minor and did not show any conspicuous, depth-related trends. Overall, most output Chl-*a* to pigment

ratios were 30-50% lower compared to the ratios in Descy (2000), but still within the ranges described in the freshwater literature (see method section).

CHEMTAX has been originally developed in marine systems and has primarily been calibrated against microscopic cell counts of marine samples (Jeffrey et al. 1997; Schlüter et al. 2000, 2003; Millie et al. 2002; Henriksen et al. 2002). There are, however, by now at least a couple of thorough methodological studies from freshwater systems, three of which found mostly good quantitative agreement between the taxon-specific biomasses estimated from microscopic and CHEMTAX-based analyses, respectively (Descy et al. 2000; Fietz et al. 2004; Buchaca et al. 2005). As microscopic 'quality control' we therefore performed only a dozen spot checks of haphazardly selected samples, mostly to confirm that chrysophytes were indeed abundant in our lakes. We found good qualitative agreement between the results of microscopic scans and CHEMTAX.

Phytoplankton and environmental variables

While comparative lake studies examining relationships of total chlorophyll concentration to environmental variables are abundant in the literature, only few comparative studies have examined such relationships for single phytoplankton taxa. We therefore comment first briefly on total Chl-*a* data and then discuss taxon-specific results in detail in order of the relative abundances of the different algal classes.

The total phosphorus content of lakes is generally a good predictor of phytoplankton biomass, the positive TP-Chl-*a* relationship being one of the most pervasive empirical patterns in limnology (Vollenweider 1976; Schindler 1978; Peters 1986; McCauley et al. 1989, Huszar et al. 1998). Total Chl-*a* was indeed significantly positively related to TP also in our study lakes. Interestingly, total nitrogen content was also retained in the multiple regression model and, when analyzed separately, explained a larger proportion of the variance in total Chl-*a* than did TP (linear regressions of log Chl-*a* vs. log TN or log TP, R^2 = 0.65 and 0.51, respectively). TN was furthermore positively related to the biomasses of four algal classes, whereas TP was retained in the multiple regression model of only a single taxon (Table 4). This apparently stronger association of algal biomass with TN than with TP is surprising, because molar TN:TP ratios suggest that phosphorus should have been the production limiting nutrient in most lakes (molar TN:TP ratios were ≥ 27 in 38 out of the 40 study lakes, Table 1). Because TN is by far not as frequently measured as TP, we are aware of only a few comparative studies that have related both nutrients

simultaneously to algal biomass. In accordance with our results, positive relationships of both TN and TP to Chl-*a* have been found in some Japanese lakes (Sakamoto 1966), in 228 northern latitude lakes (Smith 1982), in north temperate, oligo- to eutrophic lakes (McCauley and Downing 1991), and in 25 shallow New Zealand lakes (Jeppesen et al. 2000).

Although depth of the mixed surface layer strongly affects the average light climate of phytoplankton, it has been rarely considered in comparative studies of lake phytoplankton. We found a weakly unimodal relationship of total Chl-*a* to mixing depth. Such a relationship is theoretically expected for negatively buoyant phytoplankton (Diehl 2002; Berger et al. 2005); the biomass maximum is, however, predicted to occur at mixing depths < 2 m, outside the mixing depth range of our lakes. When we combined our data with data from 25 south German lakes (from which taxon-specific Chl-*a* is not available, (Kunz and Diehl 2003) in a single analysis, we did indeed find a purely negative relationship of total Chl-*a* to mixing depth (Berger et al. 2005). In this study and in the extended data set mixing depth does, however, explain only a minor portion of the variance in total algal biomass, most of the variance being related to total nutrients.

Similar to the comparative study of Jeppesen et al.(2000), who also included TN and TP in the pool of explanatory variables, zooplankton biomass was excluded from the multiple regression of total Chl-*a*. This is not surprising given that phytoplankton and zooplankton biomass are mutually interdependent and therefore dynamically linked to the same bottom-up forces (nutrients, light, Table 3). Because different algal taxa differ in susceptibility to grazing, one would, however, expect that grazing sensitive (resistant) taxa should be negatively (positively) associated with grazer biomass. In line with this, we did indeed find relationships of zooplankton biomass to the relative and absolute biomasses of several algal classes (see below).

Chrysophytes were the most abundant algal taxon in our study lakes contributing on average 27 % to total Chl-*a*. The biomass of chrysophytes was positively related to TSi and TN and unrelated to TP. The former seems plausible, given that some chrysophytes cover their cells with layers of siliceous structures (Sandgren 1988), and the latter matches observations by Reynolds (1984a) and Sandgren (1988), who noted that chrysophytes required high TN:TP ratios for growth and were more common in lakes with mean summer TN:TP ratios >30 (as was the case in 90% of our study lakes). Watson et al. (1997) also found a lack of a relationship between TP

and absolute chrysophyte biomass in a comparative study including dimictic and polymictic lakes. Most chrysophytes are flagellated and capable of regulating their vertical position in the water column during relatively calm mixing conditions, which may be one reason why large, often colonial, chrysophytes seem to be most successful during periods of stratification (Sandgren 1988). Chrysophytes have been proposed to thrive under low temperature conditions (Sandgren 1988; Huszar et al. 1998) which was not corroborated by our data. Both absolute and relative biomasses were unrelated to temperature, as was also observed in a study of Swedish lakes (Ramberg 1979). The relative biomass of chrysophytes was instead weakly negatively related to mixing depth that is the factor most strongly and negatively affecting average light levels. There is little and conflicting information concerning the light requirement of chrysophytes (Sandgren 1988).

Chlorophytes were with an average contribution of 23 % to total Chl-*a* the second most abundant algal taxon in our study lakes. The absolute biomass of chlorophytes was positively related to TN and negatively to zooplankton biomass. The former is in line with the observation that growth of larger flagellated green algae was accompanied by large decreases in the surface nitrogen pool of lakes (Happey-Wood 1988). Consistent with our data, chlorophytes dominated in experimentally enriched mesocosms with high nitrogen and phosphorus concentrations (Gonzalez Sagrario et al. 2005) .Still, the relative biomass of chlorophytes was unrelated to TN, suggesting that their response to nutrient enrichment was average compared to the majority of taxa. Similarly, chlorophytes made up relatively constant fractions of the total biomass across a broad range of TP in a set of temperate lakes (Watson et al. 1997). The relative chlorophyte biomass was unrelated to zooplankton and, in fact to any of the recorded environmental variables. This lack of pattern of relative biomass may be a consequence of the extraordinary diversity of planktonic freshwater chlorophytes. Chlorophytes are the most species-rich and morphologically diverse phytoplankton class, and many of the traits that are characteristic of certain algal classes are found among some members of the chlorophytes, too. With respect to grazing, for example, some chlorophytes are highly susceptible to daphnids, others to copepods, and yet other taxa are resistant to both types of grazers (Sarnelle 2003; Sommer et al. 2001), which may explain why chlorophytes as a group did not differ in relative sensitivity to grazing from phytoplankton as a whole.

Cyanobacteria made up 18 % of total phytoplankton biomass in our study lakes, making them the third most abundant taxon. Cyanobacteria are favored by stable stratification and low levels of turbulence (Reynolds et al. 1987; Paerl 1988; Huisman et al. 2004). Absolute and relative biomasses were strongly positively related to zooplankton biomass, whereas the relative biomass of cyanobacteria was negatively related to TP. The latter contrasts with the frequent finding of positive relationships among TP and the relative and absolute biomasses of cyanobacteria in lakes (Smith 1986; Canfield et al. 1989; Watson et al. 1997; Scheffer et al. 1997; Huszar et al. 1998). Possibly, the negative relationship to TP arose by chance as an artifact of the strong positive relation between TP and TN (Table 3) and would then rather indicate a negative relationship of the relative biomass of cyanobacteria to TN. Many (filamentous) cyanobacteria are capable of nitrogen fixation and buoyancy-regulated vertical movement into deeper, nutrient-rich water layers and have been proposed to have competitive advantages during nitrogen deficient periods (Paerl 1988; Oliver 1994; Ferber et al. 2004). Evidence of nitrogen limitation in our study lakes is, however, mixed. On the one hand, the strong positive relationship of total phytoplankton biomass to TN indicates an important role of nitrogen supply. On the other hand, average TN:TP ratios were > 30 in 90% of the study lakes, way above the Redfield ratio of 16:1. The positive relationship between the relative biomass of cyanobacteria and the zooplankton biomass is consistent with direct evidence of grazing resistance in many cyanobacterial taxa. Large sized, colonial cyanobacteria mechanically interfere with filter feeding or produce toxins that negatively affect zooplankton (Lampert 1981; Gliwicz et al. 1990; Rohrlack et al. 1999; Ghadouani et al. 2003). Cyanobacteria therefore likely benefited in relative terms from increased grazing pressure on the algal community. There is also evidence that zooplankton feed selectively on their prey (McCauley et al. 1979; Sarnelle 2003; Gliwicz et al. 1990), thus might have favored the absolute biomass of cyanobacteria.

Cryptophytes, which contributed on average 12 % to total phytoplankton biomass in the study lakes, were strongly negatively related to zooplankton biomass and positively to TN and TP. Most cryptophytes are highly sensitive to grazing by daphnids (Sarnelle 2003; Sommer et al. 2001) and their biomass seems to be regulated by large zooplankton (Watson et al. 1997) as corroborated by our data. Cryptophytes may persist under high grazing pressure solely by means of outgrowing grazing losses. Their requirements for nutrients should therefore be high under such

conditions (Klaveness 1988), which could explain the observed positive relationship of cryptophyte total biomass to both TN and TP in the current study lakes.

Dinoflagellates can be abundant in stratified lakes where many species perform vertical migrations and exploit nutrient and light gradients (Pollingher 1988; Reynolds et al. 1992). Their generation times are, however, relatively long and, in temperate lakes, large dinoflagellates frequently become abundant first in late summer (Sommer et al. 1986). In line with this, dinoflagellate biomass increased threefold from June to August/September, on average contributing 9% to total phytoplankton biomass. Similar to chrysophytes, the relative biomass of dinoflagellates was negatively related to mixing depth, suggesting that mobile taxa may benefit in relative terms from the increase in sinking losses of negatively buoyant taxa in shallow mixed layers (Ptacnik et al. 2003). The relationship of absolute dinoflagellate biomass to mixing depth was slightly more complex, with a weak tendency towards a unimodal pattern. Patterns of vertical migration of dinoflagellates seem to be altered by gradients of light and temperature (Heaney et al. 1981) and one may speculate that, in sufficiently shallow, stratified systems, dinoflagellates may stay below the thermocline where dissolved nutrient concentrations are higher and light is not strongly limiting yet. Finally, the relative biomass of dinoflagellates was positively related to TP and negatively to TN, both relationships being fairly weak. While the positive relationship to TP is consistent with the study of Watson (1997), the negative relationship to TN remains unclear.

Diatoms suffer during summer stratification from depletion of dissolved silica in combination with high sedimentation losses and slow recycling of silica (Sommer 1988). In line with this, diatoms made up only a minor proportion (on average 8%) of total biomass in the studied lakes and were strongly positively related to TSi. Overall, silica levels were not particularly low in most lakes, suggesting that sinking losses may have been primarily responsible for the low relative diatom biomasses in most lakes. Because sinking losses are inversely related to mixing depth (Ptacnik et al. 2003), we would thus have expected a positive relationship of diatom biomass to mixed layer depth. Both the relative and absolute biomasses of diatoms were, however, unrelated to mixing depth. Given that mixing depth was inversely correlated with TN (Table 3) and that TN was negatively related to the biomass of diatoms, this could be another case where a presumably causally related variable (mixing depth) was excluded from a regression model, because a correlated variable (TN) was by

chance more strongly related to the dependent variable (diatom biomass). The negative relationship of diatoms to TN (Table 4) would indeed be difficult to explain causally. Still, TN was also highly correlated with zooplankton biomass. In multiple regressions excluding TN as a predictor variable both the absolute and relative biomasses of diatoms were negatively related to zooplankton biomass, whereas mixing depth was still excluded (log diatom biomass = 0.83 + 1.24 log TSi – 0.51 log Zoo; R^2 = 0.47; pTSi < 0.001; pZoo = 0.041; asin diatom relative biomass = - 0.003 + 0.15 log TSi – 0.12 log Zoo; R^2 = 0.44; pTSi < 0.001; pZoo = 0.003). Most planktonic diatoms are indeed highly edible (Sarnelle 2003; Sommer et al. 2001) and it seems plausible that an existing negative relationship between diatoms and zooplankton was at least partially masked by the tight correlation of zooplankton biomass with TN.

With 4 % of biomass, euglenophytes only made a minor contribution to total phytoplankton biomass. As in chlorophytes, their absolute biomass was positively related to TN suggesting high nitrogen requirements for cell growth and reproduction. Contrary to chlorophytes, the relative biomass of euglenophytes showed a weak positive relationship to zooplankton biomass. Euglenophytes, a rather morphologically homogenous group, probably benefited from a more effective grazing avoidance due to relatively large size compared to algal classes which are subject to stronger grazing pressure e.g. cryptophytes, diatoms and small chlorophytes.

In conclusion, the taxon-specific analyses revealed a number of clear patterns relating different algal classes to environmental variables. Most consistent was the strong positive relationship of most algal classes to total nitrogen. A few strong, taxon-specific relationships were also found (positive: diatoms and silica, cryptophytes and phosphorus, cyanobacteria and zooplankton; negative: cryptophytes and zooplankton, chlorophytes and zooplankton). While the amount of variance in biomass explained by the multiple regression models was moderate for most taxa (Table 4a), it is, however, striking that the R^2-value of the regression model for total chlorophyll-*a* was much higher than that of any single taxon. Although this may reflect higher accuracy and less noise in the estimates of an aggregate variable (total Chl-*a*) compared to its components (single algal classes), it is also suggestive of compensatory responses among different taxa. As a consequence, aggregate phytoplankton biomass was qualitatively related to total nutrients and mixing depth as predicted by a generic, single-species model which ignores taxonomic diversity

(Diehl 2002; Berger et al. 2005), whereas single taxa showed more idiosyncratic patterns.

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References

Barlow, R.G., D.G. Cummings, and S.W. Gibb, 1997. Improved resolution of mono- and divenyl chlorophylls *a* and *b* and zeaxanthin and lutein in phytoplankton extracts using reverse phase C-8 HPLC. *Mar. Ecol.- Proc. Ser.* **161:** 303-307.

Berger, S.A., T.J. Kunz, D. Albrecht, A.M. Oucible, S. Ritzer, and S. Diehl, 2005. Light supply, plankton biomass and seston stoichiometry in a gradient of lake mixing depths. *(in preparation)*.

Bidigare, R.R., 1991. Analysis of algal chlorophylls and carotenoids. Marine particles: analysis and characterization. *Geophys. Monogr. Ser.* **63**: 119-123.

Botrell, H.H., A. Duncan, Z.M. Gliwicz, E. Grygiereck, A. Herzig, A. Hillbricht-Ilkowska, H. Kurasawa, P. Larrson, and T. Weglenska, 1976. A review of some problems in zooplankton production studies. *Nor. J. Zool.* **24:** 419-456.

Buchaca, T., M. Felip, and J. Catalan, 2005. A comparison of HPLC pigment analyses and biovolume estimates of phytoplankton groups in an oligotrophic lake. *J. Plankton Res.* **27:** 91-101.

Canfield, D.E., E. Phlips, and C.M. Duarte, 1989. Factors influencing the abundance of blue-green-algae in Florida lakes. *Can. J. Fish. Aquat. Sci.* **46:** 1232-1237.

Descy, J.P., H.W. Higgins, D.J. Mackey, J.P. Hurley, and T.M. Frost, 2000. Pigment ratios and phytoplankton assessment in northern Wisconsin lakes. *J. Phycol.* **36:** 274-286.

Deydier-Stephan, L., G. Bertru, and B. Le Rouzic, 2003. A chemotaxonomic method to quantify phytoplankton groups in freshwater lentic mesocosms: an approach including chlorophyll *a* breakdown products. *C. R. Biologies* **326:** 95-105.

Diehl, S., 2002. Phytoplankton, light, and nutrients in a gradient of mixing depths: theory. *Ecology* **83:** 386-398.

Diehl, S., S.A. Berger, R. Ptacnik, and A. Wild, 2002. Phytoplankton, light, and nutrients in a gradient of mixing depths: field experiments. *Ecology* **83:** 399-411.

Dillon, P.J. and F.H. Rigler, 1974. Phosphorus-chlorophyll relationship in lakes. *Limnol. Oceanogr.* **19:** 767-773.

Downing, J.A., S.B. Watson, and E. McCauley, 2001. Predicting Cyanobacteria dominance in lakes. *Can. J. Fish. Aquat. Sci.* **58:** 1905-1908.

Ferber, L.R., S.N. Levine, A. Lini, and G.P. Livingston, 2004. Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen? *Freshw. Biol.* **49:** 690-708.

Fietz, S. and A. Nicklisch, 2004. An HPLC analysis of the summer phytoplankton assemblage in Lake Baikal. *Freshw. Biol.* **49:** 332-345.

Ghadouani, A., B. Pinel-Alloul, and E.E. Prepas, 2003. Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. *Freshw. Biol.* **48:** 363-381.

Gliwicz, Z.M. and W. Lampert, 1990. Food thresholds in *Daphnia* species in the absence and presence of blue-green filaments. *Ecology* **71:** 691-702.

Goericke, R. and J.P. Montoya, 1998. Estimating the contribution of microalgae taxa to chlorophyll *a* in the field - variations of pigment ratios under nutrient- and light-limited growth. *Mar. Ecol. Progr. Ser.* **169:** 97-112.

Gonzalez Sagrario, M.A., E. Jeppesen, J. Goma, M. Sondergaard, J.P. Jensen, T. Lauridsen, and F. Landkildehus, 2005. Does high nitrogen loading prevent clear-water conditions in shallow lakes at moderately high phosphorus concentrations? *Freshw. Biol.* **50:** 27-41.

Happey-Wood,C.M., Ecology of freshwater planktonic green algae. p. 175-226. *In* C.D.Sandgren [ed.], *Growth and Reproductive Strategies of Freshwater Phytoplankton.* Cambridge University Press, 1988.

Heaney, S.I. and J.F. Talling, 1981. Light, temperature and nitrogen as interacting factors affecting diel vertical migrations of dinoflagellates in culture. *J. Plankton Res.* **3:** 331-344.

Hecky, R.E. and P. Kilham, 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent-evidence on the effects of enrichment. *Limnol. Oceanogr.* **33:** 796-822.

Henriksen, P., B. Riemann, H. Kaas, H.M. Sorensen, and H.L. Sorensen, 2002. Effects of nutrient-limitation and irradiance on marine phytoplankton pigments. *J. Plankton Res.* **24:** 835-858.

Higgins, H.W. and D.J. Mackey, 2000. Algal class abundances, estimated from chlorophyll and carotenoid pigments, in the western Equatorial Pacific under El Niño and non-El Niño conditions. *Deep-Sea Res. Pt I- Oceanogr. Res. Papers* **47:** 1461-1483.

Huisman, J., J. Sharples, J.M. Stroom, P.M. Visser, W.E.A. Kardinaal, J.M.H. Verspagen, and B. Sommeijer, 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. *Ecology* **85:** 2960-2970.

Huszar, V.L.D. and N.F. Caraco, 1998. The relationship between phytoplankton composition and physical-chemical variables: a comparison of taxonomic and morphological-functional descriptors in six temperate lakes. *Freshw. Biol.* **40:** 679-696.

Jeffrey, S.W., R.F.C. Mantoura, and S.W. Wright. *Phytoplankton Pigments in Oceanography*. UNESCO Publishing, 1997.

Jensen, J.P., E. Jeppesen, K. Olrik, and P. Kristensen, 1994. Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow Danish lakes. *Can. J. Fish. Aquat. Sci.* **51:** 1692-1699.

Jeppesen, E., T.L. Lauridsen, S.F. Mitchell, K. Christoffersen, and C.W. Burns, 2000. Trophic structure in the pelagial of 25 shallow New Zealand lakes: changes along nutrient and fish gradients. *J. Plankton Res.* **22:** 951-968.

Klaveness, D. Ecology of the Cryptomonadina: a first review, p. 105-133. *In* C.D. Sandgren [ed.], *Growth and Reproductive Strategies of Freshwater Phytoplankton*. Cambridge University Press, 1988.

Kunz, T.J. and S. Diehl, 2003. Phytoplankton, light and nutrients along a gradient of mixing depth: a field test of producer-resource theory. *Freshw. Biol.* **48:** 1050-1063.

Lampert, W., 1981. Inhibitory and toxic effects of blue-green-algae on Daphnia. *Int. Rev. Ges. Hydrobiol.* **66:** 285-298.

Mackey, D.J., H.W. Higgins, M.D. Mackey, and D. Holdsworth, 1998. Algal class abundances in the western equatorial Pacific: Estimation from HPLC measurements of chloroplast pigments using CHEMTAX. *Deep-Sea Res. Pt I- Oceanogr. Res. Papers* **45:** 1441-1468.

Mackey, M.D., D.J. Mackey, H.W. Higgins, and S.W. Wright, 1996. CHEMTAX - A program for estimating class abundances from chemical markers: Application to HPLC measurements of phytoplankton. *Mar. Ecol.- Proc. Ser.* **144:** 265-283.

Marinho, M.M. and S.V. Rodrigues, 2003. Phytoplankton of an eutrophic tropical reservoir: comparison of biomass estimated from counts with chlorophyll-*a* biomass from HPLC measurements. *Hydrobiologia* **505:** 77-88.

Mazumder, A., 1994. Phosphorus chlorophyll relationships under contrasting herbivory and thermal stratification - predictions and patterns. *Can. J. Fish. Aquat. Sci.* **51:** 390-400.

McCauley, E. and F. Briand, 1979. Zooplankton grazing and phytoplankton species richness: field tests of the predation hypothesis. *Limnol. Oceanogr.* **24:** 243-252.

McCauley, E. and J. Downing, 1991. Different effects of phosphorus and nitrogen on chlorophyll concentration in oligotrophic and eutrophic lakes. *Can. J. Fish. Aquat. Sci.* **48:** 2552-2553.

McCauley, E., J.A. Downing, and S.B. Watson, 1989. Sigmoid relationships between nutrients and chlorophyll among lakes. *Can. J. Fish. Aquat. Sci.* **46:** 1171-1175.

Millie, D.F., G.L. Fahnenstiel, H.J. Carrick, S.E. Lohrenz, and O.M.E. Schofield, 2002. Phytoplankton pigments in coastal Lake Michigan: Distributions during the spring isothermal period and relation with episodic sediment resuspension. *J. Phycol.* **38:** 639-648.

Oliver, R.L., 1994. Floating and sinking in gas-vacuolated cyanobacteria. *J. Phycol.* **30:** 161-173.

Paerl, H.W., Freshwater Blue-Green Algal Ecology, p. 261-315. *In* C.D. Sandgren [ed.]. *Growth and Reproductive Strategies of Freshwater Phytoplankton*. Cambridge University Press, 1988.

Peeken, I., 1997. Photosynthetic pigment fingerprints as indicators of phytoplankton biomass and development in different water masses of the Southern Ocean during austral spring. *Deep-Sea Res. Pt. II* **44:** 261-282.

Persson, L., S. Diehl, L. Johansson, G. Andersson, and S.F. Hamrin, 1992. Trophic interactions in temperate lake ecosystems - a test of food-chain theory. *Am. Nat.* **140:** 59-84.

Peters, R.H., 1986. The role of prediction in limnology. *Limnol. Oceanogr.* **31:** 1143-1159.

Pollingher, U., Freshwater armored dinoflagellates: growth, reproduction strategies, and population dynamics., p. 134-174. *In* C.D. Sandgren [ed.], *Growth and Reproductive Strategies of Freshwater Phytoplankton*. Cambridge University Press, 1988.

Ptacnik, R., S. Diehl, and S.A. Berger, 2003. Performance of sinking and non-sinking phytoplankton taxa in a gradient of mixing depths. *Limnol. Oceanogr.* **48:** 1903-1912.

Ramberg, L., 1979. Relations between phytoplankton and light climate in 2 Swedish forest lakes. *Int. Rev. Ges. Hydrobiol.* **64:** 749-782.

Reynolds, C.S. *The Ecology of Freshwater Phytoplankton*. Cambridge University Press, 1984.

Reynolds, C.S. and E.G. Bellinger, 1992. Patterns of abundance and dominance of the phytoplankton of Rhosterne Mere, England - evidence from an 18-year data set. *Aquat. Sci.* **54:** 10-36.

Reynolds, C.S., R.L. Oliver, and A.E. Walsby, 1987. Cyanobacterial dominance - the role of buoyancy regulation in dynamic lake environments. *New Zeal. J. Mar. Freshw. Res.* **21:** 379-390.

Rodriguez, F., Y. Pazos, J. Maneiro, and M. Zapata, 2003. Temporal variation in phytoplankton assemblages and pigment composition at a fixed station of the Ria of Pontevedra (NW Spain). *Estuar. Coast. Shelf Sci.* **58:** 499-515.

Rohrlack, T., E. Dittmann, M. Henning, T. Borner, and J.G. Kohl, 1999. Role of microcystins in poisoning and food ingestion inhibition of *Daphnia galeata* caused by the cyanobacterium *Microcystis aeruginosa*. *Appl. Environm. Microbiol.* **65:** 737-739.

Rowan, K.S., *Photosynthetic pigments of algae*. Cambridge University Press, 1989.

Sakamoto, M., 1966. Primary production by phytoplankton community in some Japanese lakes and its dependence on lake depth. *Hydrobiologia* **62:** 1-28.

Sakshaug, E., 1997. Parameters of photosynthesis: definitions, theory and interpretation of results. *J. Plankton Res.* **19:** 1637-1670.

Sandgren, C.D., *Growth and Reproductive Strategies of Freshwater Phytoplankton.,* first edition. Cambridge University Press, 1988.

Sarnelle, O., 1992. Nutrient enrichment and grazer effects on phytoplankton in lakes. *Ecology* **73:** 551-560.

Sarnelle, O., 1993. Herbivore effects on phytoplankton succession in a eutrophic lake. *Ecol. Monogr.* **63:** 129-149.

Sarnelle, O., 2003. Nonlinear effects of an aquatic consumer: causes and consequences. *Am. Nat.* **161:** 478-496.

Schagerl, M. and K. Donabaum, 2003a. Patterns of major photosynthetic pigments in freshwater algae. 1. Cyanoprokaryota, rhodophyta and cryptophyta. *Internat. J. Limnol.* **39:** 35-47.

Schagerl, M., C. Pichler, and K. Donabaum, 2003b. Patterns of major photosynthetic pigments in freshwater algae. 2. Dinophyta, euglenophyta, chlorophyceae and charales. *Internat. J. Limnol.* **39:** 49-62.

Scheffer, M., S. Rinaldi, A. Gragnani, L.R. Mur, and E.H. vanNes, 1997. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. *Ecology* **78:** 272-282.

Schindler, D.W., 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. *Limnol. Oceanogr.* **23:** 478-486.

Schlüter, L. and F. Mohlenberg, 2003. Detecting presence of phytoplankton groups with non-specific pigment signatures. *J. Appl. Phycol.* **15:** 465-476.

Schlüter, L., F. Mohlenberg, H. Havskum, and S. Larsen, 2000. The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: testing the influence of light and nutrients on pigment/chlorophyll a ratios. *Mar. Ecol.- Progr. Ser.* **192:** 49-63.

Smith, V., 1986. Light and nutrient effects on the relative biomass of blue-green algae in lake phytoplankton. *Can. J. Fish. Aquat. Sci.* **43:** 148-153.

Smith, V.H., 1982. The nitrogen and phosphorus dependence of algal biomass in lakes: An empirical and theoretical analysis. *Limnol. Oceanogr.* **27:** 1101-1112.

Soma, Y., T. Imaizumi, K. Yagi, and S. Kasuga, 1993. Estimation of algal succession in lake water using HPLC analysis of pigments. *Can. J. Fish. Aquat. Sci.* **50:** 1142-1146.

Sommer, U., Growth and survival strategies of planktonic diatoms, p. 227-260. *In* C.D. Sandgren [ed.], *Growth and Reproductive Strategies of Freshwater Phytoplankton*. Cambridge University Press, 1988.

Sommer, U., Z.M. Gliwicz, W. Lampert, and A. Duncan, 1986. The PEG-model of seasonal succession of planktonic events in fresh waters. *Arch. Hydrobiol.* **106:** 433-471.

Sommer, U., F. Sommer, B. Santer, C. Jamieson, M. Boersma, C. Becker, and T. Hansen, 2001. Complementary impact of copepods and cladocerans on phytoplankton. *Ecol. Lett.* **4:** 545-550.

Soto, D., 2002. Oligotrophic patterns in southern Chilean lakes: the relevance of nutrients and mixing depth. *Rev. Chil. Hist. Nat.* **75:** 377-393.

Tilman, D., R. Kiesling, R. Sterner, S.S. Kilham, and F.A. Johnson, 1986. Green, bluegreen and diatom algae - taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. *Arch. Hydrobiol.* **106:** 473-485.

Visser, P.M., L. Massaut, J. Huisman, and L.R. Mur, 1996. Sedimentation losses of Scenedesmus in relation to mixing depth. *Arch. Hydrobiol.* **136:** 289-308.

Vollenweider, R.A., 1976. Advances in defining the critical loading levels for phosphorus in lake eutrophication. *Mem. Ist. Ital. Idrobiol.* **33:** 53-83.

Watson, S.B., E. McCauley, and J.A. Downing, 1997. Patterns in phytoplankton taxonomic composition across temperate lakes of different nutrient status. *Limnol. Oceanogr.* **42:** 487-495.

Wetzel, R.G. and G.E. Likens. *Limnological Analyses*, 2nd edition, Springer Verlag,1991.

Wilhelm, C., I. Rudolph, and W. Renner, 1991. A quantitative method based on HPLC-aided pigment analysis to monitor structure and dynamics of the phytoplankton assemblage - a study from lake Meerfelder Maar (Eifel, Germany). *Arch. Hydrobiol.* **123:** 21-35.

Woitke, P., T. Schiwietz, K. Teubner, and J.G. Kohl, 1996. Annual profiles of photosynthetic lipophilic pigments in four freshwater lakes in relation to phytoplankton counts as well as to nutrient data. *Arch. Hydrobiol.* **137:** 363-384.

Wright, S.W., D.P. Thomas, H.J. Marchant, H.W. Higgins, M.D. Mackey, and D.J. Mackey, 1996. Analysis of phytoplankton of the Australian sector of the Southern Ocean: Comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using the 'CHEMTAX' matrix factorization program. *Mar. Ecol.-Progr. Ser.* **144:** 285-298.

Wright, S.W. and R.L. van den Enden, 2000. Phytoplankton community structure and stocks in the East Antarctic marginal ice zone (BROKE survey, January-March 1996) determined by CHEMTAX analysis of HPLC pigment signatures. *Deep-Sea Res. Pt. Ii- Top. Stud. Oceanogr.* **47:** 2363-2400.

Yacobi, Y.Z., U. Pollingher, Y. Gonen, V. Gerhardt, and A. Sukenik, 1996. HPLC analysis of phytoplankton pigments from Lake Kinneret with special reference to the bloom-forming dinoflagellate *Peridinium gatunense* (Dinophyceae) and chlorophyll degradation products. *J. Plankton Res.* **18:** 1781-1796.

Table 1 Limnological characteristics of the 40 study lakes in northern Germany. Data are seasonal averages of integral mixed surface layer samples collected on three occasions during summer 2001: Mixing depth (z_{mix}), total phosphorus (TP), total nitrogen (TN), total silica (TSi), water temperature (Temp), mesozooplankton biomass (Zoo), chlorophyll-*a* (Chl-*a*).

Nr	Lake	A km^2	z_{mix} m	TP $\mu g\ L^{-1}$	TN $mg\ L^{-1}$	TN:TP mol:mol	TSi $mg\ L^{-1}$	Temp °C	Zoo $\mu g\ L^{-1}$	Chl-*a* $\mu g\ L^{-1}$
1	Behler See	3.10	7.7	25	0.64	57	1.15	17.9	110	9.2
2	Blunker See	0.20	3.7	25	1.02	94	0.55	19.7	533	20.5
3	Bornhöveder See	0.73	3.5	94	1.29	34	6.07	19.8	467	57.2
4	Dieksee	3.86	9.2	27	0.60	50	1.44	17.7	103	7.1
5	Edebergsee	0.08	4.7	27	0.65	54	0.52	18.7	237	13.7
6	Garrensee	0.19	4.5	16	0.51	71	0.21	20.0	56	8.4
7	Grebiner See	0.29	4.5	19	0.67	80	0.81	18.8	190	11.4
8	Großensee	0.75	4.3	16	0.64	90	0.50	20.6	310	10.8
9	Großer Eutiner See	2.18	5.3	98	0.79	21	4.86	20.6	105	27.3
10	Großer Madebröckensee	0.07	3.7	16	0.68	99	0.37	18.8	196	6.5
11	Großer Plöner See (Ascheberg)	17.40	8.0	33	0.55	37	1.62	18.8	28	7.3
12	Großer Plöner See (Plön)	12.39	9.7	25	0.57	51	1.17	18.2	71	6.7
13	Großer Pönitzer See	1.08	4.0	25	0.73	65	2.21	20.8	195	11.2
14	Großer Segeberger See	1.70	4.2	35	0.67	44	1.37	20.5	99	10.0
15	Hemmelsdorfer See	4.10	3.5	90	1.65	41	2.49	21.2	655	52.9
16	Höftsee	0.19	7.8	24	0.58	57	0.93	17.3	210	9.4
17	Ihlsee	0.28	3.8	9	0.52	122	0.21	20.8	441	3.4
18	Kellersee	5.52	7.5	29	0.72	55	1.78	18.8	187	14.6
19	Kleiner Plöner See	2.39	7.3	50	0.60	27	1.79	17.9	53	11.2

Nr	Lake	A km^2	z_{mix} m	TP $\mu g\ L^{-1}$	TN $mg\ L^{-1}$	TN:TP mol:mol	TSi $mg\ L^{-1}$	Temp °C	Zoo $\mu g\ L^{-1}$	Chl-*a* $\mu g\ L^{-1}$
20	Kolksee	0.03	3.0	21	0.75	79	1.47	19.0	145	13.9
21	Lankauer See	0.14	3.3	18	0.69	90	7.26	20.9	55	9.0
22	Lüttauer See	0.41	3.3	32	0.76	58	3.77	19.8	294	22.2
23	Muggesfelder See	0.27	4.3	22	0.72	76	0.63	19.3	226	9.0
24	Plußsee	0.14	3.0	38	0.89	53	0.32	19.0	513	6.9
25	Ratzeburger See	16.20	7.0	30	0.57	44	1.39	18.8	89	15.4
26	Schaalsee	6.22	6.8	27	0.70	58	0.97	19.4	111	11.6
27	Schierensee (Grebin)	0.15	4.8	22	0.60	62	1.24	18.3	407	8.7
28	Schluensee	1.27	6.2	26	0.51	48	0.85	18.7	545	6.6
29	Schmarksee	0.07	3.3	35	0.74	47	10.33	18.3	485	15.8
30	Schöhsee	0.78	6.5	12	0.49	92	0.55	17.5	115	4.7
31	Selenter See	22.39	9.5	19	0.54	65	1.20	17.9	116	4.4
32	Stocksee	2.11	6.5	16	0.57	82	1.24	17.8	193	4.1
33	Stolper See	1.40	6.0	40	0.95	67	5.97	18.3	53	28.0
34	Suhrer See	1.37	7.2	11	0.63	130	1.08	17.9	201	3.8
35	Trammer See	1.63	7.3	22	0.72	73	2.31	17.8	158	7.0
36	Ukleisee	0.32	4.3	21	0.69	72	1.53	19.9	272	6.5
37	Vierer See	1.32	6.3	39	0.70	41	2.94	17.3	465	10.8
38	Westensee	7.70	5.3	58	0.84	51	3.82	19.8	295	18.6
39	Wielener See	0.25	3.8	35	1.17	81	1.54	18.6	503	24.6
40	Wittensee	10.30	9.8	122	0.55	11	3.03	18.8	57	9.1

Table 2 Pigment to chlorophyll *a* mass ratios: (a) Input matrix of pigment to chlorophyll-*a* mass ratios for the CHEMTAX calculation (modified after Descy 2001). (b) Output matrices of pigment to chlorophyll- *a* mass ratios calculated by CHEMTAX separately for samples from shallow (2 - 4 m; N = 48), intermediate (4.5 - 6.5 m; N = 32), and deep (6.5 -13.5 m; N = 40) mixed layers (z_{mix}) of the studied lakes. Pigment abbreviations are: Peri: peridinin, Fuco: fucoxanthin, Neo: neoxanthin, Viola: violaxanthin, Ddx: diadinoxanthin, Allo: alloxanthin, Zea: zeaxanthin, Lut: lutein, Dtx: diadinoxanthin; Echi: echinenone, αCar: α-carotene.

a	**Peri**	**Fuco**	**Viol**	**Neo**	**Ddx**	**Allo**	**Zea**	**Lut**	**Dtx**	**Chl b**	**Echi**	**αCar**
Chlorophytes	0	0	0.032	0.006	0	0	0.015	0.126	0	0.169	0	0.006
Chrysophytes	0	0.240	0.071	0	0	0	0.010	0	0	0	0	0
Cryptophytes	0	0	0	0	0	0.311	0	0	0	0	0	0.020
Cyanobacteria T1	0	0	0	0	0	0	0.373	0	0	0	0	0
Cyanobacteria T2	0	0	0	0	0	0	0.204	0	0	0	0.269	0
Diatoms	0	1.007	0	0	0.213	0	0	0	0.040	0	0	0
Dinoflagellates	0.649	0		0	0.231	0	0	0	0.040	0	0	0
Euglenophytes	0	0	0	0.020	0.200	0	0	0	0.002	0.390	0	0

b z_{mix} **= 2-4 m**	**Peri**	**Fuco**	**Viol**	**Neo**	**Ddx**	**Allo**	**Zea**	**Lut**	**Dtx**	**Chl *b***	**Echi**	**α Car**
Chlorophytes	0	0	0.023	0.004	0	0	0.011	0.089	0	0.146	0	0.004
Chrysophytes	0	0.128	0.036	0	0	0	0.005	0	0	0	0	0
Cryptophytes	0	0	0	0	0	0.234	0	0	0	0	0	0.015
Cyanobacteria T1	0	0	0	0	0	0	0.272	0	0	0	0	0
Cyanobacteria T2	0	0	0	0	0	0	0.138	0	0	0	0.183	0
Diatoms	0	0.446	0	0	0.094	0	0	0	0.018	0	0	0
Dinoflagellates	0.291	0	0	0	0.130	0	0	0	0.022	0	0	0
Euglenophytes	0	0	0	0.012	0.124	0	0	0	0.001	0.242	0	0
z_{mix} = 4.5-6.5 m												
Chlorophytes	0	0	0.023	0.004	0	0	0.011	0.092	0	0.136	0	0.004
Chrysophytes	0	0.121	0.032	0	0	0	0.006	0	0	0	0	0
Cryptophytes	0	0	0	0	0	0.234	0	0	0	0	0	0.015
Cyanobacteria T1	0	0	0	0	0	0	0.272	0	0	0	0	0
Cyanobacteria T2	0	0	0	0	0	0	0.138	0	0	0	0.183	0
Diatoms	0	0.484	0	0	0.088	0	0	0	0.016	0	0	0
Dinoflagellates	0.380	0	0	0	0.113	0	0	0	0.020	0	0	0
Euglenophytes	0	0	0	0.012	0.124	0	0	0	0.001	0.242	0	0
z_{mix} = 7-13.5 m												
Chlorophytes	0	0	0.024	0.004	0	0	0.011	0.075	0	0.142	0	0.004
Chrysophytes	0	0.077	0.023	0	0	0	0.004	0	0	0	0	0
Cryptophytes	0	0	0	0	0	0.234	0	0	0	0	0	0.002
Cyanobacteria T1	0	0	0	0	0	0	0.272	0	0	0	0	0
Cyanobacteria T2	0	0	0	0	0	0	0.138	0	0	0	0.183	0
Diatoms	0	0.508	0	0	0.084	0	0	0	0.016	0	0	0
Dinoflagellates	0.330	0	0	0	0.141	0	0	0	0.020	0	0	0
Euglenophytes	0	0	0	0.012	0.124	0	0	0	0.001	0.242	0	0

Table 3 Bivariate correlations of the selected environmental variables: total nitrogen (TN in µg L^{-1}), total phosphorus (TP in µg L^{-1}), total silica (TSi in µg L^{-1}), mixing depth (z_{mix} in m), water temperature (T in °C), zooplankton density (Zoo in mg dry weight L^{-1}). Shown are Pearson correlation coefficients and significance levels (** = $p < 0.01$; * = $p < 0.05$), N=40.

	TN	TP	TSi	z_{mix}	T
TP	0.50 **				
TSi	0.30	0.41 **			
z_{mix}	- 0.47 **	0.06	- 0.20		
Temp	0.38 *	0.24	0.14	- 0.62 **	
Zoo	0.57 **	0.13	0.07	- 0.53 **	0.20

Table 4 Relationships among phytoplankton variables and environmental variables in the mixed surface layers of the 40 study lakes during summer stratification. Phytoplankton variables are (a) absolute biomasses (in ng Chl-*a* L^{-1}) of total phytoplankton and of chrysophytes, chlorophytes, cyanobacteria, cryptophytes, diatoms, dinoflagellates, euglenophytes and (b) the relative contributions of the seven taxonomic groups to total chlorophyll *a*. Environmental variables are total nitrogen concentration (TN in µg L^{-1}), total phosphorus concentration (TP in µg L^{-1}), total silica concentration (TSi in µg L^{-1}), mixing depth (z_{mix}, in m), squared mixing depth (z_{mix}^2), water temperature (Temp, in °C) and mesozooplankton density (Zoo, in µg dry mass L^{-1}). Multiple regressions were performed on log-transformed data of absolute Chl-*a*, TN, TP, TSi, Zoo values. Relative biomasses were arc sine square root (asin) transformed. Regression equations, R^2-values and excluded variables are listed. Significance levels (P-values) of regression coefficients are given in parentheses, N=40. * Test of normality of studentized residuals failed (Shapiro-Wilk. = 0.001).

a Absolute biomasses

Phytoplankton variable	Regression equation					R^2	Variables excluded
log Chl-*a*	**- 0.94**	**+ 1.37 log TN**	**+ 0.52 log TP**	**+ 0.13 z_{mix}**	**- 0.012 z_{mix}^2**	**0.81**	**T, log Tsi, log Zoo**
		(0.000)	**(0.000)**	**(0.072)**	**(0.039)**		
log Chrysophytes *	- 2.78	+ 1.60 log TN (0.035)	+ 0.44 log TSi (0.033)			0.29	log TP, log Zoo, z_{mix}, z_{mix}^2, T
log Chlorophytes	- 3.10	+ 2.40 log TN (0.000)	- 0.24 log Zoo (0.017)			0.56	T, z_{mix}, z_{mix}^2, log TP
log Cyanobacteria	1.76	+ 0.59 log Zoo (0.000)				0.29	z_{mix}^2, z_{mix}, log TN, log TP, T
log Cryptophytes	- 1.45	+ 1.54 log TN (0.004)	+ 0.49 log TP (0.015)	- 0.32 log Zoo (0.008)		0.53	z_{mix}^2, z_{mix}, T
log Dinoflagellates	- 2.19	+ 0.52 z_{mix} (0.085)	- 0.05 z_{mix}^2 (0.040)	+ 0.18 T (0.082)		0.34	log TN, log Zoo, log TP
log Diatoms	4.58	- 2.60 log TN (0.008)	+ 1.53 log TSi (0.000)			0.51	T, log TP, z_{mix}^2, z_{mix}, log Zoo
log Euglenophytes	- 6.22	+ 2.89 log TN (0.000)				0.31	log TP, log Zoo, T, z_{mix}, z_{mix}^2

b Relative biomasses

Phytoplankton variable	Regression equation				R^2	Variables excluded
asin Chrysophytes	0.30	+ 0.081 log TSi (0.038)	- 0.001 z_{mix}^2 (0.067)		0.17	log Zoo, T, log TN, z_{mix}, log TP
asin Chlorophytes	0.48				0.00	log Zoo, log TN, log TP, T, z_{mix}, z_{mix}^2
asin Cyanobacteria	0.34	- 0.19 log TP (0.013)	+ 0.16 log Zoo (0.001)		0.34	log TN, z_{mix}, z_{mix}^2, T
asin Cryptophytes	0.61	- 0.13 log Zoo (0.000)			0.37	log TP, log TN, z_{mix}^2, T, z_{mix}
asin Dinoflagellates	1.50	- 0.52 log TN (0.064)	+ 0.22 log TP (0.047)	- 0.002 z_{mix}^2 (0.025)	0.16	T, log Zoo, z_{mix}
asin Diatoms	0.99	- 0.50 log TN (0.001)	+ 0.21 log TSi (0.000)		0.47	T, log TP, z_{mix}^2, z_{mix}, log Zoo
asin Euglenophytes	0.08	+ 0.04 log Zoo (0.054)			0.10	log TN, log TP, T, z_{mix}, z_{mix}^2

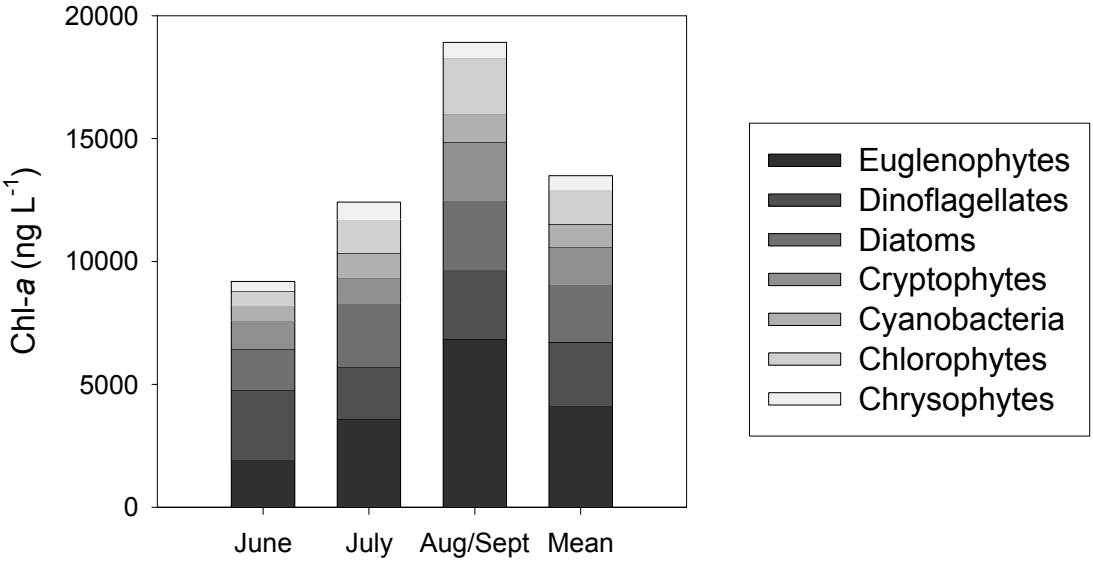

Figure 1 Total Chl-*a* amounts of seven phytoplankton taxa (see legend). Data are monthly and seasonal means from the mixed surface layers of 40 temperate lakes in Northern Germany.

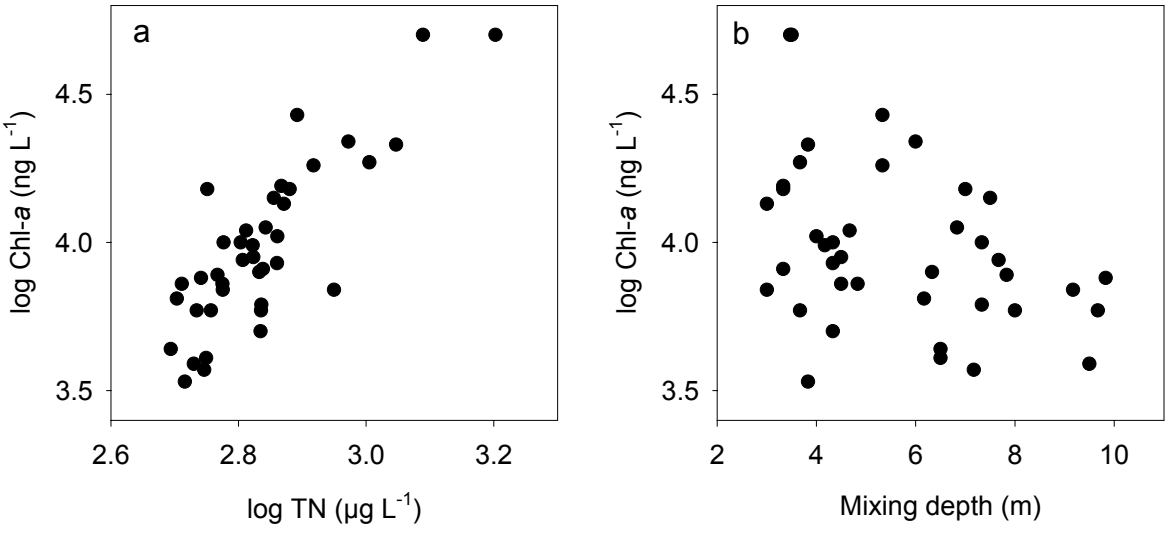

Figure 2 Relationship of total algal biomass to (a) total nitrogen content and (b) mixing depth. Data are seasonal averages from the mixed surface layers of the studied lakes.

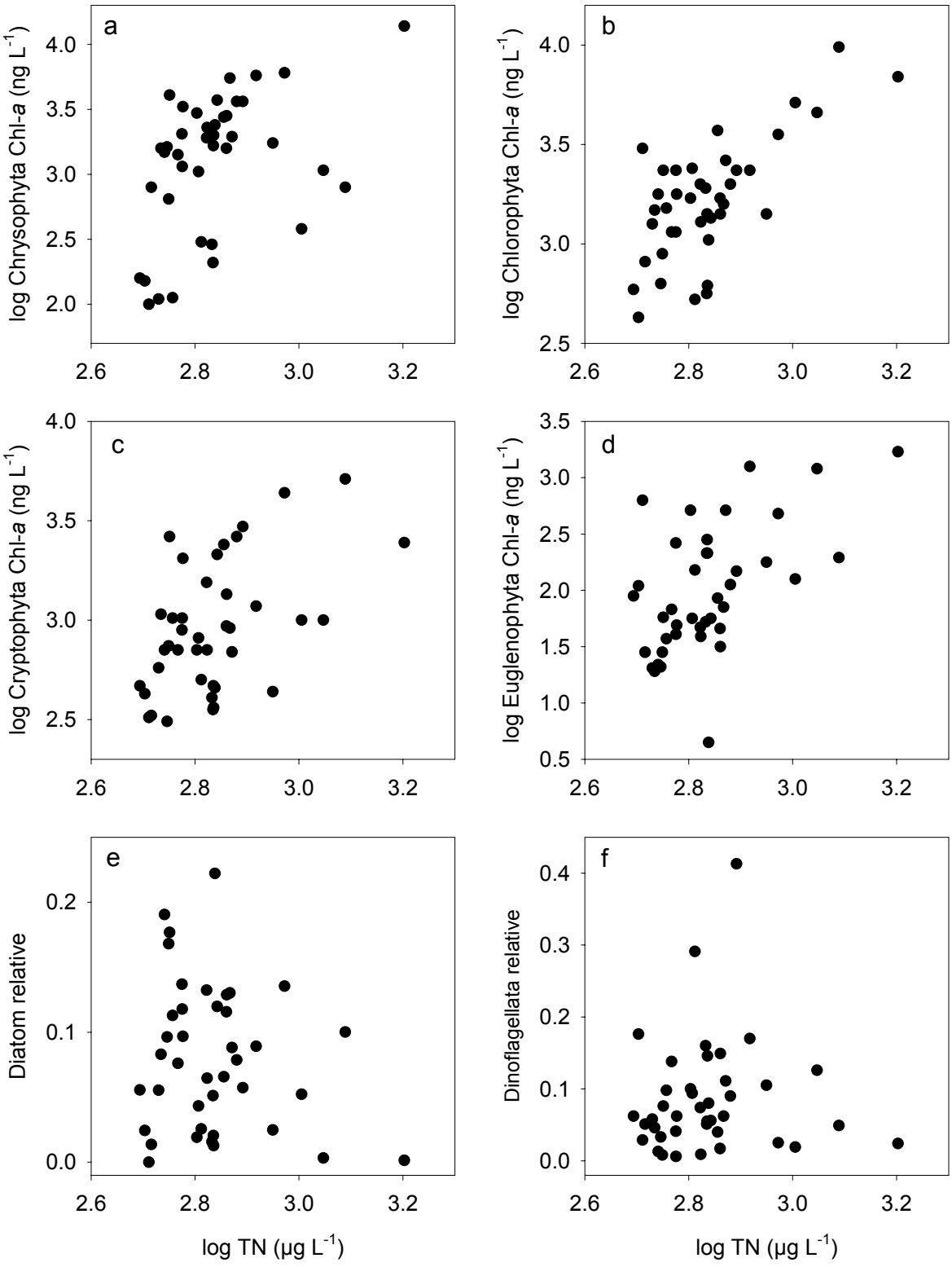

Figure 3 Relationships of absolute and relative biomasses of phytoplankton taxa to total nitrogen content. a to d: absolute biomasses of (a) chrysophytes, (b) chlorophytes, (c) cryptophytes, (d) euglenophytes; e to f: relative biomasses of (e) diatoms and (f) dinoflagellates. Data are seasonal averages from the mixed surface layers of the studied lakes.

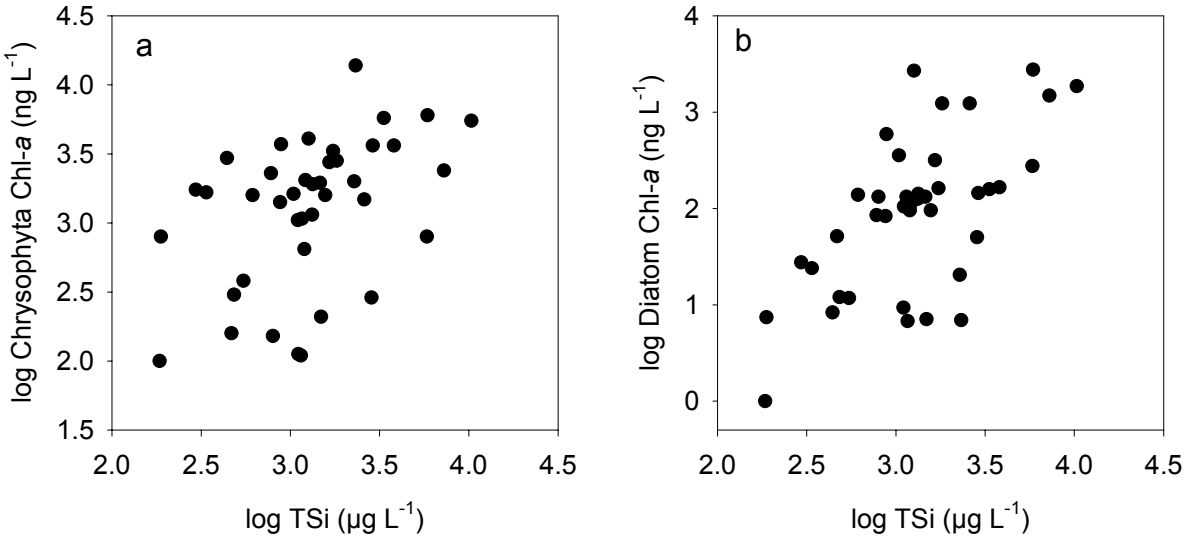

Figure 4 Relationships of absolute biomasses of phytoplankton taxa to total silica content: (a) chrysophytes and (b) diatoms. Data are seasonal averages from the mixed surface layers of the studied lakes.

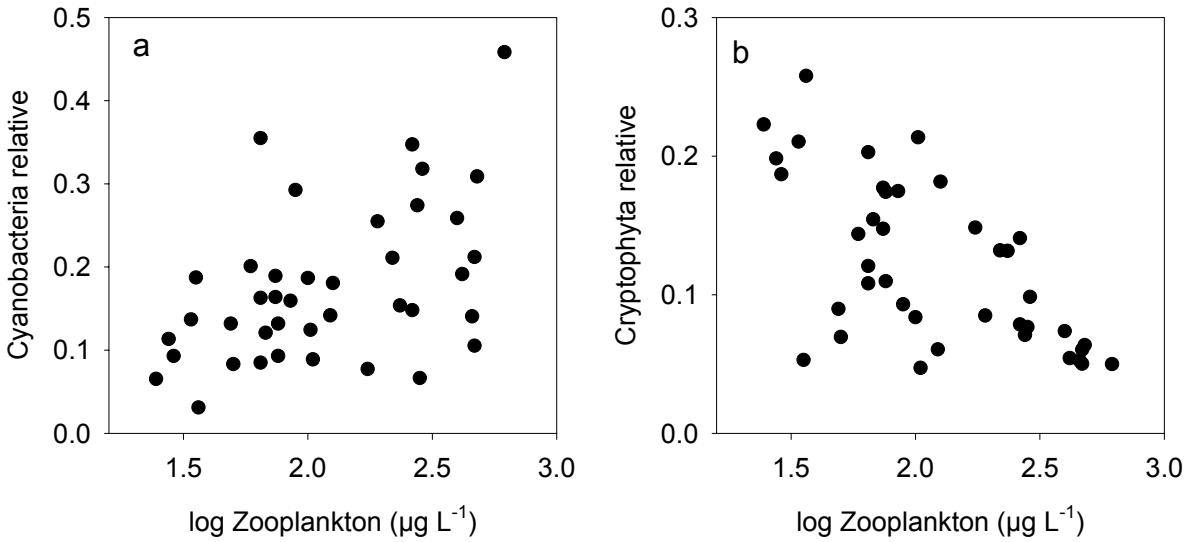

Figure 5 Relationships of relative biomasses of phytoplankton taxa to zooplankton density: (a) cyanobacteria and (b) cryptophytes. Data are seasonal averages from the mixed surface layers of the studied lakes

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Publications

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Diehl S., S.A. Berger, R. Ptacnik, A. Wild. 2002. Phytoplankton, light and nutrients in a gradient of mixing depths: field experiments. *Ecology* 83: 399-411.

Ptacnik R., S. Diehl, S.A. Berger. 2003. Performance of sinking and nonsinking phytoplankton taxa in a gradient of mixing depths. *Limnology and Oceanography* 48: 1903-1912.

Diehl, S., S.A. Berger, and R. Wöhrl, 2005. Flexible algal nutrient stoichiometry mediates environmental influences on phytoplankton and its abiotic resources. *Ecology* 86: 2931-2945.

Oral presentations and posters

Berger S.A., S. Diehl, R. Ptacnik. 2000. Effects of mixing depth and background turbidity on phytoplankton: theory and field experiments. Oral presentation. American Society of Limnology and Oceanography. June 2000, Copenhagen, Denmark.

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Berger S.A., S. Diehl. Influence of mixing depth and background turbidity on phytoplankton biomass, light and nutrients: a field enclosure experiment. Poster. European Ecological Congress. June 2002, Lund, Sweden.

Berger S.A., S. Diehl, R. Ptacnik, R. Wöhrl. 2002. Influence of mixing depth and background turbidity on phytoplankton biomass, light and nutrients: field enclosure

experiments. Oral presentation. Jahrestagung der Deutschen Gesellschaft für Limnologie. October 2002, Braunschweig, Germany.

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American Society of Limnology and Oceanography (ASLO)

All papers were written by myself.
I conducted the enclosure experiment and collected the data of 40 lakes in northern Germany.

Signed

Stella A. Berger

Approved

Prof. Dr. Sebastian Diehl

Planegg-Martinsried, March 2005