PLANKTON DYNAMICS:

THE INFLUENCE OF LIGHT, NUTRIENTS AND DIVERSITY

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften

Dr. rer. nat. der Fakultät für Biologie

der Ludwig-Maximilians-Universität München

vorgelegt von

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Zur Beurteilung eingereicht am 31. Juli 2008

Tag der mündlichen Prüfung: 07. November 2008

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CONTENTS

SUMMARY

Phytoplankton growth is controlled by the balance between reproduction and mortality. Phytoplankton reproduction is determined by environmental factors (such as temperature and pH) and by essential resources (such as light and nutrients). In my thesis, I investigated the importance of the essential resources light and nutrients for phytoplankton dynamics in laboratory and field experiments. Research questions involved topics such as: the resource use efficiency of phytoplankton communities, the role of resources for phytoplankton stoichiometry, aspects of phytoplankton food quality and grazing by zooplankton, costs of behavioural strategies of mobile phytoplankton species and the establishment of new methods to quantify growth and loss processes of phytoplankton *in situ.*

EFFECTS OF DIVERSITY ON PHYTOPLANKTON RESOURCE UPTAKE AND GROWTH

The resource use efficiency of terrestrial plant communities has been related to taxonomic diversity and a recent metaanalysis of freshwater and brackish phytoplankton communities shows that this relationship also exists in phytoplankton communities. Our experiments with natural and assembled phytoplankton communities showed a clear effect of phytoplankton biodiversity on carbon incorporation. Phytoplankton functional groups differ in their resource use attributes and exhibit different constituents of photosynthetic active pigments. We have shown that the diversity of wavelength specific photosynthetically active pigments was a function of the taxonomic diversity of the phytoplankton communities. The effect of biodiversity on carbon incorporation was related to the functional (biochemical) diversity of phytoplankton communities (Paper 1). Increasing biodiversity and thereby increasing pigment diversity resulted in a higher absorbance of light within the photosynthetic active radiation spectrum and thereby higher carbon assimilation.

EFFECTS OF DIVERSITY ON PHYTOPLANKTON RESOURCE UPTAKE AND BIOMASS COMPOSITION (STOICHIOMETRY)

Phytoplankton carbon assimilation and nutrient uptake are not tightly coupled. As a result of fluctuating resources, autotrophs can exhibit variable biomass compositions (biomass carbon to nutrient ratios). The increased efficiency of resource use in highly diverse phytoplankton communities (Paper 1) also has consequences for the biomass composition of those communities (Paper 2). Increasing biodiversity resulted in increasing carbon assimilation, but not in a comparable increase of phosphorus uptake. This resulted in increasing biomass carbon to phosphorous ratios. Phytoplankton with high biomass carbon to phosphorus ratios are considered to be low quality food for cladoceran zooplankton such as *Daphnia*. Although the stoichiometry of *Daphnia* varies somewhat with algae and diet, they maintain a relatively homeostatic composition with low carbon to nutrient (phosphorus) biomass composition compared to their food. Phytoplankton biodiversity could therefore also have consequences for freshwater phytoplankton-zooplankton interactions. The mismatch in the biomass composition between phytoplankton and *Daphnia* could lead to changed trophic transfer efficiencies between phytoplankton and zooplankton and hence affect the entire pelagic food web.

THE SUPPLY OF LIGHT AND NUTRIENTS AND ITS CONSEQUENCES FOR PHYTOPLANKTON-ZOOPLANKTON INTERACTIONS

Both, low and high light to nutrient (phosphorus) ratios in the environment can restrict herbivore growth rates by either the quantity (photosynthetically fixed carbon) of phytoplankton at low light to nutrient ratios or the nutritional quality (biomass carbon to phosphorus ratios) of phytoplankton at high light to nutrient ratios. This can result in an unimodal relationship between light intensity and zooplankton growth. In mesocosm experiments with natural phytoplankton communities from different lakes, we established

gradients of light to nutrient ratios by manipulating the light availability for phytoplankton. After two weeks we added the herbivorous zooplankter *Daphnia magna* to the mesocosms. Indeed, in treatments from phosphorus limited oligotrophic and mesotrophic lakes we found unimodal relationships between light intensity and *Daphnia* growth rates (Paper 3). At low light levels *Daphnia* growth rates were limited by food quantity and at high light levels they were limited by food quality. Light dependent variations of natural phytoplankton biomass carbon to phosphorus ratios can effect zooplankton growth.

COSTS OF BEHAVIOURAL STRATEGIES FOR PHYTOPLANKTON RESOURCES UPTAKE

In pelagic environments, light and nutrients are not equally distributed within the water column and show vertical gradients of availability. While light intensity is higher in upper water layers, nutrient concentrations are, during periods of stratification, generally higher in deeper water layers. A possibility for phytoplankton species to optimize resource uptake is mobility. Mobile species can (at least to a certain degree) migrate within the water column to choose an optimal position for nutrient uptake and photosynthesis. Mobility involves costs in terms of energy to develop, maintain and operate mobility structures. We conducted laboratory growth experiments with mobile and non-mobile green algal species along a gradient of light availability (Paper 4). Phytoplankton biomass (determined as particulate organic carbon) and biomass carbon to phosphorus ratios of non-mobile species were higher than those of mobile species. This indicates that the efficiency of resource use of mobile species was worse than that of non-mobile species. Mobile species had higher energy requirements to balance the costs of basic metabolism. Thus, the advantages of mobility are restricted to specific environmental conditions.

NEW METHODS TO ESTIMATE GROWTH AND MORTALITY OF PHYTOPLANKTON COMMUNITIES

It is difficult to measure phytoplankton growth and mortality (grazing by micro- and mesozooplankton) *in situ* in natural phytoplankton communities. However, these are important parameters to understand the dynamics of natural phytoplankton communities. We established a new method to estimate phytoplankton growth and mortality by combining existing dilution (to measure mortality) and dialysis (to measure growth) techniques (Paper 5). Experiments showed that the combination of these methods can be successfully used to quantify phytoplankton gross growth rates and micro- and mesozooplankton grazing *in situ*.

1. INTRODUCTION

PHYTOPLANKTON, PHOTOSYNTHESIS, AND PHOTOSYNTHETIC PIGMENTS

Phytoplankton is defined as the photosynthetic microorganisms, adapted to live partly or continuously in open water of the sea, of lakes, ponds and rivers, where they contribute part or most of the organic carbon available to pelagic food webs (Graham et al. 2000; Reynolds 2006). Most phytoplankton groups differ in predominant photosynthetic pigments, storage products, and cell wall components. Molecular sequence information has provided evidence for the existence of eight or nine major clades or divisions of phytoplankton (Graham et al. 2000). These are the cyanobacteria classified among the Eubacteria, and the eukaryotic phyla Glaucophyta (glaucophytes), Euglenophyta (euglenoids), Cryptophyta (cryptomonads), Haptophyta (haptophytes), Dinophyta (dinoflagellates), Ochrophyta (a diverse array of tiny flagellates, diatoms, chrysophyceans, brown algae and a host of other groups), Rhodophyta (red algae), and Chlorophyta (green algae). Members of marine and freshwater phytoplankton communities are characterized by a diversity of sizes and morphologies quite comparable to the morphological diversity of land plants. Phytoplankton communities dominate the pelagic ecosystems that cover 70% of the world's surface (Reynolds 2006) and approximately 45% of the photosynthesis on Earth occurs in aquatic environments (Falkowski 1994; Field et al. 1998).

Photosynthesis is a biological process in which light energy is captured, converted into biochemical energy and stored in the form of organic carbon compounds (Falkowski and Raven 2007). This stored energy is then used to drive cellular processes.

The task of collecting light energy from the underwater light field is carried out by the photosynthetic pigment-molecules whose structures are such that they efficiently absorb light in the 400-700nm range (the visible light spectrum) (Kirk 1994). The ability of phytoplankton to absorb light is directly related to the spectral nature of their light-harvesting capabilities given by the pigments present (Bergmann et al. 2004). There are three chemically distinct

types of photosynthetic pigment: the chlorophylls, the carotenoids and the biliproteins. All photosynthetic plants contain chlorophyll (chlorophylls *a*, *b*, *c*, and *d*) and carotenoids (Carotenes and Xanthophylls); the red-algae, the blue-green algae, and the cryptophytes contain biliproteins (Phycoerythrin and Phycocyanin) as well. Carotenoids and biliproteins are accessory pigments because the light absorbed by these pigments can be transferred to chlorophyll.

The light absorption of chlorophyll peaks in the blue-violet and red regions of the spectrum, while carotenoids absorb mainly in the blue-green and phycobilins mainly in the yellow-red regions of the light spectrum (Figure 1.1).

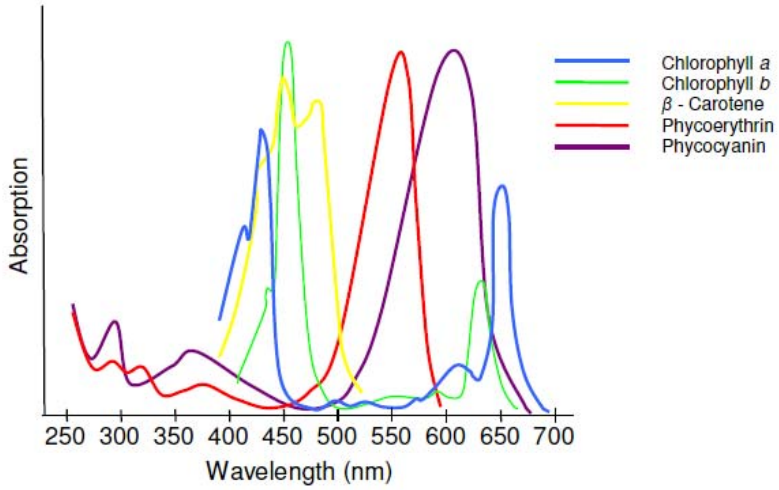


Figure 1.1: Scheme of the different absorption spectra of the three main pigment classes: chlorophylls, carotenoids, and biliproteins (redrawn after www.jochemnet.de).

The accessory pigments present in a given phytoplankton species will determine its potential for utilising particular wavelengths (Wall and Briand 1979). Therefore, one can expect a wide range of physiological responses to variations in light quality. The relative abundance of the different pigments is highly regulated in response to irradiance, nutrient availability and temperature (Geider 1987; Langdon 1988; Falkowski and Laroche 1991; Geider 1993; Cloern et al. 1995; Geider et al. 1997).

PHYTOPLANKTON, LIGHT AND NUTRIENTS

Phytoplankton species are primary producers and constitute the base of the pelagic food chain. Light and nutrients are resources that regulate the quantity, the distribution, and the structure of phytoplankton communities (Tilman 1982; Huisman and Weissing 1995; Diehl et al. 2002; Hessen et al. 2002). Light serves as the energy source for photosynthesis while nutrients have manifold functions concerning cell structure and metabolism. In freshwater systems phosphorus often limits phytoplankton growth. Light and nutrients differ fundamentally in their constitution as a resource. Nutrients can be recycled whereas absorbed light photons transformed into energy exhibit a unidirectional flow within food webs. Nutrients can either be distributed homogeneously along the water column (if mixing of the water column is sufficient) or accumulate at deeper water layers (during stratification). Light generally decreases exponentially with depth. The decline of light with depth is determined by water molecules, by the concentration of dissolved matter, and by particles (such as phytoplankton). Light that enters a natural water body is scattered, absorbed as heat, or transformed into energy sources (Figure 1.2). The exponential decrease of the light intensity with depth due to absorption and refraction is called the vertical light attenuation and can be mathematically approximated by the Lambert-Beer law:

$$E_d(z) = E_d(0)e^{-k_d z}$$

where $E_d(z)$ and $E_d(0)$ describe the light intensities at the depth z and at the surface, and k_d is the vertical attenuation coefficient. A high k_d indicates that light is absorbed rapidly. The Lambert-Beer law of light extinction generally applies to the decrease of monochromatic light with parallel light beams in pure solution. Each wavelength that passes through water has a different attenuation coefficient. Red light (620-750nm) is absorbed most rapidly in pure water while blue light (430-500nm) penetrates deepest (Figure 1.2).

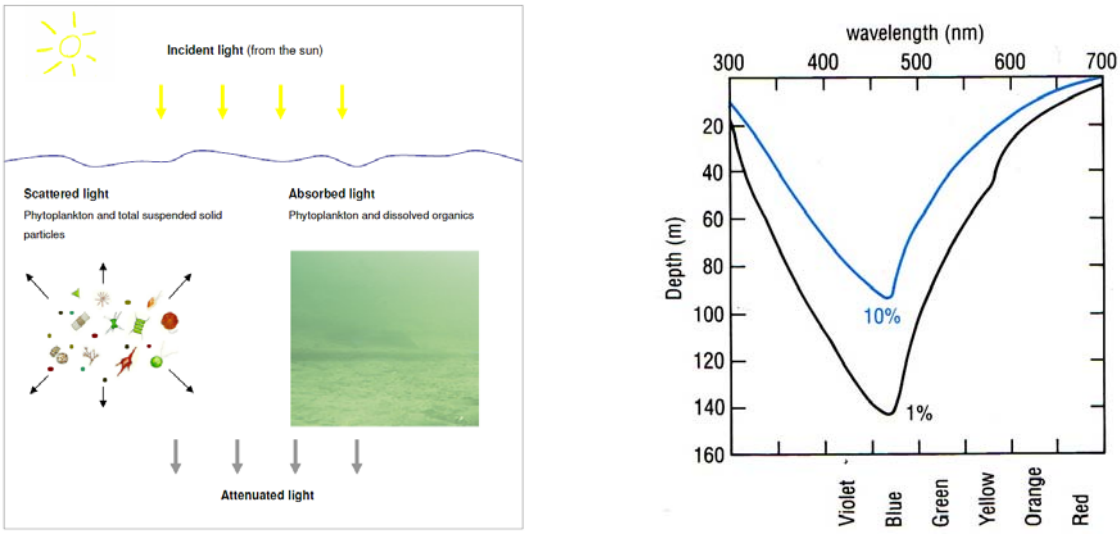


Figure 1.2: Major components of light attenuation within water (left panel) and penetration of light of different wavelength into clear oceanic water (right panel). The lines indicate the depths of penetration for 10% (blue line) and 1% (black line) of the surface light levels. Right panel from Lalli and Parsons (1997).

There is a loose coupling between the biomass carbon and nutrient content of phytoplankton. Phytoplankton carbon content generally increases with light availability. Phytoplankton biomass carbon content generally increases with light availability. Carbon to phosphorus ratios of phytoplankton are highly flexible and range between molar ratios of about 50 to 1000 (Sterner et al. 1997; Brett et al. 2000).

In contrast, the elemental composition of zooplankton biomass is largely homeostatically regulated (Andersen and Hessen 1991; Main et al. 1997; Elser et al. 2000). Zooplankton with high specific growth rates, such as *Daphnia,* need a high body phosphorus content. Therefore, their biomass carbon to phosphorus ratio is considerably lower (molar ratio of about 80) than that of phytoplankton (Elser et al. 1996; Main et al. 1997).

THE LIGHT-NUTRIENT HYPOTHESIS

A mismatch in biomass carbon to phosphorus ratios between phytoplankton and zooplankton effects the transfer efficiency of energy and matter within pelagic food webs. This relationship between light, nutrients and phytoplankton-zooplankton interactions is described in the so called 'light-nutrient hypothesis' (Urabe and Sterner 1996). A mismatch between the elemental composition of autotrophs and herbivorous consumers can result in herbivore growth becoming limited by nutrients rather than by the carbon content of phytoplankton. The elemental threshold ratio above, in which *Daphnia* growth is nutrient limited as opposed to energy (carbon) limited, is approximately a molar phytoplankton biomass carbon to phosphorus ratio of 300 (Hessen 1992; Urabe and Watanabe 1992; DeMott and Tessier 2002; Urabe et al. 2002a; DeMott and Pape 2004).

Increased light supply generally increases phytoplankton primary production and can result in higher phytoplankton biomass (up to a level where light is no longer limiting phytoplankton growth). At low phosphorus concentrations, increasing light intensities can lead to high phytoplankton biomass carbon to phosphorus ratios (Figure 1.3 A).

With increasing light intensity the carbon ingestion rate of herbivorous zooplankton (*Daphnia*) increases until saturation is reached (Figure 1.3 B). The phosphorus ingestion rates of *Daphnia* also increase with increasing light intensity, but can decrease at high light intensities due to high phytoplankton biomass carbon to phosphorus ratios (Figure 1.3).

Therefore, increased light supply may actually decrease herbivorous zooplankton growth, because any light-induced increase in food quantity may be offset by a disproportional decrease in the food's nutrient content (Andersen et al. 2004; Diehl 2007).

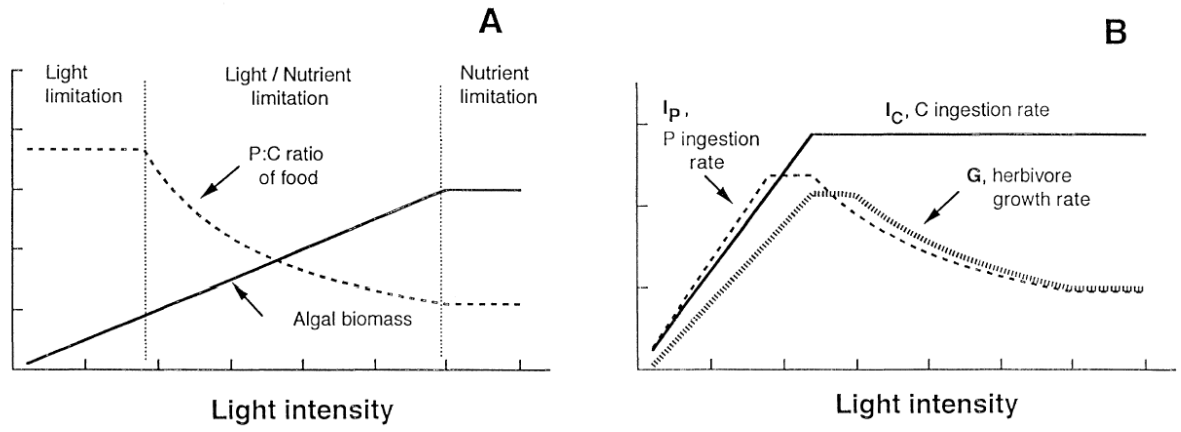


Figure 1.3: Scheme of the light-nutrient hypothesis (Urabe and Sterner 1996).
A: The responses of phytoplankton biomass and biomass phosphorus to carbon ratios to increasing light intensities (under phosphorus limitation). Notice that in this scheme phosphorus to carbon (P:C) instead of carbon to phosphorus (C:P) ratios are displayed.
B: Herbivorous ingestion and growth rates related to light intensity.

PHYTOPLANKTON BIODIVERSITY, RESOURCE USE AND PRODUCTIVITY

Biodiversity can be defined in various ways. First, in a broad sense it can be defined as a collective term for all biological differences at scales ranging from genes to ecosystems (Harper and Hawksworth 1994). The United Nations Convention on Biological Diversity (United Nations Earth Summit, 1992) defines biodiversity as the variability among living organisms from all sources (terrestrial and aquatic ecosystems) and the ecological complexes of which they are part; this includes diversity within species, between species and the diversity of ecosystems. A third definition is the variety of all forms of life, from genes to species, up to the broad scale of ecosystems, which present a unified view of the traditional three levels at which biodiversity has been identified: genetic diversity, species diversity, and ecosystem diversity (simplified after Gaston, 1996).

Research on the ecological importance of biodiversity has mostly been dealing with diversity-stability and diversity-productivity relationships. Three hypothesis of how species diversity and productivity interact have gained support from field and laboratory studies (Loreau and

Hector 2001; Hooper and Dukes 2004; Fox 2005; Cardinale et al. 2006; Tilman et al. 2006). First, the complementarity hypothesis states that more diverse communities should, by niche differentiation, be able to use resources more effectively and be more productive than less diverse ones. Secondly, the sampling effect hypothesis states that the chance for a highly productive species to be present and dominate the community would be greater at higher diversity. Both, the complementarity and the sampling effect, cause more complete utilization of limiting resources at higher diversity, which increases productivity. Thirdly, the facilitation between species (facilitation hypothesis), which may cause a positive effect on productivity, is higher in systems that are more diverse. Facilitation is a mechanism whereby certain species help or allow other species to grow by modifying the environment in a way that is favourable to a co-occurring species. In general, communities with more species should be able to use a greater variety of resource capturing characteristics, leading to a greater productivity (overyielding). Overyielding can be either transitive (mixture yield exceed yields from the most productive monoculture) or non-transitive (mixture yields exceed expectations, but not absolute yields of the most productive monoculture) (Hooper and Dukes 2004). Underyielding, in contrast, means that mixture yield is lower than expected from summing up the yields of the monocultures.

Empirical evidence supports increasingly the occurrence of increased productivity (overyielding) in species mixtures compared with monocultures (Tilman et al. 1996; Hector et al. 1999; Loreau and Hector 2001; Tilman et al. 2001). On the other hand, Jiang et al. (2008) argued that neutral or negative biodiversity and ecosystem functioning relationships may be just as likely and under certain circumstances probably more common. In a large empirical study including more than 3000 natural phytoplankton samples, Ptacnik et al. (2008) were able to show that phytoplankton diversity is the best predictor of phytoplankton resource use efficiency in freshwater and brackish environments. This is distinct evidence that a positive diversity-productivity relationship exists within pelagic communities; however such empirical studies lack of explanations for the underlying mechanisms.

MOBILITY IN PHYTOPLANKTON SPECIES: ADVANTAGES AND COSTS

Many freshwater phytoplankton species are mobile. Some of these mobile phytoplankton species are able to conduct periodic vertical migrations (for example in terms of diurnal migrations) and can, to a certain degree, choose their vertical position in the water column to optimize the availability of light and nutrients (Pick et al. 1984; Knapp et al. 2003). Mobile phytoplankton species are able to access nutrient rich water at greater depths and can, in addition, adjust for optimal irradiance (Jones 1993; Ralston et al. 2007). This can increase their competitive advantage compared to non-mobile species. The migration of mobile taxa is largely influenced by light conditions and nutrient supply levels (Knapp et al. 2003). Mobility, however, involves costs in terms of energy and nutrient expenditure. The costs of mobility for the majority of planktonic protists are the equivalent of low (<1%) or moderate (1-10%) in proportion to their total metabolic rate on mobility (Crawford 1992). Mobile phytoplankton species may therefore have a lower resource use efficiency than non-mobile phytoplankton species and this may constrict the advantages of mobility to certain environmental conditions.

ESTIMATION OF PHYTOPLANKTON GROWTH AND MORTALITY

Phytoplankton growth is controlled by the balance between reproduction and mortality. Phytoplankton reproduction is determined by environmental factors (temperature, pH) and by essential resources (light, nutrients). Mortality results from sedimentation losses, consumptions by herbivores, and physiological death of whole cells. To quantify phytoplankton reproduction and mortality *in situ* several techniques can be applied. Capriulo and Carpenter (1980) simply divided samples into two size ranges (<35µm mainly consisting of phytoplankton and >35µm mainly consisting of grazers) to separate phytoplankton from herbivorous zooplankton. Landry and Hassett (1982) diluted plankton samples, assuming that contact between phytoplankton and grazers at high dilutions

becomes almost zero. These experiments are conducted in closed bottles, where because nutrients can become limited, they are added throughout the experiment in high amounts. These nutrient additions disrupt the system in such a way that this technique not appropriate for *in situ* measurements within low-nutrient systems (Andersen et al. 1991).

Other methods for estimating mesozooplankton grazing *in situ* are various tracer methods mostly including radioactive isotopes. Due to safety regulations, it is not always possible to use such methods in the field. Additionally, radioactive tracer methods do not allow quantifying grazing rates on individual phytoplankton groups or species. Until now, simultaneous measurements of phytoplankton growth and loss rates *in situ* have not been performed. However, such simultaneous measurements would tremendously improve *in situ* estimates of phytoplankton dynamics.

My research was motivated by obvious gaps in the understanding of the above described effects of light, nutrients and diversity on phytoplankton dynamics and on phytoplankton-zooplankton interactions which are summarized as follows:

1. EFFECTS OF DIVERSITY ON PHYTOPLANKTON RESOURCE UPTAKE AND GROWTH
    - Does a coupling between biodiversity and functional diversity exist?
    - Are biodiversity and the resource use efficiency of phytoplankton communities linked?
    - If biodiversity and the resource use efficiency of phytoplankton are linked, what are possible mechanisms for this relationship?

2. EFFECTS OF DIVERSITY ON PHYTOPLANKTON RESOURCE UPTAKE AND BIOMASS COMPOSITION (STOICHIOMETRY)
    - Does a higher light use efficiency of highly diverse phytoplankton communities have consequences for their biomass composition?
    - Does increasing phytoplankton diversity result in increasing biomass carbon to phosphorous ratios of freshwater phytoplankton communities, because carbon assimilation and phosphorus uptake are only tightly coupled?

3. THE SUPPLY OF LIGHT AND NUTRIENTS AND ITS CONSEQUENCES FOR PHYTOPLANKTON-ZOOPLANKTON INTERACTIONS
    - To what extent is the ‘light-nutrient hypothesis’ useful to describe the influence of light and nutrients on natural phytoplankton communities and on herbivorous zooplankton feeding on these phytoplankton communities?
    - Can light availability influence herbivorous zooplankton growth by shifting phytoplankton food quality from energy to nutrient limitation?

4. COSTS OF BEHAVIOURAL STRATEGIES FOR PHYTOPLANKTON RESOURCES UPTAKE

- Do mobile phytoplankton species, which can optimize their resource uptake by migrating to optimal resource conditions, differ in their biomass to nutrient content from non-mobile species?
- What are possible costs of this strategy and can costs be quantified with photosynthesis and biomass composition measurements?

5. NEW METHODS TO ESTIMATE GROWTH AND MORTALITY OF PHYTOPLANKTON COMMUNITIES

- Is it possible to use a single technique to simultaneously quantify phytoplankton growth rates and the impact of grazing by meso- and microzooplankton *in situ*?

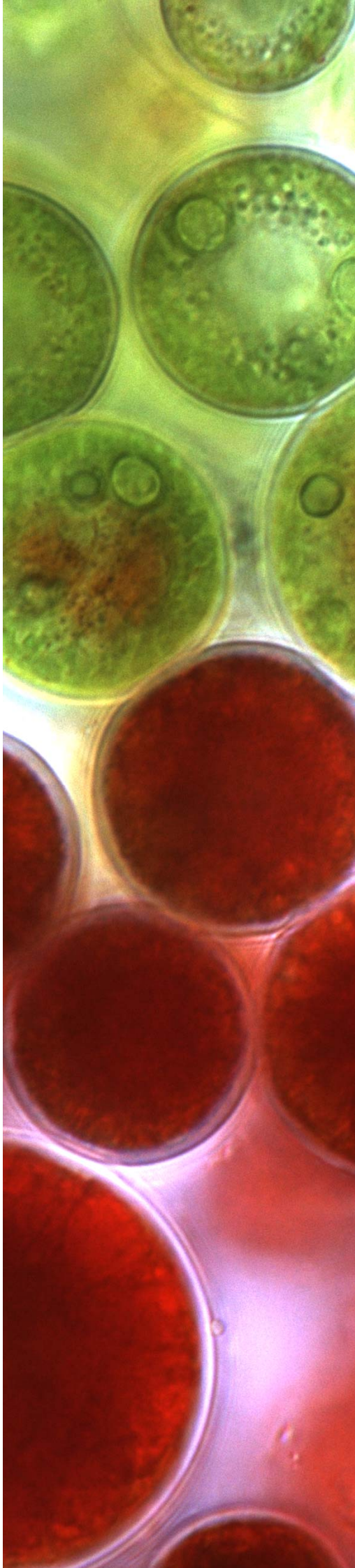
I investigated these research questions with a series of field and laboratory experiments. The results are presented in the following within two already published papers (Paper 3 and 5), two submitted papers (Paper 1 and 2) and within a manuscript prepared to be submitted (Paper 4).

PAPER 1

COLORFUL NICHES LINK BIODIVERSITY TO CARBON DYNAMICS IN PELAGIC ECOSYSTEMS

STRIEBEL, M., S. BEHL, S. DIEHL, H. STIBOR

SUBMITTED TO *ECOLOGY LETTERS*



Abstract

Positive effects of biodiversity on ecosystem function are described from an increasing number of systems, but the underlying mechanisms remain frequently elusive. A truly predictive understanding of biodiversity-ecosystem function relationships requires the *a priori* identification of traits conferring unique functions to individual species. Although planktonic primary producers are responsible for approximately half of the world's primary production, few studies have reported on the relationship between phytoplankton biodiversity and planktonic primary production. We argue that taxon-specific differential equipment with photosynthetically active pigments provides a biochemical mechanism of resource use complementarity among phototrophic microorganisms, enabling more diverse communities to more completely harvest the light spectrum. In line with this, more diverse phytoplankton communities showed higher pigment diversity, higher biomass-specific light absorbance, and higher rates of primary production and biomass accrual.

Introduction

There is growing concern that the worldwide, accelerating loss of biodiversity may impair the functioning and stability of ecosystems and, thus, the ability of the biosphere to provide critical ecosystem services to the human population (Balmford *et al.* 2002; Worm *et al.* 2006). The study of relationships between biodiversity and ecosystem function has therefore become a priority in ecological research during the last decade (Loreau & Hector 2001). Particularly well studied to date is the relationship between plant species richness and primary production, which has been found to be positive in an overwhelming majority of experimental studies (Balvanera *et al.* 2006; Cardinale *et al.* 2006; Hector *et al.* 2007). In contrast, observational studies in unmanipulated ecosystems showed inconclusive evidence of the effect of plant species richness on primary production (Thompson *et al.* 2005; Grace *et al.* 2007). Moreover, the empirical evidence is strongly biased towards temperate grassland systems, which are responsible for only a minor fraction of global primary production; studies from other terrestrial biomes and from aquatic systems are still relatively rare. Also, very few studies have conclusively documented the mechanisms mediating diversity effects. It therefore remains an open question whether positive plant diversity-primary productivity relationships are a truly general phenomenon, whether the phenomenon applies to unmanipulated, natural communities, and, if so, what are its underlying mechanisms?

Generally, two types of non-exclusive mechanisms have been made responsible for positive diversity-productivity relationships: the 'selection effect' (also called 'sampling effect'), where more diverse communities are more likely to contain and become dominated by inherently more productive species, and the 'complementarity effect', where resource partitioning and/or facilitation among species leads to increased resource use and productivity in more diverse communities (Loreau & Hector 2001; Tilman *et al.* 2006; Cardinale *et al.* 2006). In experimental studies it is now standard to assess the relative contributions of the complementarity and selection effects to total biodiversity effects by

additive variance partitioning (Loreau & Hector 2001). Many of these studies have identified complementarity as the dominant mechanism underlying positive relationships between terrestrial plant species richness and primary production (Loreau & Hector 2001; van Ruijven & Berendse 2005; Flombaum & Sala 2008). Partitioning of the physical niche space (e.g. nutrient and water uptake in different soil compartments, light harvesting in different canopy layers) among higher plants with complex morphologies has been *ad hoc* suggested to explain this pattern (Hooper 1998). Similarly, variance partitioning can only separate the contributions of selection and complementarity *ad hoc*. Prediction of the consequences of biodiversity loss requires, however, yet a deeper understanding of the mechanisms that mediate biodiversity effects. In particular, the *a priori* identification of species traits that promote selection, niche complementarity, and/or facilitation is called for.

Planktonic primary producers (phytoplankton) in the surface layers of lakes and oceans are responsible for approximately half of the world's annual primary production and are thus a major component of the global carbon cycle (Field *et al.* 1998). A recent study including more than 3000 samples from Scandinavian lakes and the Baltic Sea revealed that resource use efficiency (the biomass produced per unit of limiting resource) and phytoplankton taxonomic diversity are positively linked. Specifically, the amount of algal carbon per unit total phosphorus was positively related to genus richness of the phytoplankton communities (Ptacnik *et al.* 2008). These data are the first evidence suggesting that positive diversity-productivity relationships may be a general phenomenon extending to pelagic aquatic communities. But how is this possible? Compared to the physical complexity provided by most terrestrial environments, permanent mixing and a rather simple physical structure seem to strongly limit opportunities for niche complementarity among planktonic primary producers (Hutchinson 1961). Phytoplankton taxa do certainly differ in resource use attributes such as uptake rates and storage of nutrients, storage of carbon reserves, and light use efficiency, but models and data suggest that strong and appropriately timed temporal variability is

required to maintain even low to moderate levels of phytoplankton biodiversity (Armstrong & McGehee 1980; Passarge *et al.* 2006).

Due to its strong vertical attenuation, light often (co)limits the production and biomass of aquatic primary producers (Boyd 2002; Berger *et al.* 2006). A hitherto largely neglected aspect of algal resource partitioning is complementarity in the use of different spectral components of the photosynthetic active radiation (PAR). In addition to chlorophyll *a* most phytoplankton species possess other photosynthetic pigments in taxon-specific combinations and quantities (Rowan 1989). Complementarity effects within phytoplankton communities could then result from more diverse communities using a broader range of wavelengths and thereby increasing the effectiveness of light harvesting (Barsanti & Gualtieri 2006; Stomp *et al.* 2007a, b). Based on these arguments, a positive relationship between phytoplankton diversity and primary production could at least partly be explained by the following suite of mechanistic hypotheses: (i) Taxonomically more diverse phytoplankton communities possess a more diverse array of photosynthetically active pigments. (ii) Communities with higher pigment diversity make more efficient use of the PAR spectrum; specific absorbance (the proportion of PAR energy absorbed per unit of community biomass) should then increase with increasing taxonomic diversity. (iii) Higher specific absorbance yields higher specific primary production and thus an increase in phytoplankton biomass accrual with increasing diversity - at least in underwater climates where phytoplankton production is light limited.

We investigated this suite of hypotheses with a combination of highly controlled laboratory experiments with assembled algal communities and a comparative survey of natural phytoplankton communities in the field. In the laboratory we established monocultures of 12 algal strains (representing the major classes of freshwater algae) as well as five different, assembled algal communities (randomly drawn from the 12 strains) within each of five different levels of taxon richness (2, 3, 5, 7, and 10 species). This yielded a total of 37 assembled communities, all of which were cultured in the same growth medium. Initial total algal biovolume was identical between treatments, and different species contributed

with equal initial biovolume to treatments with two or more species. At the start of this experiment, pigment analyses were conducted from 18 assembled communities (three from each diversity level) and specific PAR absorbance per unit of community biomass was determined for 30 communities. Community biomass accrual after two weeks of exposure to continuous irradiance with 100 µmol quanta of PAR m^{-2} s^{-1} was determined as wet mass (for all 37 communities) and as particulate organic carbon (for 30 communities). In addition, we measured short-term (4 h) specific net primary production (sNPP) of 25 communities (five different, randomly drawn communities with 1, 2, 3, 5, and 7 species, respectively) in separate trials.

Methods

Experiments with assembled algal communities: Communities were assembled from the following twelve algal strains representing the major algal classes: *Chlorella* sp., *Chlamydomonas* sp., *Cryptomonas* sp., *Monoraphidium* sp., *Scenedesmus* sp., *Selenastrum* sp., *Microcystis* sp., *Synechococcus* sp., *Fragilaria crotonensis*, *Asterionella* sp., *Staurastrum tetracerum*, *Peridinium* sp. Algae are frequently cultured in taxon-specific growth media. To avoid confounding effects of a switch in growth medium between monoculture and polyculture experiments, we precultured all of the above strains in monoculture in a common growth medium (standard WC medium after (Guillard & Lorenzen 1972), which was subsequently used in all experiments) over a period of several month prior to the experiments.

We established monocultures of all 12 algal strains as well as polycultures at five levels of taxon richness (2, 3, 5, 7, and 10 species). For polycultures, five different communities (each being a unique, random draw from the available pool of strains) were established at each level of taxon richness, yielding a total of 37 different experimental communities (12 monocultures and 25 polycultures). All communities were started with the same initial total algal biovolume and different species contributed to polycultures with equal initial biovolume. All communities were kept in 1-L translucent polyethylene flasks in a climate chamber at 20°C and exposed to continuous irradiance with 100 µmol quanta of PAR m^{-2} s^{-1}. The algal growth medium was a standard WC medium (Guillard & Lorenzen 1972) with the exception that the phosphorus content was reduced to 3.1 µg P L^{-1}, comparable to an oligotrophic lake. The experiment was conducted in semibatch culture with a daily replacement of 10% of the culture volume by fresh medium.

At the start of the experiment pigment analyses (see below) were conducted from three randomly chosen communities from each diversity level (three monocultures and 15 polycultures, total n=18). Additionally, we determined the biomass-specific PAR absorbance

of all polycultures and of five randomly chosen monocultures. To do so we filtered samples of each of the mono- and polycultures (all of which had identical concentrations of algal wet biomass) onto glass-fibre filters (Whatman GF/F). The filters were extracted in 95% acetone, sonicated, mixed on a vortex mixer and allowed to extract at 4°C. To remove cell and filter debris, extracts were centrifuged and subsequently PAR absorbance by the pigment extract was measured in steps of 1nm over the range 400 to 700 nm on a Shimadzu UV-1700 spectrophotometer (Shimadzu Europe). Average PAR absorbance per nm was calculated from the resulting absorbance spectrum.

At the end of the experiment (after two weeks of incubation) we filtered water from all polycultures and five randomly chosen monocultures (total n=30) onto precombusted, acid-washed glass-fibre filters (Whatman GF/F) and determined the concentration of particulate organic carbon on an Elemental Analyser (CE Instruments, Milan, Italy). For all experimental communities (n=37) we determined final total algal wet biomass, and the contribution of each taxon to it, from microscopic algal counts. Algae in samples fixed with Lugol's iodine were counted and measured in an inverted microscope using Utermöhl chambers (Utermöhl 1958). The biovolume of each algal species was subsequently calculated according to Hillebrand *et al*. (1999) and converted to wet biomass assuming a specific wet mass of 1fg per μm^3 of algal biovolume. We used the wet biomass data to calculate the contributions of the selection and complementarity effects to the total biodiversity effect on community biomass according to the additive variance partitioning suggested by Loreau and Hector (2001). We were unable to calculate selection and complementarity effects for the communities with 10 species, because they were all contaminated by one or two species (*Desmodesmus* sp. or *Tetraedron* sp.) that we have no monoculture data on.

In a separate experiment, we established five monocultures and 20 polycultures (total n=25) at five levels of taxon richness (1, 2, 3, 5, and 7 species). Each level of taxon richness was replicated five times, each community being a random draw from the available pool of strains. For each of these 25 communities we measured the specific rates of net primary

production (sNNP) during short-term incubations. Incubation flasks were completely filled (leaving no air bubbles) with the different algal suspensions, and the dissolved oxygen concentrations in the suspensions were measured at the beginning and at the end of 4 h of incubation at 100 µmol quanta of PAR m^{-2} s^{-1} and 20°C. The net rate of carbon fixation by each algal community was calculated according to Wetzel and Likens (Wetzel & Likens 1991).

Data from natural algal communities: In September 2004 we sampled water from 46 lakes in southern Germany and Austria. Twenty-eight of the lakes were oligotrophic (TP<10µg L^{-1}), 15 were mesotrophic (10-25µg TP L^{-1}), and three were eutrophic (TP>25µg L^{-1}). From each lake we took a depth-integrated, pelagic water sample from the mixed surface layer and filtered it through a 200 µm mesh to remove crustacean zooplankton. Samples were stored cold and dark for max. 4h during transport.

We determined total phosphorus content (after sulphuric digestion) and the content of 26 different algal pigments (see below) from aliquots of the lake samples. We also determined the absolute and relative contributions of different algal taxa to phytoplankton wet biomass from microscopic counts and measurements as described above. Estimates of wet biomass were converted to algal carbon biomass assuming a carbon content of 0.14 fg C per $µm^3$ algal biovolume (Ptacnik *et al.* 2008). Additionally, we filtered water from each community onto precombusted, acid-washed glass-fibre filters (Whatman GF/F) and determined the concentration of particulate organic carbon on an Elemental Analyser (CE Instruments).

Pigment analyses: Samples from laboratory monocultures and polycultures and natural lake communities were filtered onto glass-fibre filters (Whatman GF/F) and stored at -80°C until analysis. The filters were extracted in 95% acetone, sonicated in an ice-cold bath, mixed on a vortex mixer, allowed to extract at 4°C, and vortexed again. Extracts were then filtered through 0.2 µm Teflon syringe filters to remove cell and filter debris, transferred to HPLC vials and injected (with buffer) on the HPLC (Shimadzu LC-10A HPLC system with LC Solution software, Shimadzu Europe).

Results

Laboratory experiments

There was a positive relationship between taxonomic diversity (taxon richness) and pigment richness (number of pigments) of assembled communities (Fig. 1A, B). Second, biomass-specific PAR absorbance increased with taxon richness (Fig.1C), suggesting that more diverse phytoplankton communities were able to harvest PAR more effectively than less diverse communities. Third, the specific rate of net primary production increased with taxon richness (Fig. 2). Finally, phytoplankton biomass accrual after two weeks of incubation was positively related to taxon richness (Fig. 1D). Collectively, these results suggest that the observed positive relationship between phytoplankton diversity and primary production is at least in part a consequence of algal niche complementarity with respect to the use of different spectral components of the PAR supply. Additive variance partitioning supports this interpretation. The selection effect was zero on average (mean= -0.04 mg wet mass L^{-1}; SE=0.75) and unrelated to taxon richness (Fig. 1E). In contrast, the complementarity effect was positive on average (mean=2.27 mg wet mass L^{-1}; SE=1.41) and increased with taxon richness (Fig. 1F).

Field data

A survey of 46 German and Austrian lakes covering a broad range of nutrient regimes shows that the above results extend to natural communities with shared evolutionary histories. Pigment analyses of water samples taken from the mixed surface layers of the lakes in September 2004 revealed that the relationship between taxon richness and pigment richness was similar and positive in assembled and natural phytoplankton communities and is well described by a common regression line (Fig. 1A). Because phytoplankton biomass in freshwater lakes strongly correlates with total phosphorus concentration (TP) (Schindler 1978), we used TP as an additional, independent predictor variable when assessing the

contribution of taxonomic diversity to phytoplankton biomass in the field samples. Similar to the assembled lab communities, both particulate organic carbon and algal wet mass were positively related to algal taxon richness also in the lake samples (Table 1A, B). Standard partial regression coefficients (SPRC) indicated that, over the ranges in taxon richness and TP encountered in the field, the positive impact of taxon richness on carbon biomass was more than ten times higher than the positive impact of TP (SPRC = 0.57 vs. 0.05, all variables in ln transformed units).

A clear, positive effect of biodiversity on carbon biomass in the field samples is also indicated by the positive relationship between resource use efficiency and taxon richness (Table 1C, D). We calculated resource use efficiency in two independent ways, i.e. based on POC (which includes non-algal particulate organic material) and based on algal carbon (which was estimated from microscopically derived algal biovolume under the assumption of a carbon content of 0.14 fg C per μm^3 algal biovolume (Ptacnik *et al.* 2008)). The relationship based on algal carbon (intercept = -1.3, slope = 1.35, Table 1D) is very similar to the relationship in Scandinavian lakes and the Baltic Sea reported in Ptacnik et al. (intercept = -0.96, slope = 1.12), who used the same method to estimate algal carbon.

Discussion

The idea that more diverse plant communities are more productive than less diverse ones dates back to Darwin (McNaughton 1993; Loreau 2000). While experiments with assembled communities have frequently supported this idea, the relevance of these experiments to natural communities is a matter of debate. A crucial, missing link in the quest for a conclusive, mechanistic understanding of the influence of biodiversity on ecosystem function has been the *a priori* identification of species traits conveying complementarity in resource use. We have argued that the differential equipment with photosynthetically active pigments provides a biochemical mechanism of resource use complementarity among phototrophic microorganisms, enabling more diverse communities to more completely harvest the spectrum of solar energy and thus creating a positive relationship between phytoplankton diversity and primary production. We have provided empirical support for the operation of this mechanism in both assembled and natural communities. Pigment complementarity is therefore likely to contribute to the recently described positive relationship between phytoplankton diversity and resource use efficiency in Scandinavian lakes and the Baltic Sea (Ptacnik *et al.* 2008). Interestingly, in the Scandinavian data set diversity effects on resource use efficiency were stronger in less diverse communities (Ptacnik *et al.* 2008). The latter would be expected under the pigment complementarity hypothesis because, as absorption spectra of different pigments fill up the available spectral niche space, the gain in total PAR harvesting with the addition of new pigments to the community becomes increasingly marginal. This should have constrained the evolution of pigment diversity, yielding a saturating relationship between phytoplankton biodiversity and pigment diversity. Our data do indeed clearly show that pigment richness increases most strongly with taxon richness in the least diverse communities (Fig. 1B).

Importantly, the pigment composition and absorption spectra of different phototrophic microorganisms are relatively easy to measure, allowing the *a priori* determination of spectral

niche overlap among different taxa. While the spectral component of phytoplankton niche space has for a long time received relatively little attention, it was recently shown that pigment complementarity can facilitate stable coexistence of otherwise very similar taxa (Stomp *et al.* 2004; Stomp *et al.* 2007b). The ecological consequences of photosynthetic pigment diversity may, however, extend far beyond their impact on phytoplankton community structure and diversity-productivity relationships. In particular, increased resource use efficiency, as manifested by an increase in algal carbon fixation per unit of nutrient (Ptacnik *et al.* 2008) (Table 1C, D), affects the carbon to nutrient stoichiometry of suspended particles, with consequences for ecosystem processes such as the sequestration and storage of atmospheric carbon dioxide in aquatic systems and the transfer of energy and matter along the food chain (Hessen *et al.* 2004; Diehl *et al.* 2005; Striebel *et al.* 2008). Future studies on the ecological consequences of photosynthetic pigment diversity therefore hold promise to greatly improve our understanding of the carbon dynamics of pelagic ecosystems, which cover 70% of the earth's surface.

Acknowledgments

This study was supported by funding from Deutsche Forschungsgemeinschaft (STI 180/2-1). We thank A. Wild and A. Weigert for technical support and Olav Vadstein for helpful comments on an earlier version of this manuscript.

References

Armstrong R.A. & McGehee R. (1980). Competitive-exclusion. *Am. Nat.,* 115, 151-170.

Balmford A., Bruner A., Cooper P., Costanza R., Farber S., Green R.E., Jenkins M., Jefferiss P., Jessamy V., Madden J., Munro K., Myers N., Naeem S., Paavola J., Rayment M., Rosendo S., Roughgarden J., Trumper K. & Turner R.K. (2002). Ecology - Economic reasons for conserving wild nature. *Science,* 297, 950-953.

Balvanera P., Pfisterer A.B., Buchmann N., He J.S., Nakashizuka T., Raffaelli D. & Schmid B. (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecol. Letters,* 9, 1146-1156.

Barsanti L. & Gualtieri P. (2006). *Algae: Anatomy, Biochemistry, and Biotechnology*. CRC Press Taylor & Francis Group, Boca Raton.

Berger S.A., Diehl S., Kunz T.J., Albrecht D., Oucible A.M. & Ritzer S. (2006). Light supply, plankton biomass, and seston stoichiometry in a gradient of lake mixing depths. *Limnol. Oceanogr.,* 51, 1898-1905.

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

Cardinale B.J., Srivastava D.S., Duffy J.E., Wright J.P., Downing A.L., Sankaran M. & Jouseau C. (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature,* 443, 989-992.

Diehl S., Berger S. & Wohrl R. (2005). Flexible nutrient stoichiometry mediates environmental influences, on phytoplankton and its resources. *Ecology,* 86, 2931-2945.

Field C.B., Behrenfeld M.J., Randerson J.T. & Falkowski P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science,* 281, 237-240.

Flombaum P. & Sala O.E. (2008). Higher effect of plant species diversity on productivity in natural than artificial ecosystems. *Proc. Natl. Acad. Sci. USA,* 105, 6087-6090.

Grace J.B., Anderson T.M., Smith M.D., Seabloom E., Andelman S.J., Meche G., Weiher E., Allain L.K., Jutila H., Sankaran M., Knops J., Ritchie M. & Willig M.R. (2007). Does species diversity limit productivity in natural grassland communities? *Ecol. Letters,* 10, 680-689.

Guillard R.R. & Lorenzen C.J. (1972). Yellow-green algae with chlorophyllide c. *J. Phycol.,* 8, 10-14.

Hector A., Joshi J., Scherer-Lorenzen M., Schmid B., Spehn E.M., Wacker L., Weilenmann M., Bazeley-White E., Beierkuhnlein C., Caldeira M.C., Dimitrakopoulos P.G., Finn J.A., Huss-Danell K., Jumpponen A., Leadley P.W., Loreau M., Mulder C.P.H., Nesshoover C., Palmborg C., Read D.J., Siamantziouras A.S.D., Terry A.C. & Troumbis A.Y. (2007). Biodiversity and ecosystem functioning: reconciling the results of experimental and observational studies. *Funct. Ecol.,* 21, 998-1002.

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

Hillebrand H., Dürselen D., Kirschtel D., Pollingher U. & Zohary T. (1999). Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.,* 35, 403-424.

Hooper D.U. (1998). The role of complementarity and competition in ecosystem responses to variation in plant diversity. *Ecology,* 79, 704-719.

Hutchinson G.E. (1961). The Paradox of the plankton. *Am. Nat.,* 95, 137-145.

Loreau M. (2000). Biodiversity and ecosystem functioning: recent theoretical advances. *Oikos,* 91, 3-17.

Loreau M. & Hector A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature,* 413, 548.

McNaughton S.J. (1993). Biodiversity and function of grazing ecosystems. In: *Biodiversity and ecosystem function* Springer, pp. 361-383.

Passarge J., Hol S., Escher M. & Huisman J. (2006). Competition for nutrients and light: stable coexistance, alternative stable states, or competitive exclusion? *Ecol. Monogr.,* 76, 57-72.

Ptacnik R., Solimini A.G., Andersen T., Tamminen T., Brettum P., Lepistö L., Willén E. & Rekolainen S. (2008). Diversity predicts stability and resource use efficiency in natural phytoplankton communities. *Proc. Natl. Acad. Sci. USA,* 105, 5134-5138.

Rowan K.S. (1989). *Photosnthetic pigments of algae*. Cambridge University Press.

Schindler D.W. (1978). Factors regulating phytoplankton production and standing crop in worlds freshwaters. *Limnol. Oceanogr.,* 23, 478-486.

Stomp M., Huisman J., de Jongh F., Veraart A.J., Gerla D., Rijkeboer M., Ibelings B.W., Wollenzien U.I.A. & Stal L.J. (2004). Adaptive divergence in pigment composition promotes phytoplankton biodiversity. *Nature,* 432, 104-107.

Stomp M., Huisman J., Stal L.J. & Matthijs H.C.P. (2007a). Colorful niches of phototrophic microorganisms shaped by vibrations of the water molecule. *Isme Journal,* 1, 271-282.

Stomp M., Huisman J., Voros L., Pick F.R., Laamanen M., Haverkamp T. & Stal L.J. (2007b). Colourful coexistence of red and green picocyanobacteria in lakes and seas. *Ecol. Letters,* 10, 290-298.

Striebel M., Spörl G. & Stibor H. (2008). Light-induced changes of plankton growth and stoichiometry: Experiments with natural phytoplankton communities. *Limnol. Oceanogr.,* 53, 513-522.

Thompson K., Askew A.P., Grime J.P., Dunnett N.P. & Willis A.J. (2005). Biodiversity, ecosystem function and plant traits in mature and immature plant communities. *Funct. Ecology,* 19, 355-358.

Tilman D., Reich P.B. & Knops J.M.H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature,* 441, 629-632.

Utermöhl H. (1958). Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. Int. Ver. Limnol.,* 9, 1-38.

van Ruijven J. & Berendse F. (2005). Diversity-productivity relationships: Initial effects, long-term patterns, and underlying mechanisms. *Proc. Natl. Acad. Sci. USA,* 102, 695-700.

Wetzel R.G. & Likens G.E. (1991). *Limnological Analyses*, Second edn. Springer Verlag, New York.

Worm B., Barbier E.B., Beaumont N., Duffy J.E., Folke C., Halpern B.S., Jackson J.B.C., Lotze H.K., Micheli F., Palumbi S.R., Sala E., Selkoe K.A., Stachowicz J.J. & Watson R. (2006). Impacts of biodiversity loss on ocean ecosystem services. *Science,* 314, 787-790.

Table 1:

Regression statistics describing the relationships between phytoplankton taxon richness and measures of production and resource use efficiency in 46 natural lakes. Multiple regressions of (A) particular organic carbon (POC in mg L^{-1}) and of (B) algal wet mass (mg L^{-1}) against taxon richness and total phosphorus concentration (TP in µg L^{-1}). (C, D) Linear regressions of resource use efficiency (RUE) against taxon richness. (C) RUE_{POC} is defined as POC (µmol L^{-1}) per unit TP (µmol L^{-1}). (D) $RUE_{biovolume}$ is defined as algal carbon (µmol L^{-1}, calculated from microscopic counts) per unit TP (µmol L^{-1}).

	Overall regression		Regression parameters		
	r^2	*p*	Coefficient	SE	*p*
(A) ln POC = -5.6+1.3*ln taxon richness+0.24*lnTP	0.51	<0.0001	taxon richness: 1.3	0.28	<0.0001
			TP: 0.24	0.12	0.05
(B) ln wet mass = -4.7+1.0*ln taxon richness+0.38*lnTP	0.29	0.0006	taxon richness: 1.0	0.43	0.02
			TP: 0.38	0.19	0.05
(C) ln $RUE_{(POC)}$ = 1.85+0.9*ln taxon richness	0.1	0.03	taxon richness: 0.9	0.42	0.03
(D) ln $RUE_{(biovolume)}$ = -1.3+1.35*ln taxon richness	0.14	0.01	taxon richness: 1.35	0.5	0.01

Figure legends

Figure 1:

A: Relationship between taxon richness (no. of phytoplankton taxa) and pigment richness (no. of photosynthetically active pigments) in assembled (filled circles) and natural (open circles) algal communities. Linear regression statistics are (i) assembled communities: pigment richness = 8.49+3.63*ln taxon richness; r^2=0.69; p<0.0001; n=18; (ii) natural communities: pigment richness = 9.2+2.69*ln taxon richness; r^2=0.17; p=0.0045; n=45 [outlier (= open triangle) excluded]; (iii) assembled and natural communities combined: pigment richness = 9.43+2.66*ln taxon richness; r^2=0.68; p<0.0001; n=63 [outlier (= open triangle) excluded]. Relationships are similar and statistically significant whether the outlier is excluded or not. Shown is the common regression line for the combined assembled and natural communities.

B: Same relationship as in A, but with taxon richness on an arithmetic scale and a nonlinear curve fit to the combined data from assembled and natural communities. Regression statistics are: pigment richness = 19.1*taxon richness/(1.5+ taxon richness); r^2=0.68; p<0.0001 for both coefficients; n=63 [outlier (= open triangle) excluded]. The relationship is similar and statistically significant whether the outlier is excluded or not.

C: Relationship between taxon richness and biomass-specific average PAR absorbance (per nm and mg C L^{-1}) of assembled communities. Linear regression statistics are: average absorbance = 0.048+0.016*ln taxon richness; r^2=0.22; p=0.0085; n=30.

D: Relationship between taxon richness and particulate organic carbon concentration (POC in mg L^{-1}, black dots and solid regression line) and phytoplankton wet mass concentration (in mg L^{-1}, open dots and dashed regression line) of assembled communities after two weeks of incubation. Linear regression statistics are: (i) lnPOC = 0.26+0.24*ln taxon richness; r^2=0.37; p=0.0004; n=30; (ii) ln wet mass = 1.58+0.5*ln taxon richness; r^2=0.27; p=0.0009; n=37.

E: Relationship between taxon richness and the selection effect (expressed in mg phytoplankton wet mass L^{-1}) in assembled communities. Linear regression statistics are: selection effect = -1.5+1.13*ln taxon richness; r^2=0.03; *p*=0.48; n=20.

F: Relationship between taxon richness and the complementarity effect (expressed in mg phytoplankton wet mass L^{-1}) in assembled communities. Linear regression statistics are: complementarity effect = -5.6+6.1*ln taxon richness; r^2=0.25; *p*=0.03; n=20.

Figure 2:

Relationship between taxon richness and specific net primary production (sNPP in µg C mg $C^{-1}h^{-1}$) of assembled algal communities during short-term incubations. Linear regression statistics are: sNPP = 1.4+0.99*ln taxon richness; r^2=0.19; *p*=0.03; n=25.

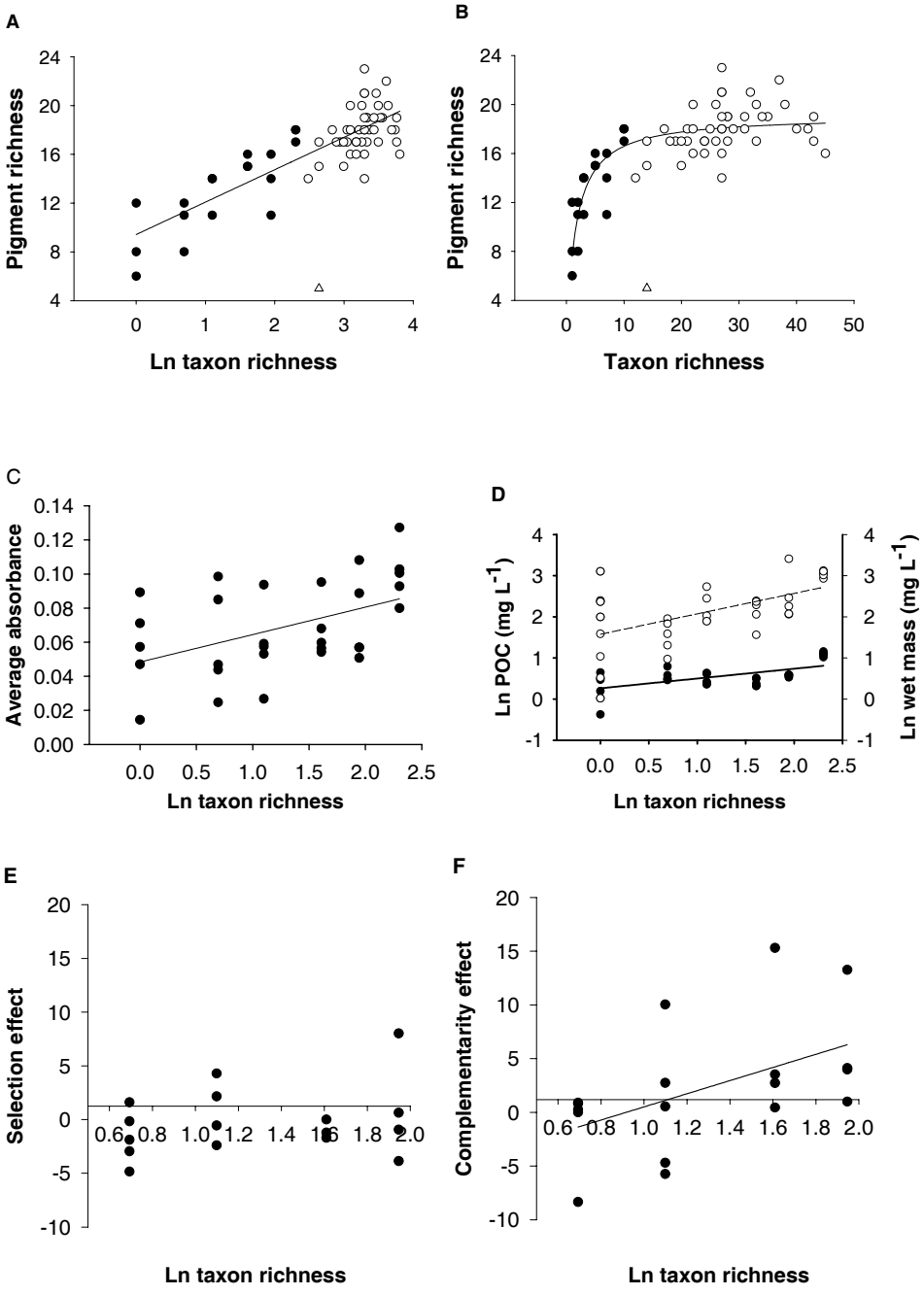

A
Pigment richness
Ln taxon richness
B
Pigment richness
Taxon richness
C
Average absorbance
Ln taxon richness
D
Ln POC (mg L-1)
Ln wet mass (mg L-1)
Ln taxon richness
E
Selection effect
Ln taxon richness
F
Complementarity effect
Ln taxon richness

Figure 1

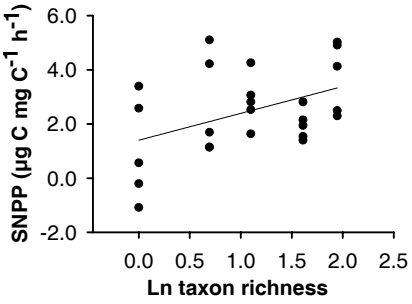

SNPP (µg C mg C-1 h-1)
6.0
4.0
2.0
0.0
-2.0
0.0
0.5
1.0
1.5
2.0
2.5
Ln taxon richness

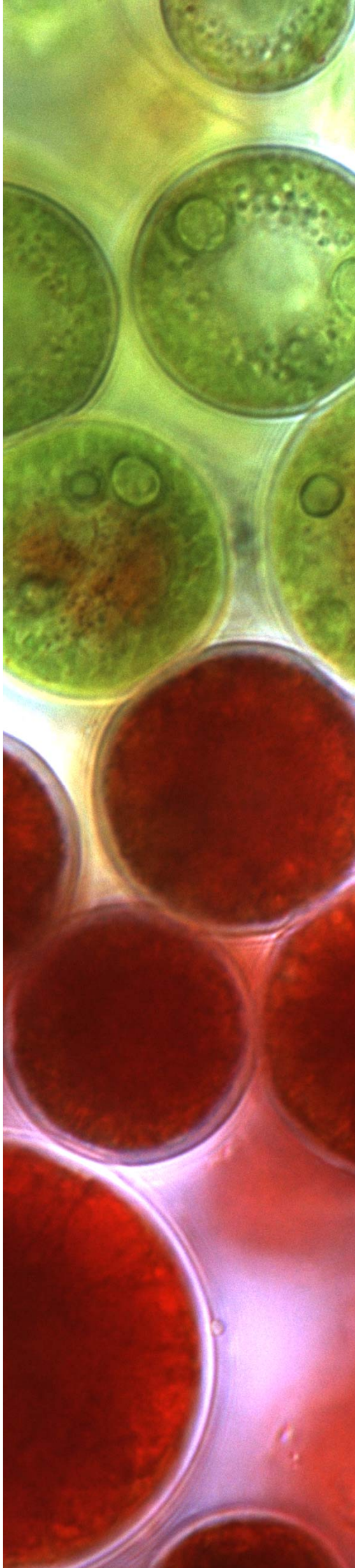
Figure 2

PAPER 2

THE COUPLING OF BIODIVERSITY AND PRODUCTIVITY IN PHYTOPLANKTON COMMUNITIES: CONSEQUENCES FOR BIOMASS STOICHIOMETRY

STRIEBEL, M., S. BEHL, H. STIBOR

SUBMITTED TO *ECOLOGY*



Abstract

There is widespread concern that loss of biodiversity can influence important ecosystem services. A positive relationship between diversity and productivity has been observed in investigations of terrestrial and aquatic plant communities. However, an increase in primary production (carbon assimilation) does not necessarily result in higher nutrient uptake by primary producers. There is a loose coupling between carbon assimilation and nutrient uptake in autotrophs, and their biomass carbon-to-nutrient ratios (stoichiometry) are flexible. We performed controlled laboratory experiments to investigate the effect of phytoplankton biodiversity on phytoplankton stoichiometry. Our results indicate that biodiversity influences carbon assimilation and nutrient uptake of phytoplankton communities in different ways, resulting in variations of biomass stoichiometry. Data from 46 lake communities also support this link. Shifts in the biomass stoichiometry of phytoplankton communities are generally attributed to environmental fluctuations in resources. However, our results show that biodiversity is also important in determining their stoichiometry.

Introduction

The rapid loss of the Earth's biodiversity has generated great interest in the influence of diversity on ecosystem processes, and research on diversity–ecosystem functioning relationships now occupies a central position in ecology. Many studies, mostly in terrestrial systems, have investigated the impact of biodiversity on population dynamics and ecosystem functioning (MacArthur 1955, Tilman & Downing 1994). However, even if the ability to make generalizations about biodiversity effects is quite limited, there is a general trend that plant biodiversity can enhance primary production (Giller *et al.* 2004). A diversity–productivity relationship has been supported by theoretical models and by laboratory and field experiments (Tilman *et al.* 1999, Tilman 1999, Tilman *et al.* 2001, Downing & Leibold 2002). Studies that investigate plant diversity–productivity relationships have mainly focused on terrestrial plant communities (Hooper *et al.* 2005). Mechanisms explaining the observed plant diversity–productivity relationships include: complementary use of resources (complementarity effect), facilitation between species in highly diverse communities (facilitation hypotheses) and higher probability that highly diverse communities include a highly productive species (sampling or selection effect). However, the consequences of diversity– productivity relationships for food web dynamics remain mostly unexplored. Primary production determines the amount of organic carbon that is available for higher trophic levels. Bottom-up effects can transfer fluctuations of primary production up to all trophic levels of the food web.

Carbon is the standard unit used to quantify plant biomass production. However, research in the field of ecological stoichiometry shows that not only the carbon content, but also the carbon-to-nutrient ratio (stoichiometry) of plant biomass is an important determent of ecological dynamics (Andersen *et al.* 2004). Most autotrophs seem to be nutrient limited, valuated from their low nutrient quotas (Elser *et al.* 2000). An increase in carbon assimilation does not necessarily imply an increase in nutrient uptake, and the nutrient availability available for autotrophs is normally much lower than the availability of CO_2. Accordingly,

autotrophs often show flexible and generally high carbon-to-nutrient ratios in their biomass. By contrast, herbivores are generally less flexible in their biomass composition and possess lower carbon-to-nutrient ratios than their food. This can result in a mismatch between elemental ratios of resources and consumers (Sterner & Hessen 1994).

Pelagic communities are one of the best-studied examples of how the flexible biomass carbon-to-nutrient content of autotrophs can affect phytoplankton-herbivorous zooplankton dynamics. The ratio of light supply to dissolved phosphorus is a good predictor of phytoplankton biomass, which determines the food quality for herbivorous zooplankton. Herbivorous zooplankton can be either energy (carbon) limited, at low phytoplankton biomass carbon-to-phosphorous (C:P) ratios, or nutrient limited, at high phytoplankton biomass C:P ratios (Urabe & Sterner 1996). In general, phytoplankton biomass C:P ratios above 300 can result in phosphorus limitation of *Daphnia* growth (DeMott *et al.* 2001, DeMott 2003, DeMott *et al.* 2004a, DeMott *et al.* 2004b).

Biodiversity is a good predictor of the resource use efficiency (RUE-the amount of carbon that can be assimilated per unit of limiting nutrient) in phytoplankton communities (Ptacnik *et al.* 2008). We have previously shown that the biodiversity of phytoplankton communities affects their carbon assimilation positively, the underlying mechanism most likely being complementarity in the use of light (Striebel *et al.* 2008a). Variations in phytoplankton biodiversity could therefore result in variations in biomass carbon-to-nutrient ratios.

We hypothesize that not only shifts in the supply ratios of light and nutrients, but also phytoplankton biodiversity affects phytoplankton stoichiometry. The following chain of arguments explains our reasoning:

First, phytoplankton biodiversity and biomass-specific carbon production are positively linked. Therefore, the biomass carbon content of phytoplankton communities can increase with biodiversity. Secondly, the effect of biodiversity on nutrient uptake is different from the effect of biodiversity on carbon assimilation. Nutrients (such as phosphorus) are often scarce and

limit phytoplankton growth. Further, most of the nutrients are bound in biomass and no longer available in a dissolved form. Therefore, an increase in carbon assimilation is not necessarily accompanied by a comparable increase in nutrient uptake of phytoplankton.

It is impossible to control biodiversity of phytoplankton communities in field experiments, making it difficult to separate diversity effects from correlating environmental factors. Therefore, we investigated the possible relationship between biodiversity, productivity and stoichiometry in phytoplankton communities within diversity-controlled laboratory experiments. We established different phytoplankton diversity levels and performed growth experiments at high and low phosphorus concentrations. To include natural phytoplankton communities with shared evolutionary histories we also performed field experiments with natural phytoplankton communities from 46 lakes.

Methods

Laboratory experiment

We did experiments with artificial algal communities at low (0.1 µMol P L^{-1}) and high (50 µMol P L^{-1}) phosphorus concentrations that were supplemented to phosphorus-free algal growth medium (WC medium (Guillard & Lorenzen 1972)). To establish algal communities with different levels of diversity (taxon richness ranging from 1–10 species), we used the following algal strains (*Chlorella* sp., *Chlamydomonas* sp., *Cryptomonas* sp., *Monoraphidium* sp., *Scenedesmus* sp., *Selenastrum* sp., *Microcystis* sp., *Synechococcus* sp., *Fragilaria crotonensis*, *Asterionella* sp., *Staurastrum tetracerum*, *Peridinium* sp.), representing the major classes of freshwater phytoplankton. To avoid confounding effects of different growth media, we precultured all of the above-mentioned strains in monoculture in a common growth medium (standard WC medium) over a period of several months prior to the experiments. We established six diversity levels (taxon richness 1, 2, 3, 5, 7, 10) with low phosphorus supplementation and five diversity levels (taxon richness 1, 2, 3, 5, 7) with high phosphorus supplementation. Each was replicated five times with randomly chosen phytoplankton species, resulting in a total of 30 and 25 different experimental phytoplankton communities with low and high phosphorus supplementation, respectively. All treatments started with the same initial total algal biovolume ($2*10^6$ fl ml^{-1}). The experiments were arranged as semibatch cultures (10% exchange day^{-1}) in 1 L translucent polyethylene flasks. They were kept at 20 °C for two weeks and exposed to a continuous irradiance of 100 µmol quanta of photosynthetic active radiation (PAR, m^{-2} s^{-1}).

Field experiment

We took depth-integrated phytoplankton samples from 46 lakes in southern Germany and Austria in September 2004. We filtered them through a 200 µm mesh nylon screen to remove mesozooplankton. We filled 1 L translucent polyethylene flasks with these samples and

submerged them in Lake Brunnensee at a depth of one meter to ensure equal temperature and light conditions for phytoplankton growth. The total time from collection to submersion was within 4 hours. The experiment lasted for seven days.

Measurements

Phytoplankton particulate organic carbon (POC), particulate phosphorus (PP) and total phosphorus (TP) were analyzed at the start of the experiments and after one week of incubation for field experiments and two weeks of incubation for laboratory experiments. To determine POC and PP, we filtered water from each sample onto precombusted and acid-washed glass-fiber filters (Whatman GF/F). POC was measured with an Elemental Analyzer (CE Instruments, Milan, Italy) and PP was measured after sulfuric acid digestion followed by molybdate reaction. Total phosphorus was quantified by persulfate digestion followed by molybdate reaction.

Phytoplankton taxon richness of the natural phytoplankton communities was determined at the start of the experiment by counting the number of algal species from samples fixed with Lugol's iodine in an inverted microscope using Utermöhl chambers (Utermöhl 1958). We counted at least 100 units (cells or colonies) of every species by scanning a minimum of two perpendicular transects or 20 distinct fields randomly distributed on two such transects to keep the counting error at <10% (Lund *et al.* 1958).

RUE, resource assimilation efficiency and biomass C:P ratios

RUE of the natural phytoplankton communities was determined after seven days of incubation, following Ptacnik et al. (2008), by calculating the amount of phytoplankton POC (in µmol L^{-1}) per unit TP (in µmol P L^{-1}). The resource assimilation efficiency (RAE) was calculated by determining phytoplankton PP (in µmol L^{-1}) per unit TP (in µmol P L^{-1}) and plotted against taxon richness. To determine the impact of taxon richness on biomass molar

C:P ratios, we used the residuals from the linear regression of phytoplankton biomass C:P ratios against the amount of TP in the environment. We plotted the residuals against taxon richness to analyze whether the deviations between the measured and the calculated (regression-based) C:P ratios were a function of taxon richness.

Results

Laboratory experiments

POC content of phytoplankton biomass ranged from 0.7–3.2 mg C L^{-1} in the samples with low phosphorus supplementation and from 1.0–15.4 mg C L^{-1} in the samples with high phosphorus supplementation. At both phosphorus concentrations, POC significantly increased with higher taxon richness (p<0.001; Figs. 1A and 2A).

The mean concentration of PP in algal biomass was about 11.2 µg P L^{-1} (SE=2.2) in samples with low phosphorus supplementation and about 137.8 µg P L^{-1} (SE=41.7) in samples with high phosphorus supplementation. In contrast to carbon assimilation, phosphorus incorporation increased with higher taxon richness only at low phosphorus supplementation (p<0.05; Figs. 1B and 2B).

The molar C:P ratios ranged from 189–754 in treatments with low phosphorus supplementation and from 25–384 in treatments with high phosphorus supplementation. At both phosphorus concentrations, the C:P ratios increased significantly with higher taxon richness (p≤0.05; Figs. 1C and 2C).

The two-way ANOVA of the laboratory experiments showed statistically significant effects of both, taxon richness (p<0.001) and phosphorus concentration (p<0.001) on the algal biomass C:P ratios (Table 1). No interaction between the effects of taxon richness and the phosphorus level existed (p=0.18).

Field experiment

RUE of various natural phytoplankton communities increased with higher taxon richness (Fig. 3A, p=0.06). RAE, on the other hand, did not increase with higher taxon richness (Fig. 3B, p=0.9). Biomass C:P ratios decreased with increasing TP concentrations (ln C:P ratio=6.05-0.008*TP; r^2=0.1; p<0.05). The residuals of the linear regression between TP and molar C:P ratios were influenced by the taxon richness of the algal communities: at low taxon richness the residuals were mostly negative, meaning that measured C:P ratios were lower

than expected, while at high taxon richness the residuals became mostly positive, meaning that measured C:P ratios were higher as expected (Fig. 3C).

Discussion

Biodiversity, productivity and phytoplankton stoichiometry

An increasing number of empirical and experimental studies provide evidence that the diversity of plants can influence primary production (Tilman *et al.* 1999, Tilman 1999, Tilman *et al.* 2001, Downing & Leibold 2002). Effects of biodiversity on primary production could also have consequences beyond productivity. Primary productivity is a determinant of various ecosystem characteristics, such as food web efficiency and the length of food chains (Begon *et al.* 1990). Recently, it has become clear that qualitative (stoichiometry) as well as quantitative (carbon) aspects have to be considered when evaluating productivity–ecosystem functioning relations (Andersen *et al.* 2004). While carbon is very seldom a limiting element for primary production, key nutrients such as phosphorus and nitrogen often limit autotroph production (Hecky & Kilham 1988, Vitousek & Howarth 1991). If carbon assimilation and uptake of nutrients are influenced by biodiversity to differing extents, the biomass carbon-to-nutrient ratio can also vary with biodiversity.

In pelagic systems, changes in phytoplankton stoichiometry are commonly explained by fluctuations in the light-to-nutrient ratio (Urabe & Sterner 1996, Sterner *et al.* 1997, Hessen *et al.* 2002). These fluctuations can affect the stoichiometry of single phytoplankton species and, as a consequence, also phytoplankton communities. We can exclude resource fluctuations as reasons for changes in phytoplankton stoichiometry because we exposed the different phytoplankton communities to constant light-to-nutrient conditions. Hence, changes in phytoplankton stoichiometry can only occur if the RUE between these phytoplankton communities differs. Higher RUEs in highly diverse phytoplankton communities are founded on species-specific traits of the constituent members. We have previously shown that photosynthetically active pigments are species-specific traits that determine the phytoplankton community's response to light (Striebel *et al.* 2008a). The pigmental constitutions of the various phytoplankton species determine which part of the PAR spectrum

can be used for photosynthesis. A highly diverse pigmental composition of a phytoplankton community will therefore allow a more effective utilization of the PAR spectrum (Barsanti & Gualtieri 2006, Stomp *et al.* 2007a, Stomp *et al.* 2007b). The pigment diversity of phytoplankton communities is related to their taxon richness (Striebel *et al.* 2008a). However, while biodiversity-dependent pigment composition influences carbon assimilation, pigments are not directly involved in nutrient uptake. Therefore, we expect phytoplankton stoichiometric shifts within phytoplankton communities not only along gradients of light-to-nutrient ratios, but also along a gradient of biodiversity.

Studies of natural phytoplankton communities from three American (Dickman *et al.* 2006) and six European (Striebel *et al.* 2008b) lakes showed a relationship between the biomass C:P ratio of natural phytoplankton communities and their biodiversity. However, these studies lack an explanation for the relation. Our laboratory results bring clear evidence that biodiversity of phytoplankton communities can influence their carbon assimilation and thereby their biomass stoichiometry. Highly diverse communities had a higher carbon content per unit of limiting nutrient in their biomass (biomass C:P ratios). Biodiversity-mediated shifts of biomass carbon-to-nutrient ratios occurred at both, low and high phosphorus availability. We found a non-significant interaction between the effects of phytoplankton diversity and phosphorus availability on biomass C:P ratios.

Another possible mechanism for how diversity can influence phytoplankton stoichiometry is a sampling effect, where the chance that a highly productive species dominates is greater in highly diverse communities. However, partitioning of the net biodiversity effect (Loreau & Hector 2001) on carbon assimilation within laboratory phytoplankton communities has indicated that complementarity effects are always positive and a function of diversity, whereas selection (species) effects are not related to diversity and are similar across all diversity treatments (Striebel *et al.* 2008a). We therefore conclude that a link between biodiversity and carbon assimilation underlies the positive effect between biodiversity and C:P ratios.

C:P ratios and phytoplankton food quality

Biodiversity-mediated shifts in the biomass stoichiometry of our laboratory communities were in a range that affects zooplankton growth. Several laboratory and field experiments have shown that variations in phytoplankton C:P ratios comparable to our results would have measurable consequences on zooplankton dynamics (Sterner *et al.* 1998, Urabe *et al.* 2002a, Urabe *et al.* 2002b, Striebel *et al.* 2008b). Observed algal C:P ratios in our laboratory experiments showed that phytoplankton food quality shifted along the gradient of biodiversity, from energy limitation (C:P<300) to nutrient limitation (C:P>300), for herbivorous zooplankton (*Daphnia*) growth (Urabe & Watanabe 1992).

Our field experiments showed that diversity influenced RUE of phytoplankton communities. The increase of carbon uptake per unit phosphorus was in a comparable range to data from a study including a multitude of Scandinavian lakes (Ptacnik *et al.* 2008). However, biodiversity was not linked to a comparable (or significant) shift in the uptake efficiency of phosphorus; the uptake of phosphorus per unit TP in the environment was similar across all lake communities. This resulted in biodiversity-dependent changes in the C:P ratio. Higher taxon richness resulted in higher biomass C:P ratios than predicted from the total amount of phosphorus in the environment. Phosphorus and the availability of light determine the physiologically possible C:P ratios for a phytoplankton community, while taxon richness determines whether this ratio is near the upper or the lower limit of this range.

Implications of biodiversity–stoichiometry relationships for food web dynamics

High quantities of resources can result in fast growth rates of consumer populations, leading to population fluctuations near environmental capacities (Begon *et al.* 1990). Such fluctuations can produce instabilities within predator–prey relationships (Diehl 2007). However, in the presence of decreasing food quality (such as observed in our experiments), high food quantity will not necessarily result in high population growth rates of consumers. Food quantity of primary producers may frequently be inversely related to food quality, since

high biomass and low turnover commonly yield autotroph biomass with suboptimal nutrient content. Biodiversity-mediated shifts in the food quality of primary producers could therefore also result in a stabilization of autotroph–herbivore systems. Theoretical calculations of phytoplankton–zooplankton dynamics, parameterized within an ecologically meaningful frame, show that shifts in phytoplankton C:P ratios, similar to those observed in our experiments, can have consequences for the stability of phytoplankton-zooplankton interactions (Andersen *et al.* 2004).

Additionally, highly diverse phytoplankton communities with higher biomass C:P ratios could also influence nutrient recycling by zooplankton. Zooplankton feeding on phytoplankton communities with high biomass C:P ratios have to extract as much phosphorus as possible. This will reduce the release rate of phosphorus by zooplankton and diminish the recycling of phosphorus in the lower pelagic food web.

Biodiversity effects on autotroph biomass stoichiometry will have far-reaching consequences, including effects on herbivores and on nutrient cycling within food webs. These examples demonstrate that, in addition to investigations of important quantitative effects of autotroph biodiversity on productivity, the almost unexplored stoichiometric effects need more study across a broad range of autotroph–herbivore systems.

Acknowledgments

This study was supported by funding from Deutsche Forschungsgemeinschaft (STI 180/2-1). We thank Margit Feißel, Angelika Wild and Achim Weigert for technical support during the experiments.

Literature cited

Andersen, T., J. J. Elser, and D. O. Hessen, 2004. Stoichiometry and population dynamics. Ecology Letters **7**:884-900.

Barsanti L., and P. Gualtieri. 2006. Algae: Anatomy, Biochemistry, and Biotechnology. CRC Press Taylor & Francis Group, Boca Raton.

Begon M., J. L. Harper, and C. R. Townsend. 1990. Ecology - Individuals, Populations and Communities., 2nd edition. Blackwell Scientific Publications, Boston.

DeMott, W. R. 2003. Implications of element deficits for zooplankton growth. Hydrobiologia **491**:177-184.

DeMott, W. R., J. R. Edington, and A. J. Tessier. 2004a. Testing zooplankton food limitation across gradients of depth and productivity in small stratified lakes. Limnology and Oceanography **49**:1408-1416.

DeMott, W. R., R. D. Gulati, and E. Van Donk. 2001. Daphnia food limitation in three hypereutrophic Dutch lakes: Evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. Limnology and Oceanography **46**:2054-2060.

DeMott, W. R., B. J. Pape, and A. J. Tessier. 2004b. Patterns and sources of variation in Daphnia phosphorus content in nature. Aquatic Ecology **38**:433-440.

Dickman, E. M., M. J. Vanni, and M. J. Horgan. 2006. Interactive effects of light and nutrients on phytoplankton stoichiometry. Oecologia **149**:676-689.

Diehl, S. 2007. Paradoxes of enrichment: Effects of increased light versus nutrient supply on pelagic producer-grazer systems. American Naturalist **169**:E173-E191.

Downing, A. L., and M. A. Leibold. 2002. Ecosystem consequences of species richness and composition in pond food webs. Nature **416**:837-841.

Elser, J. J., W. F. Fagan, R. F. Denno, D. R. Dobberfuhl, A. Folarin, A. Huberty, S. Interlandi, S. S. Kilham, E. McCauley, K. L. Schulz, E. H. Siemann, and R. W. Sterner. 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature **408**:578-580.

Giller, P. S., H. Hillebrand, U. G. Berninger, M. O. Gessner, S. Hawkins, P. Inchausti, C. Inglis, H. Leslie, B. Malmqvist, M. T. Monaghan, P. J. Morin, and G. O'Mullan. 2004. Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. Oikos **104**:423-436.

Guillard, R.R., and C. J. Lorenzen. 1972. Yellow-green algae with chlorophyllide c. Journal of Phycology **8**:10-14.

Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in fresh-water and marine environments—A review of recent-evidence on the effects of enrichment. Limnology and Oceanography **33**:796-822.

Hessen, D. O., P. J. Faerovig, and T. Andersen. 2002. Light, nutrients, and P : C ratios in algae: Grazer performance related to food quality and quantity. Ecology **83**:1886-1898.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setala, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs **75**:3-35.

Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature **413**:72-76.

Lund, J. W. G., G. Kipling, and E. D. Le Creen. 1958. The inverted microscope method of estimating algae numbers and the statistical basis of estimation by counting. Hydrobiologia **11**:143-170.

MacArthur, R. 1955. Fluctuations of animal populations, and a measure of community stability. Ecology **36**:533-536.

Ptacnik, R., A. G. Solimini, T. Andersen, T. Tamminen, P. Brettum, L. Lepistö, E. Willén, and S. Rekolainen. 2008. Diversity predicts stability and resource use efficiency in natural phytoplankton communities. Proceedings of the National Academy of Sciences of the United States of America **105**:5134-5138.

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

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. American Naturalist **150**:663-684.

Sterner, R. W., and D. O. Hesse. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics **25**:1-29.

Stomp, M., J. Huisman, L. J. Stal, and H. C. P. Matthijs. 2007a. Colorful niches of phototrophic microorganisms shaped by vibrations of the water molecule. ISME Journal **1**:271-282.

Stomp, M., J. Huisman, L. Voros, F. R. Pick, M. Laamanen, T. Haverkamp, and L. J. Stal. 2007b. Colourful coexistence of red and green picocyanobacteria in lakes and seas. Ecology Letters **10**:290-298.

Striebel M., Behl S., Diehl S. & Stibor H. Colorful niches link biodiversity to carbon dynamics in pelagic ecosystems. 2008a. Submitted.

Striebel, M., G. Spörl, and H. Stibor. 2008b. Light-induced changes of plankton growth and stoichiometry: Experiments with natural phytoplankton communities. Limnology and Oceanography **53**:513-522.

Tilman, D. 1999. Ecology - Diversity and production in European grasslands. Science **286**:1099-1100.

Tilman, D., and J. A. Downing. 1994. Biodiversity and stability in grasslands. Nature **367**:363-365.

Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. Science **294**:843-845.

Tilman, E. A., D. Tilman, M. J. Crawley, and A. E. Johnston. 1999. Biological weed control via nutrient competition: Potassium limitation of dandelions. Ecological Applications **9**:103-111.

Urabe, J., J. J. Elser, M. Kyle, T. Yoshida, T. Sekino, and Z. Kawabata. 2002a. Herbivorous animals can mitigate unfavourable ratios of energy and material supplies by enhancing nutrient recycling. Ecology Letters **5**:177-185.

Urabe, J., M. Kyle, W. Makino, T. Yoshida, T. Andersen, and J. J. Elser. 2002b. Reduced light increases herbivore production due to stoichiometric effects of light/nutrient balance. Ecology **83**:619-627.

Urabe, J., and R. W. Sterner. 1996. Regulation of herbivore growth by the balance of light and nutrients. Proceedings of the National Academy of Sciences of the United States of America **93**:8465-8469.

Urabe, J., and Y. Watanabe. 1992. Possibility of N-limitation or P-limitation for planktonic cladocerans: an experimental test. Limnology and Oceanography **37**:244-251.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt.Int.Ver.Limnol. **9**:1-38.

Vitousek, P. M., and R. W. Howart. 1991. Nitrogen limitation on land and in the sea-how can it occur. Biogeochemistry **13**:87-115.

Table 1:

Results from 2-way ANOVA for biomass carbon to phosphorus (C:P) ratio from laboratory experiments.

Dependent Variable	Source of variation					
	Phosphorus concentration		Taxon richness		Interaction	
C:P	$F_{1,41}$=90.8	p <0.001	$F_{5,41}$=9.3	p<0.001	$F_{4,41}$=1.7	p=0.18

Figure Legends

Figure 1: Data from laboratory experiments with low (0.1 µMol) phosphorus supplementation.

A: Relationship between taxon richness and particulate organic carbon (POC) content of phytoplankton communities (POC=1.27+0.45*ln taxon richness; r^2=0.38; p=0.0003).

B: Relationship between taxon richness and particulate phosphorus (PP) of phytoplankton communities (PP=9.9+1.0*ln taxon richness; r^2=0.14; p=0.047).

C: Relationship between taxon richness and molar biomass carbon-to-phosphorus (C:P) ratio (C:P ratio=352.9+61.7*ln taxon richness; r^2=0.13; p=0.05) of phytoplankton communities. Linear regressions are displayed as solid lines when statistically significant ($p \leq 0.05$).

Figure 2: Data from laboratory experiments with high (50 µmMol) phosphorus supplementation.

A: Relationship between taxon richness and particulate organic carbon (POC) content of phytoplankton communities (POC=4.2+3.4*ln taxon richness; r^2=0.4; p=0.0009).

B: Relationship between taxon richness and particulate phosphorus (PP) of phytoplankton communities (PP=119.5+17.2*ln taxon richness; r^2=0.09; p=0.16).

C: Relationship between taxon richness and molar biomass carbon-to-phosphorus (C:P) ratios (C:P ratio=99.5+49.2*ln taxon richness; r^2=0.19; p=0.04) of phytoplankton communities. Linear regressions are displayed as solid lines when statistically significant ($p \leq 0.05$).

Figure 3: Data from field experiment with natural phytoplankton communities.

A: Relationship between taxon richness and resource use efficiency (RUE) of phytoplankton communities (ln RUE=3.3+0.58*ln taxon richness; r^2=0.08; p=0.06).

B: relationship between taxon richness and resource assimilation efficiency (RAE) of phytoplankton communities (ln RAE=-0.8+0.02*ln taxon richness; r^2=0.0003; p=0.9).
C: Relationship between taxon richness and residuals of the linear regression between TP and ln biomass carbon-to-phosphorus (C:P) ratio of phytoplankton communities (residuals=-2.05+0.63*ln taxon richness; r^2=0.13; p=0.016; linear regression without outlier: residuals=-1.59+0.5*ln taxon richness; r^2=0.16; p=0.007). Linear regressions are displayed as solid lines when statistically significant ($p \leq 0.05$) and as dashed lines when marginally significant ($p < 0.1$).

Figures

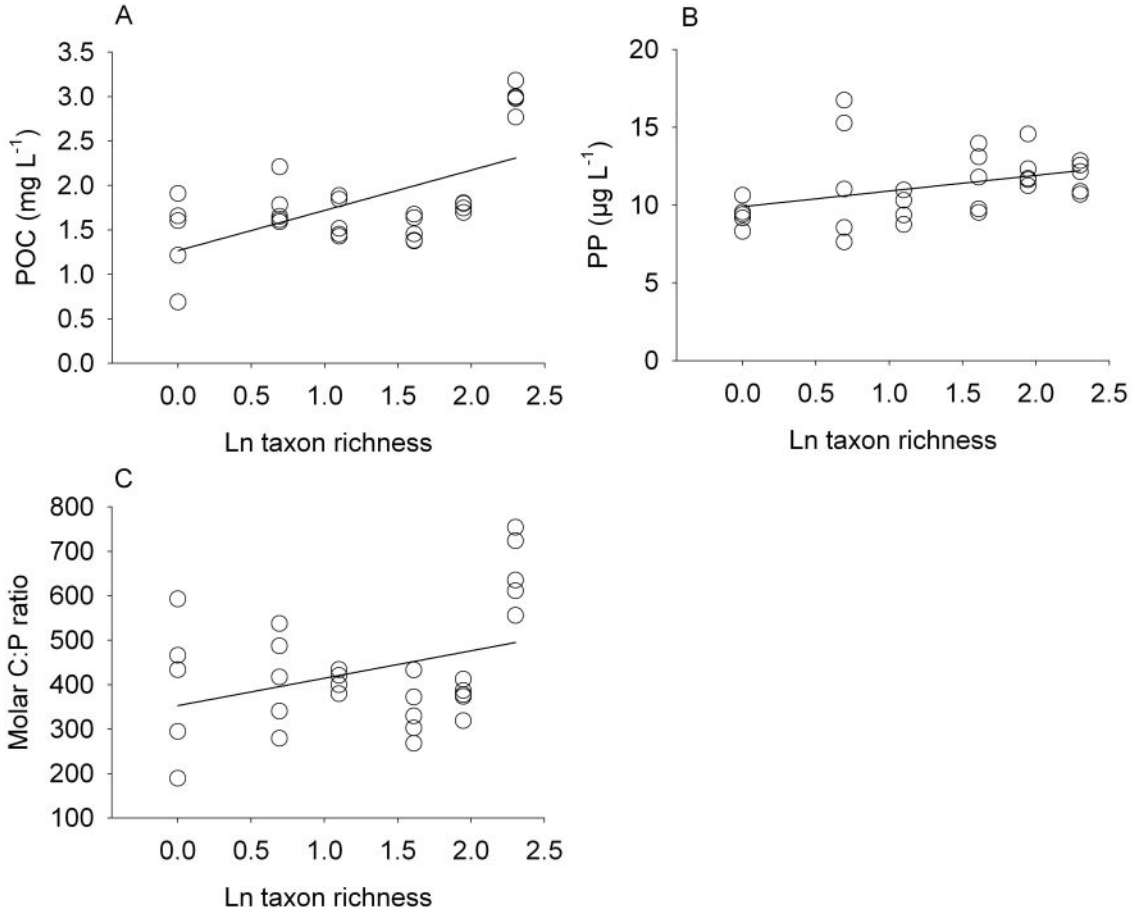


Figure 1

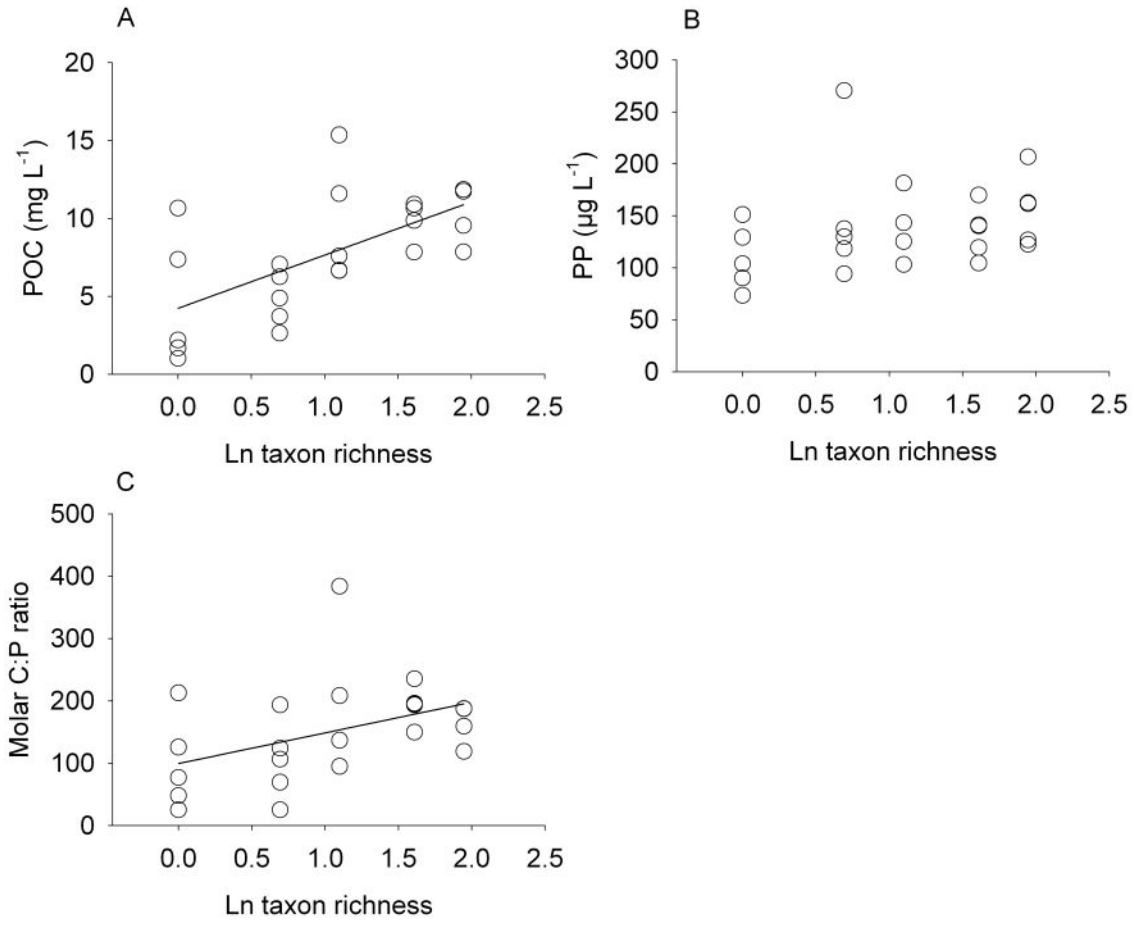

A
POC (mg L-1)
20
15
10
5
0
0.0
0.5
1.0
1.5
2.0
2.5
Ln taxon richness
B
PP (µg L-1)
300
250
200
150
100
50
0
0.0
0.5
1.0
1.5
2.0
2.5
Ln taxon richness
C
Molar C:P ratio
500
400
300
200
100
0
0.0
0.5
1.0
1.5
2.0
2.5
Ln taxon richness

Figure 2

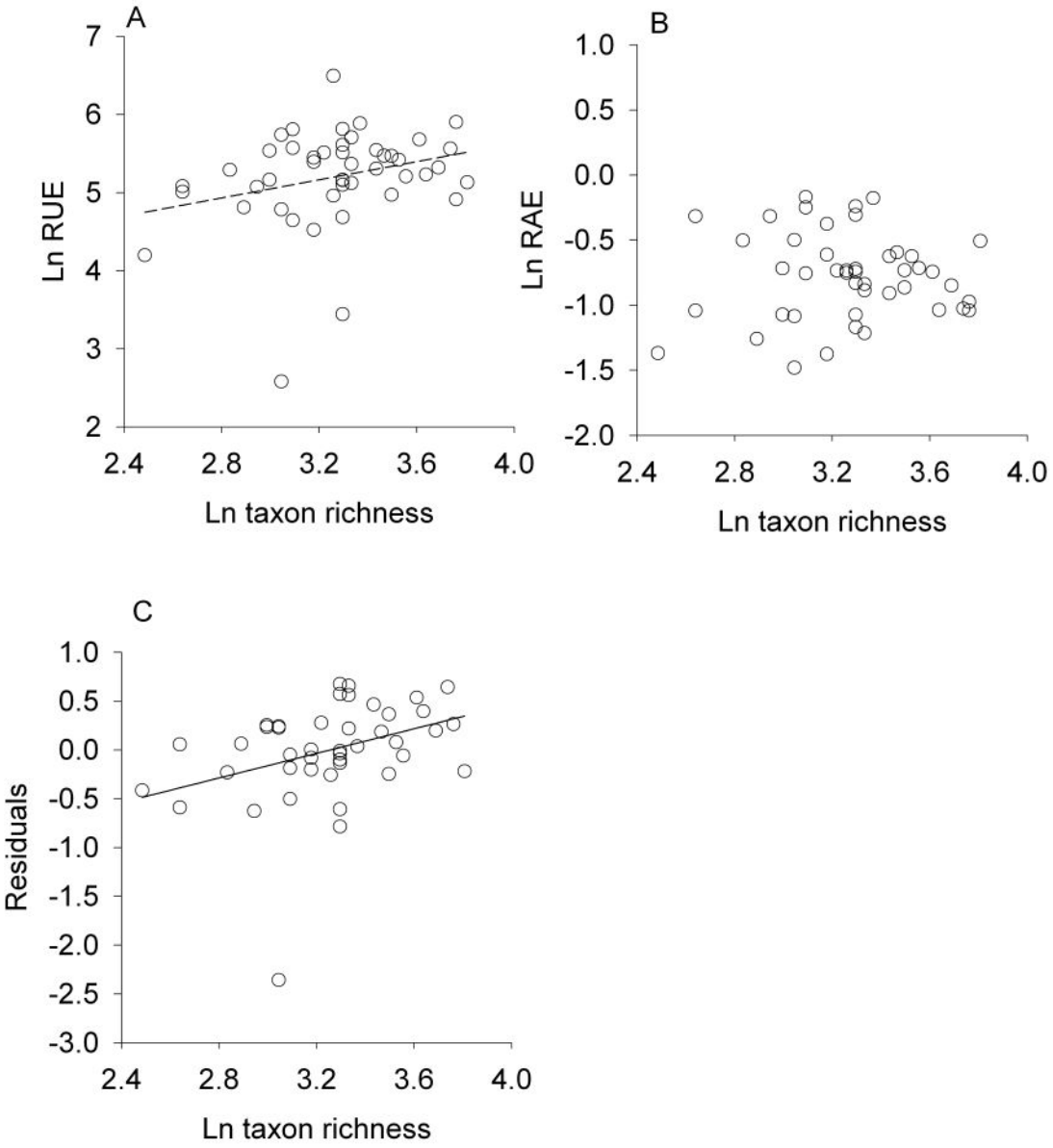

A
Ln RUE
7
6
5
4
3
2
2.4
2.8
3.2
3.6
4.0
Ln taxon richness
B
Ln RAE
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
2.4
2.8
3.2
3.6
4.0
Ln taxon richness
C
Residuals
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
2.4
2.8
3.2
3.6
4.0
Ln taxon richness

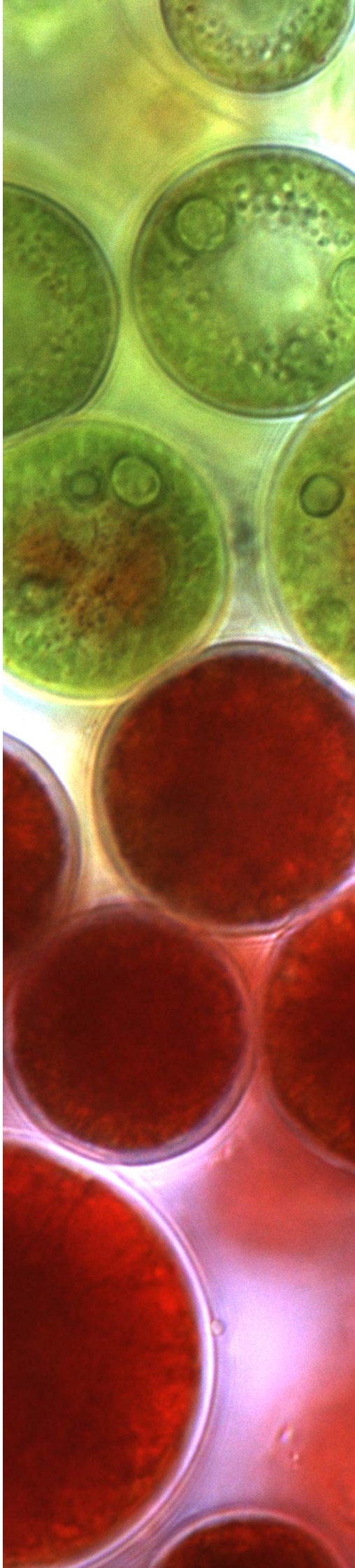
Figure 3

PAPER 3

LIGHT INDUCED CHANGES OF PLANKTON GROWTH AND STOICHIOMETRY: EXPERIMENTS WITH NATURAL PHYTOPLANKTON COMMUNITIES

STRIEBEL, M., G. SPÖRL, H. STIBOR. 2008.

LIMNOLOGY AND OCEANOGRAPHY. 53(2): 513-522.



Abstract

Both low and high rates of light supply can restrict herbivore growth rates by limiting either the quantity (photosynthetically fixed carbon) or the nutritional quality (nutrient content per fixed carbon) of the herbivores' food. The 'light-nutrient hypothesis' therefore predicts that, if phosphorus supply is sufficiently low, production of herbivorous zooplankton should be unimodally related to light intensity. We manipulated the light regime of six different algal communities in a field experiment and investigated the effect of these manipulations on *Daphnia* growth. The algal communities came from six lakes having different total phosphorus concentrations ranging from oligotrophic to eutrophic. Seston carbon and seston carbon: phosphorus ratios in communities from oligotrophic and mesotrophic lakes increased with higher light availability. Across all lakes, the strength of these responses was related to algal diversity. More diverse algal communities showed a stronger increase in both their carbon biomass and their C:P ratio with increasing light than less diverse communities. Furthermore, in oligotrophic and mesotrophic treatments *Daphnia* growth was highest at intermediate light intensities. In contrast, seston parameters and *Daphnia* growth were only weakly related to light supply in communities from eutrophic lakes.

Introduction

In pelagic ecosystems, primary production is determined by the supply with light and dissolved mineral nutrients. In lakes, the nutrient limiting primary production is often phosphorus (P) (Vollenweider 1976). The loose coupling between algal nutrient uptake and photosynthesis allows for highly flexible C:P ratios of algal biomass. It is therefore common that the P content of algal biomass relative to carbon (C) fixed by photosynthesis decreases with increasing light input (Sterner et al. 1997, Diehl et al. 2005, Berger et al. 2006). In contrast, the elemental composition of herbivorous zooplankton is largely homeostatically regulated (Andersen and Hessen 1991, Main et al. 1997, Elser et al. 2000). Zooplankton with high specific growth rates, such as *Daphnia,* tend to have a high body phosphorus content and, therefore, a C:P ratio that is considerably lower than that of phytoplankton (Elser et al. 1996, Main et al. 1997, Weider et al. 2004). If this mismatch in the elemental composition between autotrophs and herbivores becomes sufficiently strong, herbivore growth may become limited by the nutrient rather than by the carbon content of their food. For example, *Daphnia* growth has been reported to be limited by P at molar seston C:P ratios above 300 (Hessen 1992, Urabe and Watanabe 1992, Urabe et al. 2002*a*) whereas only weak P-limitation and stronger energy limitation is usually observed at molar seston C:P ratios below 300 (DeMott and Tessier 2002, DeMott et al. 2004).

The degree of mismatch in the elemental composition between autotrophs and herbivores has implications for the efficiency with which biomass and energy are transferred up the food chain, summarized in the 'light-nutrient hypothesis' (Sterner et al. 1997). In short, while increased light supply usually promotes phytoplankton growth, the resulting increase in primary production can only be fully transferred to the herbivore level if herbivore growth is predominantly carbon (energy) limited. In contrast, if herbivore growth is predominantly nutrient limited, increased light supply may actually decrease herbivore production, because any light-induced increase in food quantity may be offset by a disproportional decrease in the

food's nutrient content (Andersen et al. 2004, Diehl 2007). The latter phenomenon has been termed the 'paradox of energy enrichment' (Loladze et al. 2000) and has been observed in several laboratory experiments with *Daphnia* and monocultures of chlorophytes (Urabe and Sterner 1996, Sterner et al. 1998, Urabe et al. 2002*a*). Still, to date there have been only very few attempts to investigate experimentally how widely applicable the light-nutrient hypothesis (LNH) is to the description of natural phytoplankton-zooplankton-interactions with diverse algal communities.

To our knowledge there is only one field experiment to date (Urabe et al. 2002*b*) that investigated the response of the plankton community of an oligotrophic lake to the factorial manipulation of light (shading) and nutrients (P-enrichment) in field mesocosms. These manipulations had major effects on seston C:P stoichiometry and on zooplankton production and growth over the four week experiment, consistent with the predictions of the LNH. However, the short-term response of an algal community to an experimental manipulation may be constrained by its initial characteristics, such as species richness and taxonomic composition.

Here, we report from a field study in which we exposed different natural algal communities to a gradient of light intensities. The algal communities originated from six lakes chosen along a gradient of total phosphorus (TP) concentration. By using different natural algal communities, we furthermore assured realistic species combinations and algal communities with shared evolutionary histories as experimental systems. The LNH predicts a unimodal relation between light intensity and *Daphnia* growth over some range of the light-nutrient supply space. Such a response can only be captured with a gradient design. We therefore exposed the algal communities and the herbivores to a gradient of five light levels. We investigated how the different algal communities responded to light manipulations and to which extent these responses and subsequent effects of the light manipulations on *Daphnia* growth could be described by the LNH and other, alternative or complementary, mechanisms.

Material and methods

Algal communities

We exposed natural algal communities originating from six lakes in Bavaria covering a broad range of nutrient conditions (Table 1) to different light intensities in outdoor mesocosms. Based on total phosphorus concentration, Lake Förchensee and Lake Brunnensee can be defined as oligotrophic, Lake Klostersee and Lake Langbürgenersee as mesotrophic, and Lake Thalersee and Lake Bansee as eutrophic lakes. All lakes are located near the Limnological Research Station of the University of Munich at Seeon (Bavaria, Southern Germany).

Experimental design

On 26 August 2004, we took from each lake a pooled sample of the epi- and hypolimnion and filtered it through a 224-µm mesh nylon screen to remove mesozooplankton. The samples were immediately transferred into rectangular 20-liter mesocosms of clear polyethylene. To ensure equal temperature and light conditions, all mesocosms were exposed in Lake Brunnensee at a water depth of one meter. At that depth, water temperature was about 21°C during the experiment, which lasted from 26 August to 23 September 2006. We wrapped the mesocosms with one or more layers of white PE-foil (Renoplan foil) to establish the following light gradient for each algal community: 90%, 70%, 45%, 25%, and 5% of ambient light. Each treatment was replicated twice (yielding a total of 60 mesocosms). Light intensity one meter below water surface at noon ranged between 150 µmol quanta (m^{-2} s^{-1}) on cloudy days and 1400 µmol quanta (m^{-2} s^{-1}) on clear, sunny days. The mesocosms were exposed for two weeks to allow the algal communities to respond to the different light intensities in the absence of grazing from mesozooplankton. After these two weeks, we stocked juvenile *Daphnia magna* (one individual $liter^{-1}$) into the mesocosms and continued the experiment for another two weeks. The juvenile *Daphnia* came from a

synchronized stock culture and were put into the mesocosms 12 hours after they were released from their mothers. We obtained an initial value of *Daphnia* biomass by determining the particular organic carbon (POC) content of an aliquot of the juvenile *Daphnia*.

Sampling and measurement

We took samples from each mesocosm at the beginning and at the end of the experiment and once per week during the experiment. These samples were immediately filtered through a 224 µm mesh nylon screen. We measured particular organic carbon (POC), particular phosphorus (PP), total phosphorus (TP), total algal biovolume, and algal species composition. To estimate POC and PP, we filtered water from each mesocosm onto precombusted and acid-washed glass-fibre filters (Whatman GF/F). POC was determined after filtration and combustion using infrared spectrometry (C-Mat 500, Ströhlein; Korschenbroich, Germany). PP was measured after sulphuric acid digestion followed by molybdate reaction. TP was quantified by persulfate digestion followed by molybdate reaction.

We identified and counted different algal species from samples fixed with Lugol's iodine in an inverted microscope using Utermöhl chambers (Utermöhl 1958). To determine phytoplankton diversity of the initial samples from the six algal communities, the entire counting chamber was screened and each algal species occurring in these samples was recorded. If present, at least 400 units (cells or colonies) of each species were counted to keep the counting error at <10% (Lund et al. 1958). Because the total number of counted algal units differed among the six lakes, we conducted a rarefaction analysis to check the robustness of our diversity measures. The results of this analysis showed that our diversity estimates were robust against sampling efforts. In the samples from weeks two and four, we counted at least 400 units (cells or colonies) of every abundant species by scanning a minimum of two perpendicular transects or 20 distinct fields randomly distributed on two such transects. Biovolume of the different algal species was calculated according to Hillebrand et

al. (1999). The taxonomic composition of an algal community may affect its quality as food for *Daphnia*. While some species are completely inedible, others can be ingested by *Daphnia* but are digestion resistant. We categorized the algal species in our mesocosms as edible or inedible algae. We defined inedible algae as those, which, according to literature, either can not be easily ingested or digested by *Daphnia*. According to these criteria, we assumed that *Cyanophyceae*, large diatoms, defended (spiny) algae and filamentous or gelatinous green algae were inedible (Burns 1968, Burns et al. 1989, DeMott et al. 2001).

At the end of the experiment, we counted the *Daphnia* in each mesocosm and determined their carbon biomass by using infrared spectrometry after combustion. We calculated the population growth rate (*r*) of *Daphnia* using the following equation:

$$r = \left(\frac{\ln N_{End} - \ln N_{Start}}{t_{End} - t_{Start}} \right) \quad (1)$$

Where N_{Start} and N_{End} are the biomasses of *Daphnia* (measured as POC) on the day of *Daphnia* stocking (t_{Start} = day 14) and the final day (t_{End} = day 28) of the experiment. Using stepwise multiple regression (with backward elimination), we related *Daphnia* growth rates to seston biomass (as POC and as POC^2 to account the possibility of saturation of the growth response), the seston C:P ratio, and the proportion of edible algae. For these parameters we used the average of days 14, 21, and 28. Standardized coefficients for the regression parameters were estimated according to Sokal and Rohlf (1981). We analyzed the responses of algal communities and *Daphnia* to the light manipulations with the regression analysis tools of Sigma Plot (8.0). When visual inspection of the data suggested a unimodal relationship between a response variable and the light gradient we fitted the following Weibull function:

$$y(x) = m \left(\frac{b}{a} \left(\frac{x}{a} \right)^{b-1} e^{-\left(\frac{x}{a}\right)^b} \right) \quad (2)$$

Where *y* is the response variable, *x* is the light treatment (percent of ambient light intensity) and *a*, *b*, and *m* are fitted constants. We used this equation because it can describe unimodal distributions with both symmetric and asymmetric peaks.

Results

Seston biomass and C:P ratio

Initial concentrations of seston POC ranged from 0.18 to 1.79 mg C L^{-1}. They differed among the initial communities and were positively related to lake phosphorus status (Table 1; Pearson correlation of POC vs. log (TP), $r = 0.966$, $p = 0.002$). The relative ranking of the lake communities with respect to seston POC remained similar throughout the experiment, with Förchensee and Brunnensee having the lowest and Thalersee and Bansee having the highest values (Fig. 1). With one exception, seston POC concentrations in the treatments from the different lakes were positively related to the experimentally manipulated light supply both on day 14 (prior to *Daphnia* stocking) and at the end of the experiment (Figs. 1A, B, E, F; Table 2). Described by linear regression, these relationships were statistically significant or marginally significant in treatments from Brunnensee, Klostersee, and Langbürgenersee on both dates and in treatments from Förchensee and Bansee on the final date (Table 2).

The initial molar seston C:P ratios ranged from 103.5 to 559.2. They differed among the starting communities and were negatively related to lake phosphorus status (Table 1; Pearson correlation of C:P vs. log (TP), $r = -0.837$, $p = 0.038$). Seston C:P ratios increased in many treatments over time, extending the overall range of molar C:P values to 67 (Brunnensee, 5% ambient light) – 986 (Thalersee, 70% ambient light) on day 28 (Fig. 1G, H). In all lake treatments, seston C:P ratios were positively related to the experimentally manipulated light supply on both day 14 (prior to *Daphnia* stocking) and at the end of the experiment, but the statistical support for these relationships was weaker in most treatments

on day 14 (Figs. 1C, D, G, H; Table 2). Described by linear regression, the positive relationships were statistically significant in Klostersee treatments on both dates and in treatments from Förchensee, Brunnensee, and Langbürgenersee on the final date, but not in treatments from eutrophic Bansee and Thalersee (Table 2).

Daphnia *abundance and growth*

Daphnia biomass increased in most treatments from an initial value of 6.7 µg C L^{-1} to final values ranging from 16.5 µg to 173.1 µg C L^{-1} corresponding to daily growth rates of 0.076 to 0.243 (Fig. 2). As suggested by the good fit of Weibull functions, *Daphnia* growth rates were unimodally related to light availability in the treatments from Förchensee, Klostersee, and Langbürgenersee (Fig. 2A), the latter one being only marginally statistically significant (Table 3). In all these treatments, the highest *Daphnia* growth rates occurred at intermediate light availability (35-45% of available light). The treatments from Bansee suggested a U-shaped relationship between light availability and *Daphnia* growth rates, but the variance among replicates was very high (Fig. 2B). Because zero survival occurred in several replicates of the Brunnensee and Thalersee treatments, we did not relate *Daphnia* growth rates to light availability in these treatments.

To ascertain whether the relationships of *Daphnia* growth to seston biomass and seston C:P stoichiometry could have been confounded by differences in algal ingestibility and digestibility, we categorized algae as edible and inedible, as described in the Methods section. In spite of large differences in the initial composition of the algal communities from the different lakes, between 80% and 95% of total algal biovolume was categorized as edible. The proportional contribution of edible algae to total algal biovolume decreased in several treatments during the first two weeks of incubation (Fig. 3A, B). In each of the three lake treatments where we found a unimodal relationship between *Daphnia* growth and light availability (Förchensee, Klostersee, Langbürgenersee), the proportion of edible algae tended to be weakly positively related to light availability before and two weeks after *Daphnia*

stocking but these relationships were not statistically significant (Fig. 3). Given that food quantity (seston POC) was positively related to light availability in these treatments, light dependent differences in algal ingestibility and digestibility seem unlikely to explain the declining limbs of the *Daphnia* growth responses to light availability.

The latter was confirmed in the stepwise multiple regression in which we regressed *Daphnia* growth rates from all treatments on seston C:P ratio, seston POC content, and the proportion of edible algae. The proportion of edible algae was not retained in the final model. Instead, *Daphnia* growth rate was negatively related to seston C:P ratios, in an upward convex manner to seston POC content, suggesting saturation of *Daphnia* growth rate at higher POC-values (*Daphnia* growth rate day^{-1} = 0.14 -0.0001 x C:P ratio +0.08 x POC -0.03 x POC^2; r^2 = 0.32; $F_{3,46}$ = 6.76; p=0.0008, Fig. 4).

Algal diversity and seston C:P ratios

Taxon richness (S), diversity (Shannon-Wiener Index, H) and evenness (H/ln S) of the initial algal communities differed among the lakes (Table 1). The highest species richness occurred in mesotrophic lakes and both Shannon diversity and evenness were highest in the initial algal community from mesotrophic Lake Klostersee (Table 1). Diversity and evenness of the initial algal communities were positively related to their stoichiometric responses to light enrichment in the absence of *Daphnia*. We calculated the size of the effect of light enrichment on the molar C:P ratios of the algal communities as the slopes of the regressions of light availability versus seston C:P ratio on day 14 (see Table 2). This measure of effect size was positively related to both Shannon diversity (Fig. 5A; effect size = 2.2 x Diversity +0.13; r^2=0.74; $F_{1,4}$=11,3; p<0.05) and to evenness (Fig. 5B; effect size = 2.9 x Evenness -0.02; r^2=0.79; $F_{1,4}$=15,1; p<0.05) of the algal communities. A similar relationship between initial algal diversity and the stoichiometric response to the light gradient was no longer found after two weeks of *Daphnia* grazing.

Discussion

Possible mechanisms of unimodal relationships between Daphnia *growth and light supply*

Urabe and Sterner (1996) have demonstrated unimodal relationships between light availability and zooplankton production in laboratory experiments. Our experiment, which covers a very broad range of natural algal communities, suggests that such patterns of light-nutrient interactions may also occur under field conditions. In the two eutrophic treatments, seston POC and seston C:P were only weakly (and mostly non-significantly) related to the light gradient. Consistent with this lack of clear light-seston relationships, *Daphnia* growth rates were unrelated to light intensity in the eutrophic treatments. In contrast, seston POC and seston C:P were positively related to the light gradient in most oligotrophic and mesotrophic treatments, and *Daphnia* growth rates in Förchensee and Klostersee were best described by unimodal relationships with light availability. In addition, mesotrophic Langbürgenersee treatments suggested a unimodal relationship between *Daphnia* growth rate and light supply, but here the descending limb of the relationship hinges critically on a single data point.

The rising limbs of these relationships are likely a consequence of food (carbon) limitation being alleviated with increasing light availability in the oligotrophic and mesotrophic lake communities. The quantity of food (amount of seston POC) increased with higher light availability and more food resulted in faster zooplankton growth. Using natural lake seston as food for *Daphnia*, Müller-Navarra and Lampert (1996) observed moderate food (carbon) limitation at 0.3-0.6 mg C L^{-1}. When estimates of food quantity are based on the amount of edible algae (see Figs. 1 and 3), carbon limitation of *Daphnia* might have occurred in oligotrophic and mesotrophic treatments, especially in mesocosms with low light availability. In contrast, carbon limitation seems unlikely in eutrophic treatments (Figs. 1 and 3), which could explain the absence of a positive effect of light supply on *Daphnia* growth rates in these treatments.

Several mechanisms can be put forward that could explain the observed decline in *Daphnia* growth rates at high light availabilities in Förchensee, Klostersee, and Langbürgenersee treatments. Our data support a significant contribution of elemental food quality (C:P ratio) with changing light availability, as indicated by the multiple regression analysis of *Daphnia* growth rates across all treatments (Fig. 4). Light and nutrient supply may, however, affect other algal traits than just C:P ratios. For example, light inhibition may cause a unimodal response of algal photosynthesis to light availability. In addition, light dependent changes in phytoplankton taxonomic composition towards less nutritious or less edible taxa at high light levels should be considered.

Our data show no evidence for a saturation of photosynthetic activity at higher light intensities. Carbon to chlorophyll *a* ratios (as an indicator of photosynthetic characteristics) showed no significant relationships to light availability (Striebel, unpubl. data). Additionally, phytoplankton biomass increased with increasing light levels in all treatments except for those from eutrophic Thalersee. Similarly, we found no evidence that edibility was negatively related to light availability in any of the treatments. If anything, the contribution of edible algae to total phytoplankton biomass increased at light levels beyond 50% ambient light in Förchensee, Klostersee, and Langbürgenersee, which is opposite to the observed decline in *Daphnia* growth rates over the same range of light supplies (Fig. 2 and 3).

We considered the proportional contributions of three important algal classes (Chrysophyceae, Bacillariophyceae, and Chlorophyceae) to the total biovolume of each algal community as an additional potential food quality parameter and investigated how these were related to light availability. Cyanophyceae and Cryptophyceae represented at the time of *Daphnia* stocking less than 1% of total algal biovolume in all treatments. The proportional contribution of presumably less edible Chlorophyceae to total algal biovolume was either unrelated (four cases) or negatively related (two cases) to light availability; the potentially more edible classes Bacillariophyceae and Chrysophyceae were instead mostly unrelated (nine cases) or positively related (three cases) to light availability (Fig. 6). Accordingly,

changes in the taxonomic composition of the algal communities seem an unlikely explanation for declining *Daphnia* growth rates at the highest light availabilities.

Additional aspects of food quality

It is clearly insufficient to characterize the food quality of different algal communities only by the seston C:P ratio, which may explain the relatively weak explanatory power of the multiple regression relating *Daphnia* growth to seston C:P and POC (r^2=0.32, Fig. 4). Most probably, variation in the assimilation efficiency of *Daphnia* for the carbon fraction of their food will determine the food's critical C:P ratio. If much of algal carbon is located in structures (such as cell wall structures with a high amount of cellulose) that *Daphnia* cannot assimilate with high efficiency (Van Donk et al. 1997), P-limitation should occur at higher C:P ratios compared to communities where algal carbon is easier to assimilate. The assimilation efficiency for carbon is probably considerably more variable than the assimilation efficiency for the phosphorous fraction of the algal biomass. Therefore, C:P ratios might be most valuable as a component measure of algal food quality once the suitability of the carbon fraction has been characterized. Obviously, this should be studied in more detail in further studies.

Responsiveness of different algal communities to the light supply

Our approach of using different algal communities from lakes of different nutrient status (rather than manipulating nutrient supply to a single algal community) brought additional insights into how different algal communities respond to light manipulations. In particular, we found that, in the absence of *Daphnia*, the size of light effects on seston C:P ratios (measured as the slope of the relationship between seston C:P ratio and light supply) was positively related to two measures of diversity, the Shannon Wiener indices of diversity and evenness of the algal communities. Light mediated changes in seston C:P ratios were stronger in more diverse algal communities (Fig. 5). A similar pattern was observed by

Dickman et al. (2006) in a study of light mediated changes in seston C:P ratios of three different natural algal communities. Such relatively small datasets cannot give conclusive evidence that the observed relationship is general. Characterizing these relationships and unraveling the mechanisms behind them requires the study of a broader range of natural algal communities.

References

Andersen, T., and D.O. Hessen. 1991. Carbon, nitrogen and phosphorus content of freshwater zooplankton. Limnol. Oceanogr. **36**: 807-814.

Andersen, T., J.J. Elser, and D.O. Hessen. 2004. Stoichiometry and population dynamics. Ecology Letters **7**: 884–900.

Berger, S. A., S. Diehl, T. J. Kunz, D. Albrecht, A. M. Oucible, and S. Ritzer 2006. Light supply, plankton biomass, and seston stoichiometry in a gradient of lake mixing depths. Limnol. Oceanogr. **51**: 1898-1905.

Burns, C.W. 1968. The relationship between body size of filter-feeding cladocera and the maximum size of particle ingested. Limnol. Oceanogr. **13**: 675-678.

Burns, C.W., D.J. Forsyth, J.F. Haney, M.R. James, W. Lampert, and R.D. Pridmore. 1989. Coexistence and exclusion of zooplankton by *Anabaena minutissima* var. *attenuate* in Lake Rotongaio, New Zealand. Arch. Hydrobiol. Erg. Limnol. **32**: 63-82.

DeMott, W.R., R.D. Gulati, and E. Van Donk. 2001. Effects of dietary phosphorus deficiency on the abundance, phosphorus balance and growth of *Daphnia cucullata* in three hypereutrophic Dutch lakes. Limnol. Oceanogr. **46**: 1871–1880.

DeMott, W.R., and A.J. Tessier. 2002. Stoichiometric constraints vs. algal defences: testing mechanisms of zooplankton food limitation. Ecology **83**: 3426–3433.

DeMott, W.R., B. J. Pape, and A. J. Tessier. 2004. Patterns and sources of variation in *Daphnia* phosphorus content in nature. Aquatic Ecology **38**: 433–440.

Dickman, E. M., M. J. Vanni, and M. J. Horgan. 2006. Interactive effects of light and nutrients on phytoplankton Stoichiometry. Oecologia **149**: 676–689.

Diehl, S. 2007. Paradoxes of enrichment: Effects of increased light versus nutrient supply on pelagic producer-grazer systems. Am. Nat. **169**: E173-E191.

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

Elser, J. J., D. Dobberfuhl, N.A. MacKay, and J.H. Schampel. 1996. Organism size, life history, and N:P stoichiometry: towards a unified view of cellular and ecosystem processes. BioScience **46**: 674–684.

Elser, J. J., W. F. Fagan, R.F. Denno, D.R. Dobberfuhl, A. Folarin, A. Huberty, S. Interlandi, S.S. Kilham, E. McCauley, K.L. Schulz, E.H. Siemann, and R.W. Sterner. 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature **408**: 578–580.

Hall, S.R., M.A. Leibold, D.A. Lytle, and V.H. Smith. 2004. Stoichiometry and planktonic grazer composition over gradients of light, nutrients, and predation risk. Ecology **85**: 2291-2301.

Hall, S.R., M. A. Leibold, D. A. Lytle, and V. H. Smith. 2007. Grazing and the light:nutrient hypothesis: revisiting bottom-up and top-down effects on producer stoichiometry. Ecology **88**: 1142-1152.

Hessen, D.O. 1992. Nutrient element limitation of zooplankton production. Am. Nat. **140**: 799-814.

Hillebrand, H., C.-D. Dürselen, D. Kirschtel, U. Pollingher, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology **35**: 403-424.

Leibold, M.A. 1989. Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. Am. Nat. **134**: 922-949.

Loladze, I., Y. Kuang, and J.J. Elser. 2000. Stoichiometry in producer-grazer systems: Linking energy flow with element cycling. Bulletin of Mathematical Biology **62**: 1137-1162.

Lund, J.W.G., G. Kipling, and E.D. Le Creen. 1958. The inverted microscope method of estimating algae numbers and the statistical basis of estimation by counting. Hydrobiologia **11**:143–170.

Main, T., D. R. Dobberfuhl, and J.J. Elser. 1997. N:P stoichiometry and ontogeny in crustacean zooplankton: a test of the growth rate hypothesis. Limnol. Oceanogr. **42**: 1474-1478.

Müller-Navarra, and D., W. Lampert. 1996. Seasonal patterns of food limitation in *Daphnia galeata*: Separating food quantity and food quality effects. J. Plankton Res. **18**: 1137-1157.

Sokal, R.R., and F.J. Rohlf. 1981. Biometry. W.H. Freeman and Company.

Sterner, R.W., J.J. Elser, E.J. Fee, S.J. Guildford, and T.H. Chrazanowski. 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. Ecology Letters **1**: 146-150.

Urabe, J., and Y. Watanabe. 1992. Possibility of N or P limitation for planktonic cladocerans: an experimental test. Limnol. Oceanogr. **37**: 244-251.

Urabe, J., and R.W. Sterner. 1996. Regulations of herbivore growth by the balance of light and nutrients. Proc. Natl. Acad. Sci. USA **93**: 8465-8469.

Urabe, J., J.J. Elser, M. Kyle, T. Yoshida, T. Sekino, and Z. Kawabata. 2002*a*. Herbivorous animals can mitigate unfavourable ratios of energy and material supplies by enhancing nutrient recycling. Ecol. Lett. **5**: 177-185.

Urabe, J., M. Kyle, W. Makino, T. Yoshida, T. Andersen, and J.J. Elser. 2002*b*. Reduced light increases herbivore production due to stoichiometric effects of light:nutrient balance. Ecology **83**: 619-627.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik**.** Mitt. Verein. Theor. Angew. Limnol. **9**: 1-38.

Van Donk, E., M. Lürling, D.O. Hessen, G.M. Lokhorst. 1997. Altered cell wall morphology in nutrient-deficient phytoplankton and its impacts on grazers. Limnol. Oceanogr. **42**: 357-364.

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

Weider, L. J., K. L. Glenn, M. Kyle, and J. J. Elser. 2004. Associations among ribosomal

(r)DNA intergenic C:N:P stoichiometry in the genus *Daphnia.* Limnol. Oceanogr. **49**: 1417-1423.

Table 1

Characteristics of the lakes and the algal communities deriving from these lakes at the start of the experiment. Seston N:P ratios (marked with *) are from samples obtained in 2005.

Lake	Förchen-see	Brunnen-see	Kloster-see	Langbürgener-see	Thaler-see	Bansee
Total						
Phosphorus (µg L^{-1})	6.0	9.4	14.2	17.2	55.0	81.0
Chlorophyll *a* (µg L^{-1})	0.2	2.5	3.1	4.5	26.9	53.4
POC (mg L^{-1})	0.18	0.46	0.54	1.10	1.38	1.79
Seston C:P (molar)	559	278	178	299	104	107
Seston N:P (molar)*	42	40	38	29	29	16
Mean depth (m)	4.0	8.5	5.9	9.2	4.4	2.4
Surface area (km^2)	0.04	0.06	0.47	1.04	0.04	0.03
Diversity (H)	0.07	0.29	0.85	0.53	0.59	0.39
Evenness (H* ln S^{-1})	0.07	0.28	0.68	0.42	0.54	0.33
Proportions of algal biovolume						
Cyanophyta	0.0	8.5	0.0	0.4	60.0	0.0
Chlorophyceae	97.9	84.1	72.7	83.3	33.6	2.5
Dinophyceae	0.0	2.0	6.9	1.2	0.0	0.0
Bacillariophyceae	0.3	0.9	1.9	3.2	1.6	1.6
Cryptophyceae	0.0	1.3	3.4	0.4	0.0	84.4
Chrysophyceae	0.0	0.0	3.2	2.7	0.0	1.5
Other Flagellates	1.8	3.0	11.6	8.8	4.0	9.7

Table 2

Summary of linear regression statistics of algal biomass (mg C L^{-1}) and molar seston C:P ratio against light treatment (% of ambient light intensity) after two weeks of exposure without *Daphnia* and after two weeks of exposure with *Daphnia*. Values in brackets are ±SE.

	After two weeks of exposure and prior to the introduction of *Daphnia*					After two weeks of exposure with *Daphnia*				
Lake	Intercept	Slope	r^2	$F_{1,8}$	p	Intercept	Slope	r^2	$F_{1,8}$	p
Förchensee										
Biomass (mg C L^{-1})	0.2 (0.03)	-0.0001 (0.0004)	0.003	0.02	0.89	0.2 (0.02)	0.002 (0.0003)	0.89	65.1	<0.0001
C:P molar	319.9 (65.5)	0.5 (1.2)	0.02	0.15	0.71	485.6 (101.5)	4.5 (1.8)	0.44	6.2	0.04
Brunnensee										
Biomass (mg C L^{-1})	0.3 (0.03)	0.002 (0.0006)	0.56	10.0	0.01	0.2 (0.03)	0.002 (0.0006)	0.56	10.1	0.01
C:P molar	357.1 (49.7)	0.8 (0.9)	0.08	0.7	0.42	48.7 (48.6)	3.7 (0.9)	0.69	17.7	0.003
Klostersee										
Biomass (mg C L^{-1})	0.2 (0.03)	0.003 (0.0006)	0.74	22.7	0.001	0.2 (0.05)	0.004 (0.0009)	0.75	23.4	0.001
C:P molar	164.3 (30.0)	2.2 (0.5)	0.67	16.6	0.004	159.9 (21.3)	1.9 (0.4)	0.76	25.4	0.001
Langbürgenersee										
Biomass (mg C L^{-1})	0.8 (0.006)	0.003 (0.001)	0.45	6.6	0.03	0.4 (0.1)	0.005 (0.002)	0.37	4.74	0.06
C:P molar	253.3 (38.8)	0.7 (0.7)	0.12	1.1	0.32	297.8 (72.9)	4.5 (1.3)	0.60	12.11	0.008
Thalersee										
Biomass (mg C L^{-1})	3.4 (0.4)	0.01 (0.008)	0.17	1.6	0.24	2.7 (1.2)	0.03 (0.02)	0.18	1.7	0.23
C:P molar	372.1 (71.2)	1.8 (1.3)	0.21	2.1	0.19	364.9 (172.3)	1.9 (3.1)	0.05	0.4	0.55
Bansee										
Biomass (mg C L^{-1})	2.3 (0.4)	0.01 (0.007)	0.23	2.4	0.16	2.3 (0.3)	0.01 (0.005)	0.50	8.1	0.02
C:P molar	195.4 (23.6)	0.8 (0.4)	0.31	3.6	0.09	547.4 (96.9)	0.4 (1.7)	0.005	0.04	0.85

Table 3

Standard Weibull regression (Eq. 2): Parameters of unimodal relationships between *Daphnia* growth rates (day^{-1}) and light availability (per cent of incident light). Values in brackets are standard errors.

		m	a	b	r^2	$F_{2,7}$	p	peak
Förchensee	Coefficient	26.0 (5.9)	134.4 (36.0)	1.3 (0.07)	0.65	6.4	0.02	38.9
	p	0.003	0.007	<0.0001				
Klostersee	Coefficient	28.3 (7.1)	145.7 (45.6)	1.3 (0.07)	0.67	7.1	0.02	42.2
	p	0.005	0.001	<0.0001				
Langbürgenersee	Coefficient	44.3 (19.5)	202.2 (101.2)	1.2 (0.08)	0.53	3.9	0.07	44.8
	p	0.06	0.09	<0.0001				

Figure legends:

Figure 1: Effects of different light treatments on the seston biomasses (POC in mg L^{-1}) of the communities from (A) oligotrophic Förchensee and Brunnensee, mesotrophic Klostersee and Langbürgenersee and (B) from eutrophic Thalersee and Bansee after two weeks of exposure. Effects of different light treatments on the molar seston C:P ratios of the communities from (C) oligotrophic Förchensee and Brunnensee, mesotrophic Klostersee and Langbürgenersee and (D) from eutrophic Thalersee and Bansee after two weeks of exposure. Effects of different light treatments on the seston biomasses (POC in mg L^{-1}) after four weeks of exposure in communities from (E) Förchensee, Brunnensee, Klostersee, and Langbürgenersee and (F) from eutrophic Thalersee and Bansee. Effects of different light treatments on the molar seston C:P ratios after four weeks of exposure in communities from (G) Förchensee, Brunnensee, Klostersee, and Langbürgenersee and (H) from Thalersee and Bansee. Linear regressions are shown when they are statistically significant ($p \leq 0.05$). Förchensee (FOS), Brunnensee (BRU), Klostersee (KLS), Langbürgenersee (LAN), Thalersee (THA), Bansee (BAN).

Figure 2: Effects of different light treatments on *Daphnia* growth rates (d^{-1}) in treatments from (A) oligotrophic Förchensee and Brunnensee, mesotrophic Klostersee and Langbürgenersee and (B) from eutrophic Thalersee and Bansee. Fits of nonlinear Weibull functions are shown when $p<0.1$. Förchensee (FOS), Brunnensee (BRU), Klostersee (KLS), Langbürgenersee (LAN), Thalersee (THA), Bansee (BAN).

Figure 3: Proportions of edible algae in different light treatments prior to *Daphnia* stocking (A, B) and after two weeks of exposure with *Daphnia* (C, D). Communities from oligotrophic Förchensee (FOS) and Brunnensee (BRU) and mesotrophic Klostersee (KLS) and

Langbürgenersee (LAN) are shown in panel A and C. Communities from eutrophic Thalersee (THA) and Bansee (BAN) are shown in panels B and D.

Figure 4: *Daphnia* growth rates (d^{-1}) from all lake treatments plotted against abundance (POC in mg L^{-1}) and C:P ratio (molar) of seston in week three. The plane describes the multiple regression, *Daphnia* growth rate day^{-1} = 0.14 -0.0001 x C:P ratio +0.08 x POC -0.03 x POC^2 (r^2 = 0.32; $F_{3,46}$ = 6.76; p=0.0008).

Figure 5: Relationships between the size of light enrichment effects on seston C:P ratios after two weeks of exposure without *Daphnia* and (A) diversity and (B) evenness of the algal communities on the day prior to *Daphnia* stocking. The effect of light enrichment on the C:P ratios of the algal communities (effect size) was calculated by using the slopes (with standard error) of the light availability versus C:P ratio regressions before the addition of *Daphnia*. Förchensee (FOS), Brunnensee (BRU), Klostersee (KLS), Langbürgenersee (LAN), Thalersee (THA), Bansee (BAN).

Figure 6: Proportions of the three dominant algal classes in the treatments from Lake Förchensee (A,B), Lake Brunnensee (C,D), Lake Klostersee (E,F), Lake Langbürgenersee (G,H), Lake Thalersee (I,J), and Lake Bansee (K,L). Data after two weeks of exposure are displayed as Figures A,C,E,G,I,K and data after four weeks of exposure are displayed as Figures B,D,F,H,J,L. Statistically significant relationships (linear regression, P<0.05) are indicated by straight lines.

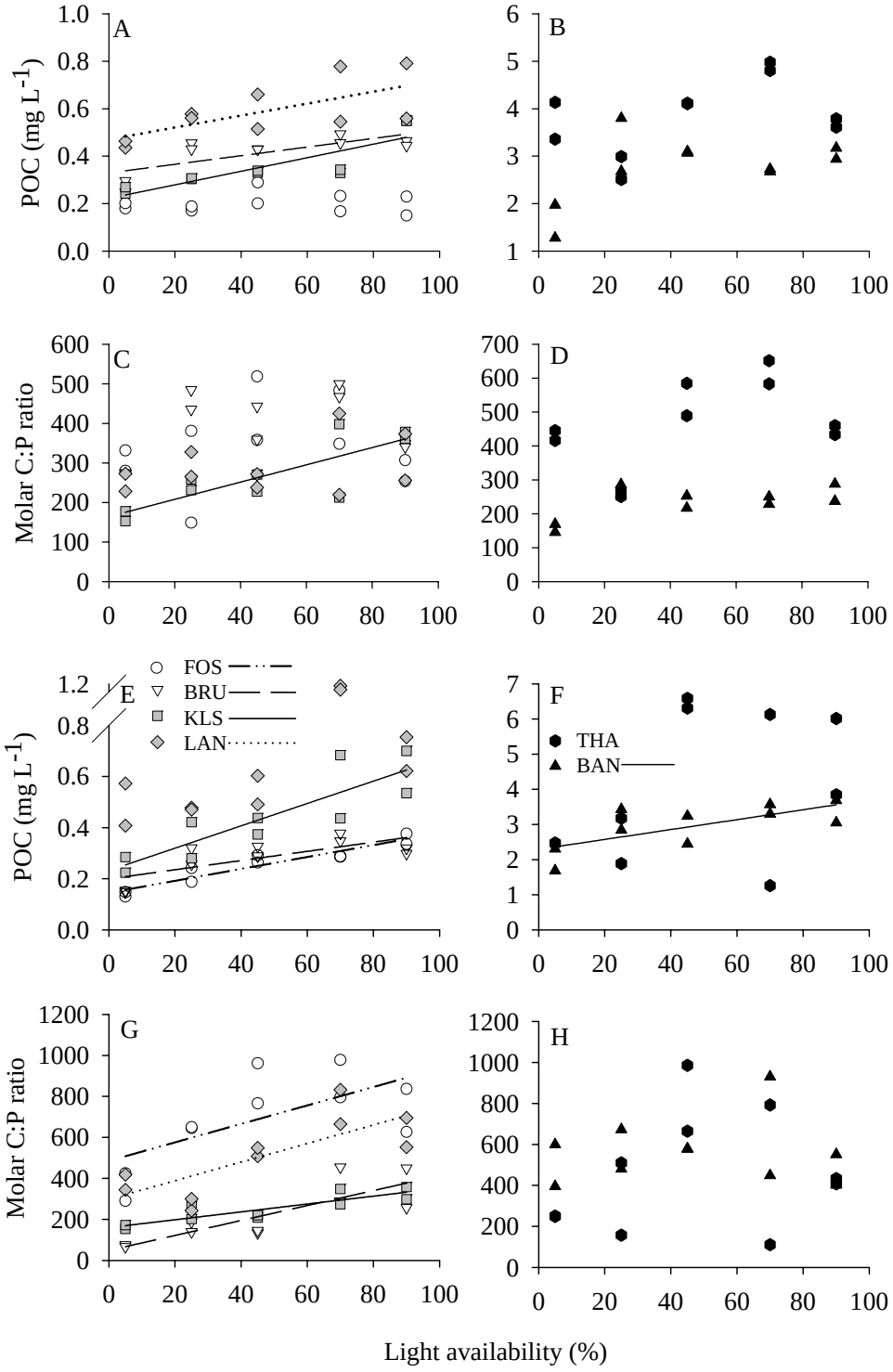

A
B
C
D
E
F
G
H
POC (mg L-1)
Molar C:P ratio
FOS
BRU
KLS
LAN
THA
BAN
Light availability (%)

Fig. 1

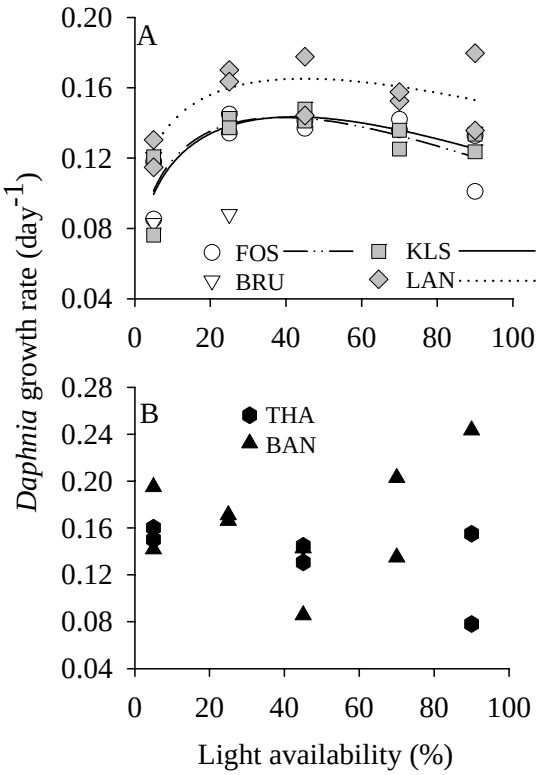

A
B
FOS
KLS
BRU
LAN
THA
BAN
Daphnia growth rate (day-1)
Light availability (%)
0.20
0.16
0.12
0.08
0.04
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0
20
40
60
80
100

Fig. 2

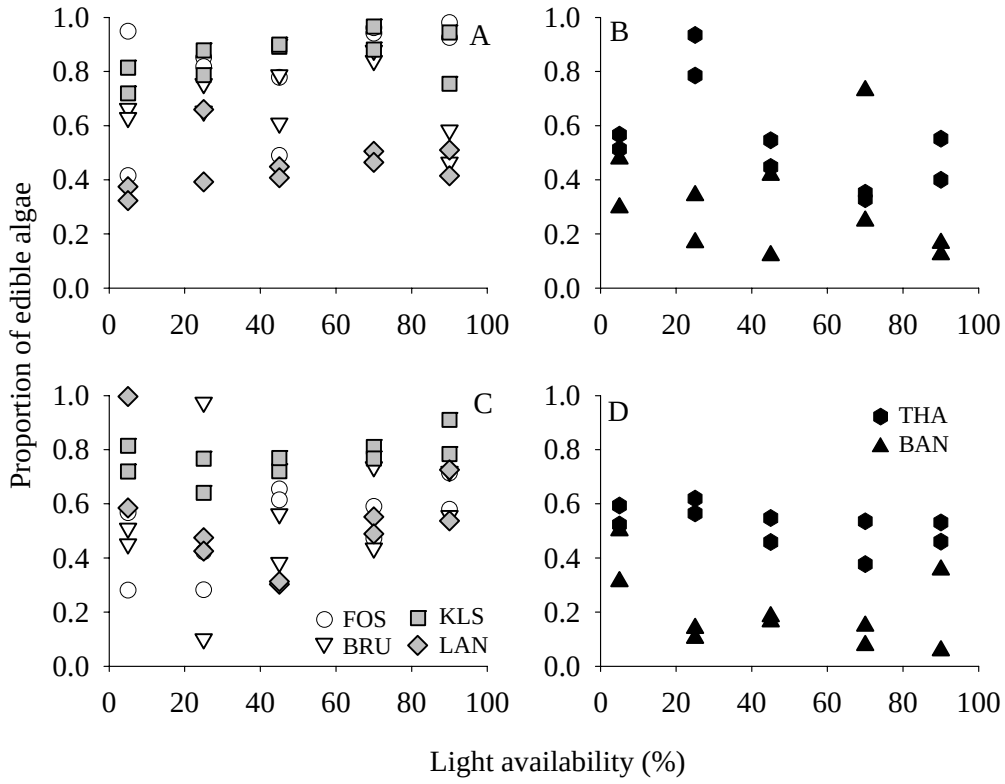

Proportion of edible algae
Light availability (%)
A
B
C
D
FOS
KLS
BRU
LAN
THA
BAN
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100

Fig. 3

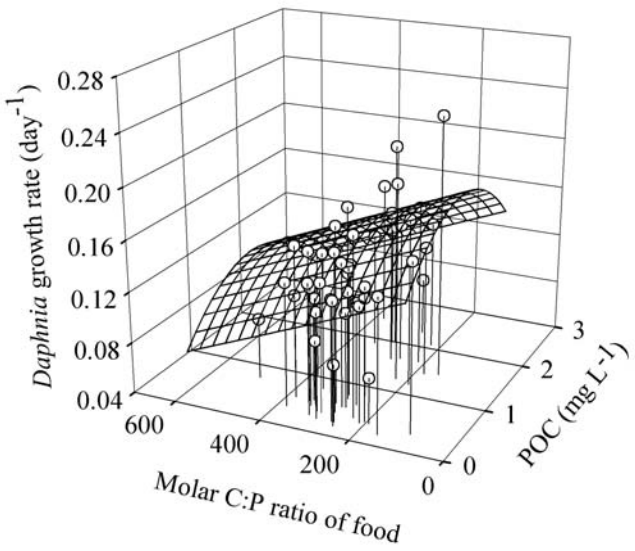

Daphnia growth rate (day-1)
0.28
0.24
0.20
0.16
0.12
0.08
0.04
600
400
200
0
Molar C:P ratio of food
0
1
2
3
POC (mg L-1)

Fig. 4

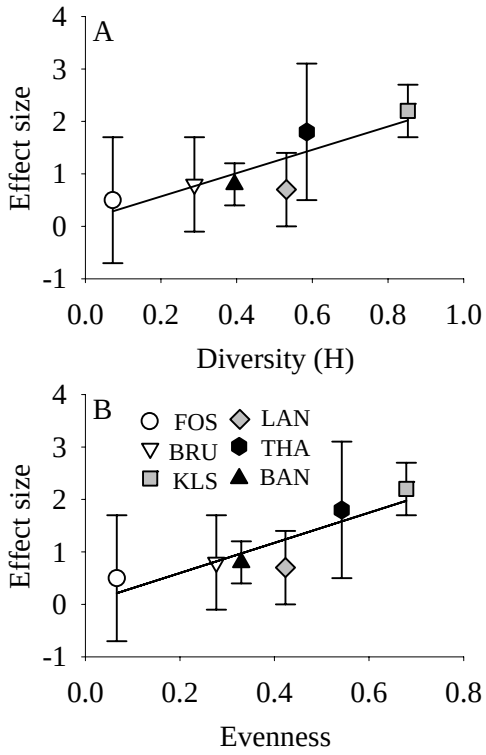

A
Effect size
Diversity (H)
B
FOS
BRU
KLS
LAN
THA
BAN
Effect size
Evenness

Fig. 5

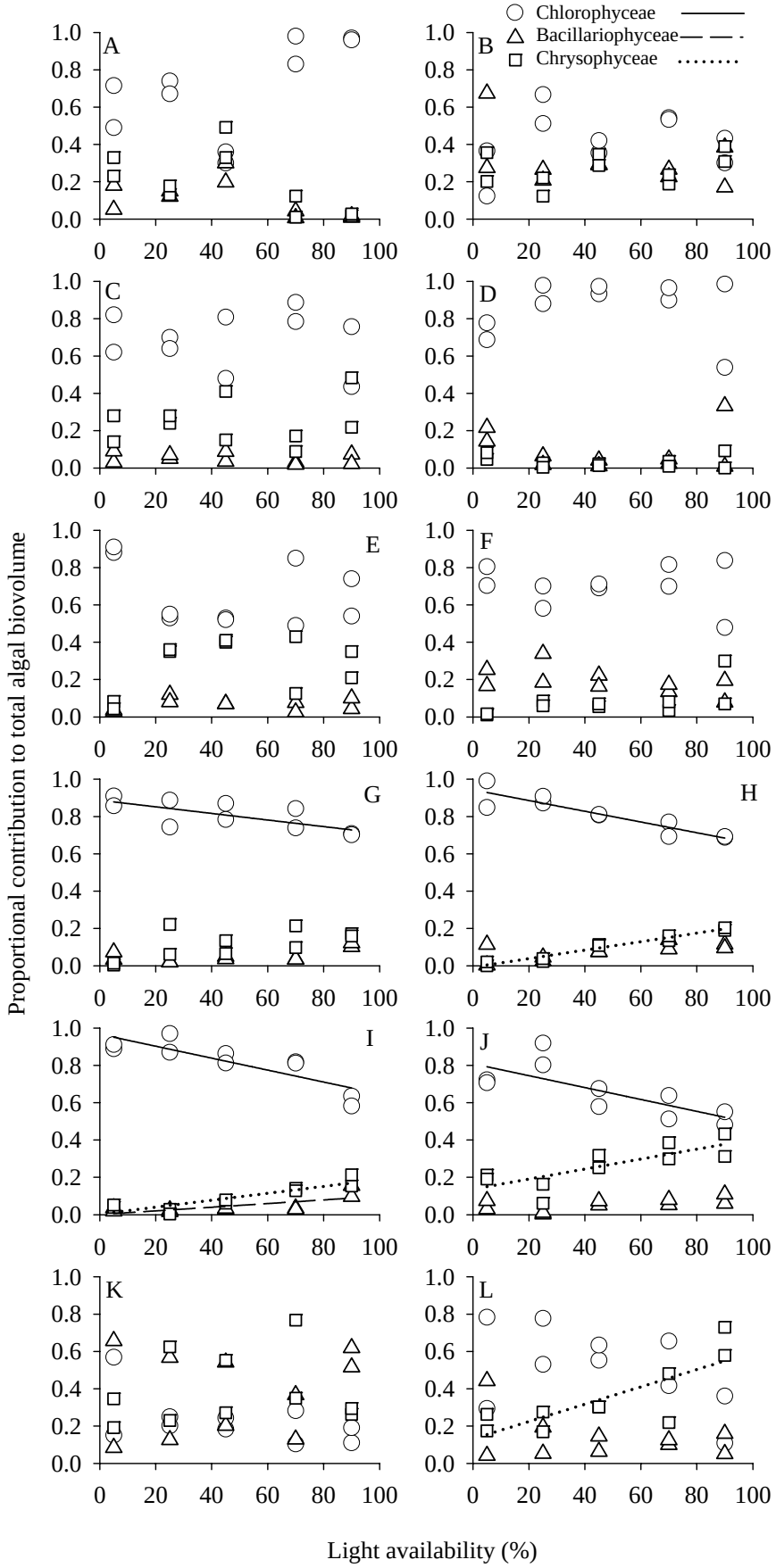

Chlorophyceae
Bacillariophyceae
Chrysophyceae
A
B
C
D
E
F
G
H
I
J
K
L
Proportional contribution to total algal biovolume
Light availability (%)

Fig. 6

PAPER 4

CARBON SEQUESTRATION AND STOICHIOMETRY OF MOBILE AND NON-MOBILE GREEN ALGAE

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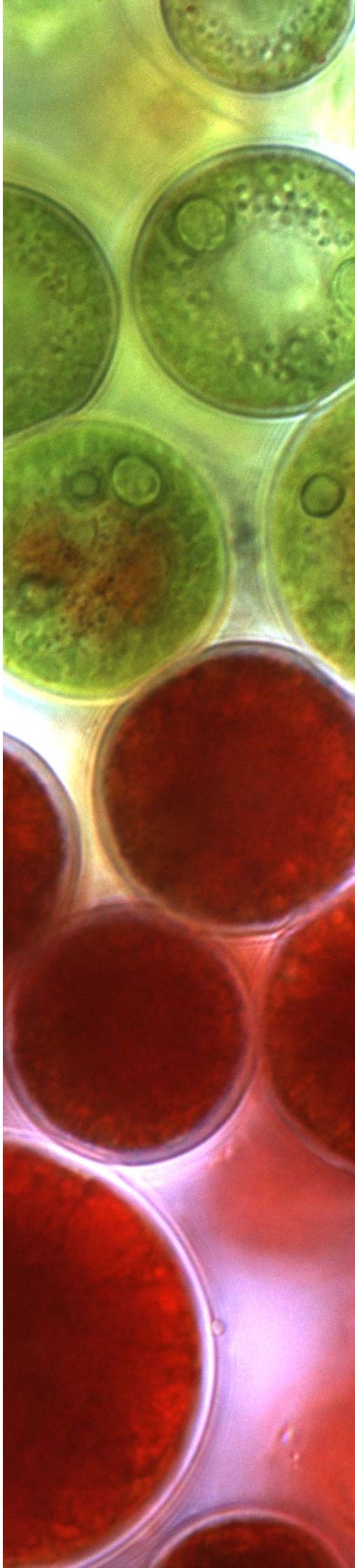
MANUSCRIPT PREPARED FOR SUBMISSION TO *LIMNOLOGY AND OCEANOGRAPHY*



Abstract

Growth of phytoplankton mainly depends on the availability of light and nutrients, which usually exhibit vertically opposing gradients in pelagic ecosystems. Mobile phytoplankton species are, to a certain degree, able to migrate, allowing them to optimize the availability of light and nutrients by actively choosing their vertical position in the water column. However, mobility involves costs in terms of energy and nutrient expenditures. Thus, mobile phytoplankton species may have higher energy expenditures to maintain their metabolic rate and higher phosphorus demands for energy storage (in the form of adenosine triphosphate, ATP). In contrast, non-mobile species have to cope with temporally variable ratios of light and nutrients because their position in the water column is mainly determined by passive floating and sinking. These different strategies may result in differences in carbon dynamics and biomass composition (stoichiometry) between mobile and non-mobile species.

We conducted experiments with nine green algae species (four mobile and five non-mobile) over a gradient of light availability and quantified algal primary production, biomass accrual and algal biomass carbon-to-phosphorus ratios.

Phytoplankton primary production and biomass carbon-to-phosphorus ratios differed between mobile and non-mobile species. The relationship between respiration and maximal production (indicating higher metabolic costs) was higher and biomass carbon-to-phosphorus ratios were lower in mobile species than in non-mobile species. We conclude that higher energy demands and the necessity to maintain high biomass phosphorus content limit the advantages of mobility to specific environmental conditions.

Introduction

Primary production in aquatic systems depends on environmental conditions. The most important and also the best studied factors affecting the dynamics of phytoplankton communities are the availability of light and the amount of limiting nutrients, such as phosphorus in freshwater systems (Tilman 1982, Huisman and Weissing 1995, Hessen et al. 2002). The availability of light declines exponentially with depth (Wall and Briand 1979, Flöder and Burns 2005). Phosphorus, on the other hand, is often scarce in upper, illuminated water layers but abundant in the depths. Thus, the supplies of the two most important resources for phytoplankton photosynthesis and growth are spatially separated and usually exhibit opposing vertical gradients (Huisman and Weissing 1995).

The uptake of dissolved inorganic phosphorus and the amount of light-dependent carbon fixation by phytoplankton are not tightly coupled. Therefore, the carbon-to-phosphorus ratios of phytoplankton biomass are often highly flexible (molar ratios between 50 and 1000) and alter with changing environmental conditions (Sterner et al. 1997, Berger et al. 2006). At high light availability, phytoplankton species are able to fix high amounts of carbon photosynthetically. Under low phosphorus supplies, this can lead to high biomass carbon-to-phosphorus ratios of phytoplankton (Urabe and Sterner 1996, Sterner et al. 1997, Striebel et al. 2008). On the other hand, low light-to-phosphorus ratios can lead to relatively low biomass carbon-to-phosphorus ratios (Urabe and Sterner 1996, Urabe et al. 2002, Striebel et al. 2008). Among other factors, such as size or biochemical composition (e.g. fatty acid content), biomass carbon-to-phosphorus ratios of phytoplankton serve as a measure of their food quality for herbivorous zooplankton. Phytoplankton species with high biomass carbon-to-phosphorus ratios are considered to be low-quality food for fast-growing herbivorous zooplankton with high phosphorus demands. Conversely, phytoplankton with relatively low biomass carbon-to-phosphorus ratios are considered to be a relatively good food source

(Sterner et al. 1997, Diehl et al. 2002, Park et al. 2002). Thus, changes in biomass carbon-to-phosphorus ratios of phytoplankton may affect herbivorous zooplankton growth.

Mobile phytoplankton species are able to conduct periodic vertical migrations (for example, diurnal migrations) and are, to a certain degree, able to choose their vertical position in the water column in order to optimize the availability of light and nutrients (Pick et al. 1984, Flynn and Fasham 2002, Knapp et al. 2003). Those migrations allow mobile algae to access deeper, nutrient-rich water and to adjust for optimal irradiance. Mobile species may therefore be less susceptible to shifts in biomass composition in response to resource fluctuations. Hence, mobile species exhibit a considerable advantage over non-mobile species, especially at low turbidity or in stratified water columns (Jones 1993, Ralston et al. 2007).

However, mobility also involves costs in terms of energy expenditure. The majority of planktonic protists expend a low (<1%) to moderate (1-10%) proportion of their total metabolic activity on mobility (Crawford 1992). Mobile species have higher energy needs to survive and growth. Mobility also requires high amounts of phosphorus because biochemical reactions involved in all kinds of energy-demanding processes use the phosphate-rich molecule ATP for energy storage. Alternative nutrition modes may be employed to satisfy the high phosphorus requirements of mobility. Grazing of algae on phosphorus-rich bacteria (phagotrophy) provides an example of alternative phosphorus uptake (Vadstein 2000).

Non-mobile phytoplankton species have to cope with temporally variable ratios of light and nutrients as their vertical position in the water column fluctuates due to passive floating and sinking. Biomass production of non-mobile phytoplankton species is highest in the euphotic zone, where growth is mostly limited by phosphorus and light levels are high, resulting in high phytoplankton biomass carbon-to-phosphorus ratios. Additionally, non-mobile species of (green) algae often possess cell walls containing large amounts of structural carbon compounds such as cellulose, which will further increase their biomass carbon-to-phosphorus ratios.

Therefore, we hypothesize that mobile and non-mobile phytoplankton species differ in their biomass composition, and that mobile phytoplankton species have to maintain lower biomass carbon-to-phosphorus ratios compared to non-mobile phytoplankton species. To test this hypothesis, we conducted growth experiments with nine different mobile and non-mobile phytoplankton species over a gradient of light availability. To exclude variability due to different photosynthetic pigment composition among the phytoplankton species, we used only green algae for this experiment. We determined the biomass accrual and stoichiometric composition of the various mobile and non-mobile phytoplankton species under different light conditions. To estimate the minimal light requirements for a positive carbon balance, we also measured the specific net primary production of mobile and non-mobile phytoplankton species.

Methods

Experimental design

We used nine different green algae species, four mobile (*Chlamydomonas* sp., *Haematococcus pluvialis*, *Phacotus lenticularis*, and *Carteria* sp.) and five non-mobile (*Scenedesmus* sp., *Staurastrum tetracerum*, *Golenkinia brevispicula*, *Tetraedron minimum*, and *Monoraphidium* sp.). The algae were precultivated over a period of several weeks prior to the experiments in a phosphorus-reduced growth medium (WC medium, after (Guillard and Lorenzen 1972) containing 10 µg P L^{-1}). The same medium was subsequently used in all growth experiments. We established five light levels: 3, 7, 30, 130 and 300 µmol quanta m^{-2} s^{-1}. Each algal treatment was established with the same initial biovolume (2×10^6 fl mL^{-1}) and replicated three times. The treatments were arranged as semibatch cultures (10% exchange day^{-1}) in 250 mL translucent cell culture bottles in a climate chamber at 20°C. The growth experiments lasted for fourteen days, at which point phytoplankton growth had reached the stationary phase. Particulate organic carbon and particulate phosphorus were analyzed at the start of the experiment and after fourteen days of incubation. To determine particulate organic carbon and particulate phosphorus, we filtered samples from each culture bottle onto precombusted, acid-washed glass-fiber filters (Whatman GF/F). Particulate organic carbon was measured with an elemental analyzer (CE Instruments, Milan, Italy) and particulate phosphorus was measured photometrically after sulfuric acid digestion followed by molybdate reaction.

Specific net primary production

We measured the specific net primary production of mobile and non-mobile green algal species over the gradient of light intensities. We used the oxygen method with 4 hours of incubation (Wetzel and Likens 2003) to quantify primary production.

Based on the data from specific net primary production at different light levels, we determined primary production–light intensity curves. We used a modified Michaelis–Menten–type model (Equation 1) to estimate the highest specific photosynthetic rates (P_{max}), the half-saturation coefficient (k_s) for P_{max} and the Respiration (y_0). The point intercepted by the net photosynthesis curve along the x-axis ($light_{comp}$) defines the light intensity at which the gross photosynthesis is sufficient to compensate for respiratory losses:

$$sNPP = y_0 + \frac{(P_{\max} \text{ x light intensity})}{(k_S + \text{light intensity})} \quad (1)$$

Results

Phytoplankton carbon assimilation and biomass carbon-to-phosphorus ratios

Phytoplankton biomass of mobile and non-mobile species, measured as particulate organic carbon, increased with increasing light availability (Fig. 1). The light levels at which the different species reached maximal biomass ranged from 30–300 µmol quanta $m^{-2}s^{-1}$. Two-way ANOVA showed clear effects of light availability and mobility on phytoplankton biomass (Table 1). We also found a significant interaction between light availability and mobility on phytoplankton biomass (p<0.05). Mobile algal species had lower biomasses than non-mobile species; however, the differences appeared only at light intensities of 30 µmol quanta $m^{-2}s^{-1}$ and above (Fig. 1j). At the end of the experiment, mean carbon content of mobile species ranged between 0.65 mg C L^{-1} (SE=0.07) and 1.57 mg C L^{-1} (SE=0.25), while mean carbon content of non-mobile species ranged between 0.78 mg C L^{-1} (SE=0.11) and 2.98 mg C L^{-1} (SE=0.33).

Phytoplankton biomass carbon-to-phosphorus ratios also increased with increasing light availability, with the highest biomass carbon-to-phosphorus ratios occurring at light intensities above 30 µmol quanta $m^{-2}s^{-1}$ (Fig. 2). A two-way ANOVA revealed a significant effect of light and mobility on algal biomass carbon-to-phosphorus ratios (Table 1). Non-mobile species had higher biomass carbon-to-phosphorus ratios than mobile ones (Fig. 2j). No significant interaction between mobility and light intensity on biomass stoichiometry was found.

Specific net primary production

Haematococcus pluvialis, *Staurastrum tetracerum*, *Golenkinia brevispicula* and *Monoraphidium* sp. had too low primary production to be quantified with the oxygen method. We therefore excluded them from further analyses. Specific net primary production of the remaining species increased with light availability (Fig. 3a-e). We performed a two-way

ANOVA to analyze the effects of light and mobility on primary production. Both light intensity and mobility had significant effects on primary production (Table 1). Non-mobile species showed higher primary production than mobile ones (Fig. 3f). A significant interaction between light availability and mobility on phytoplankton primary production existed (Table 1). Differences in primary production between mobile and non-mobile species increased with increasing light intensity (Fig. 3j).

Mobile species had, on average, lower maximal photosynthetic rates (P_{max}= 86.75; Table 2) and higher half-saturation coefficients for P_{max} (k_s= 84.66; Table 2) than non-mobile species (P_{max}= 140.85; k_s = 52.36; Table 2). The relationship between respiration and maximal production of mobile species was higher than that of non-mobile species (Table 2). All mobile species needed higher light intensities to balance respiratory losses (Table 2). On average, the minimal light intensity to achieve a positive net primary production in non-mobile species was 6.04 µmol quanta $m^{-2}s^{-1}$, compared to 17.47 µmol quanta $m^{-2}s^{-1}$ in mobile species (Table 2).

Discussion

Sinking losses are a main cause of phytoplankton mortality. Photosynthetic organisms must maintain themselves in the euphotic zone, where light intensity is strong enough to yield a positive carbon balance. Phytoplankton sinking velocity is influenced by species-specific parameters such as its particle radius, its relative density in comparison to water and its deviation from the shape of a volume-equivalent sphere (the so-called form resistance) (Lampert and Sommer 2007). Environment-specific factors influencing sinking losses are the depth of the mixed layer of a lake and the intensity of turbulences within the water column. For non-mobile species under natural conditions, it is left to chance which light intensity and resource concentrations they are exposed to. Mobile species, in contrast, are, to a certain degree, able to perform directional migrations to obtain optimal resource availability. Such migrations may result from opposing gradients of light and nutrient availability, allowing mobile species to choose the optimal vertical position in a (poorly mixed) water column (Pick et al. 1984, Knapp et al. 2003). Additionally, mobility can result in a more efficient nutrient uptake because laminar layers around algae, which hinder nutrient diffusion, are reduced with increasing swimming speed (Lampert and Sommer 2007). Therefore, the question arises why a certain degree of mobility is not found in all phytoplankton species.

Costs of mobility consist of higher losses of carbon for basic metabolism and higher demand for nutrients. Mobility leads to an increased demand for phosphorus because of an augmented need for the phosphorus-containing molecule ATP. Possible advantages of mobility depend, therefore, on environment factors such as the amount and spatial distribution of resources and the resulting trade-offs for growth and reproduction.

Our data support the assumption that mobility may affect algal biomass stoichiometry. We found clear differences in phytoplankton biomass carbon-to-phosphorus ratios between mobile and non-mobile species (Fig. 2j). Both mobile and non-mobile species showed higher biomass carbon-to-phosphorus ratios with increasing light availability. However, on average,

mobile species kept their biomass carbon-to-nutrient ratio lower than non-mobile species (Fig. 2j). The high amounts of structural carbon in the cell walls of non-mobile species may additionally intensify differences in biomass stoichiometry between mobile and non-mobile phytoplankton species.

To keep biomass carbon-to-phosphorus ratios low, mobile species must incorporate less carbon, have higher respiration losses or incorporate higher amounts of phosphorus. Indeed, our photosynthetic measurements pointed out that all mobile species needed higher light intensities to compensate for respiratory losses and had, on average, lower carbon incorporation at light saturation. This supports earlier measurements (Harris 1978, Cushing 1989) showing that the relationship between respiration and maximal production of mobile species is worse than that of non-mobile species.

All samples received the same amount of the limiting nutrient, phosphorus. Therefore, the lower biomass carbon production of mobile species reflects their lower resource use efficiency, which is a measure of how much carbon per unit phosphorus can be produced (Ptacnik et al. 2008).

Combining mobility and mixotrophic nutrition (nearly all flagellates use a combination of phototrophic and phagotrophic production (Raven 1997)) would be a means to gain sufficient phosphorus for mobility in environments where dissolved phosphorus is scarce. Phosphorus is often several orders of magnitude more concentrated in the biomass of bacteria than in the water (Vadstein 2000), and mixotrophic algae grazing on bacteria could access this substantial phosphorus source. A study dealing with the effect of mixotrophic species on phytoplankton biomass stoichiometry reported similar results (Katechakis et al. 2005) as observed in our study with mobile species. Mixotrophic species showed much lower biomass carbon-to-phosphorus ratios than purely autotrophic algae. This resulted in a constant high-quality food for herbivores. Mixotrophic species in the study by Katechakis et al. (2005) were all mobile; thus, the question remains whether the low biomass carbon-to-phosphorus ratios were a consequence of mobility, mixotrophic nutrition or both. Therefore, further research

should examine the amount of mixotrophic nutrition within mobile phytoplankton species and the contribution of mixotrophy and mobility to phytoplankton biomass stoichiometry.

Mobility involves costs in terms of structures and higher energy expenditures necessary for movement. Our results show that mobile species needed higher minimal light intensities to have a positive carbon balance (Table 2), indicating that mobile species needed more energy to compensate for respiration losses. Our laboratory experiment, conducted in vessels with limited volume, where mobile species had no possibility to profit from their ability to move, pointed out the costs and disadvantages of this strategy. In pelagic environments with deep mixed layers, where sinking losses are low and the spatial separation of light and nutrients is often less pronounced, mobile species are probably worse competitors. Experiments investigating the effects of turbidity and mixing depth on the proportion of mobile algae within phytoplankton communities were in agreement with these assumptions (Jäger et al. 2008). High mixing depth and high turbidity resulted in a competitive advantage of non-mobile species such as diatoms.

Experiments where resources have been manipulated to large extents often result in large shifts in biomass stoichiometry. Major variations of light availability generally result in strong effects on phytoplankton biomass composition (Urabe et al. 2002, Striebel et al. 2008), but sometimes such manipulations induce only weak stoichiometric responses (Diehl, personal communication). The proportion of mobile species with low biomass carbon-to-phosphorus ratios within phytoplankton communities may be an important factor helping to explain the large variation in phytoplankton stoichiometric responses to environmental resource fluctuation.

Additionally, the strength of shifts in biomass composition of phytoplankton communities has consequences beyond phytoplankton ecophysiology. Phytoplankton–zooplankton interactions can be strongly influenced by phytoplankton stoichiometry. Fast-growing herbivorous zooplankton species, such as *Daphnia*, have high demands for phosphorus and therefore exhibit relatively low biomass carbon-to-phosphorus ratios. The low biomass

carbon-to-phosphorus ratios and the absence of distinct cell walls of mobile phytoplankton species can result in high assimilation efficiencies from mobile species when grazed by *Daphnia*. The proportion of mobile species within phytoplankton communities may therefore influence the transfer efficiency between phytoplankton and fast-growing zooplankton, thus influencing the whole food web dynamic in limnetic pelagic ecosystems. Further studies that manipulate the proportion of mobile species in phytoplankton communities grazed by *Daphnia* may allow a detailed investigation of this possible link between phytoplankton composition, mobile phytoplankton species and herbivorous zooplankton dynamics.

References

Berger, S. A., S. Diehl, T. J. Kunz, D. Albrecht, A. M. Oucible, and S. Ritzer. 2006. Light supply, plankton biomass, and seston stoichiometry in a gradient of lake mixing depths. Limnol. Oceanogr. **51**:1898-1905.

Crawford, D. W. 1992. Metabolic cost of motility in planktonic protists—theoretical considerations on size scaling and swimming speed. Microbial Ecol. **24**:1-10.

Cushing, D. H. 1989. A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. J. Plankton Res. **11**:1-13.

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

Flöder, S., and C. W. Burns. 2005. The influence of fluctuating light on diversity and species number of nutrients-limited phytoplankton. J. Phycol. **41**:950-956.

Flynn, K. J., and M. J. R. Fasham. 2002. A modelling exploration of vertical migration by phytoplankton. J. Theor. Biol. **218**:471-484.

Guillard, R. R., and C. J. Lorenzen. 1972. Yellow-green algae with chlorophyllide c. J. Phycol. **8**:10-14.

Harris, G. P. 1978. Photosynthesis, productivity and growth: the physiological ecology of phytoplankton. Arch. Hydrobiol. Berl. Ergebn. Limnol. **10**:1-171.

Hessen, D. O., P. J. Faerovig, and T. Andersen. 2002. Light, nutrients, and P : C ratios in algae: Grazer performance related to food quality and quantity. Ecology **83**:1886-1898.

Huisman, J., and F. J. Weissing. 1995. Competition for nutrients and light among phytoplankton species in a mixed water column: Theoretical studies. Water Sci. Technol. **32**:143-147.

Jäger, C. G., S. Diehl, and M. G. Schmidt. 2008. Influence of water depth and mixing intensity on phytoplankton biomass and functional community composition. Limnol. Oceanogr. **in press**.

Jones, R. I. 1993. Phytoplankton migrations: Patterns, processes and profits. Arch. Hydrobiol. Beiheft Ergebnisse der Limnologie **39**:67-77.

Katechakis, A., T. Haseneder, R. Kling, and H. Stibor. 2005. Mixotrophic versus photoautotrophic specialist algae as food for zooplankton: The light: nutrient hypothesis might not hold for mixotrophs. Limnol. Oceanogr. **50**:1290-1299.

Knapp, C. W., F. deNoyelles, D. W. Graham, and S. Bergin. 2003. Physical and chemical conditions surrounding the diurnal vertical migration of *Cryptomonas* spp. (Cryptophyceae) in a seasonally stratified midwestern reservior (USA). J. Phycol. **39**:855-861.

Lampert W., and U. Sommer. 2007. Limnoecology., Second edition. Oxford University Press Inc., New York.

Park, S., M. T. Brett, D. C. Müller-Navarra, and C. R. Goldman. 2002. Essential fatty acid content and the phosphorus to carbon ratio in cultured algae as indicators of food quality for *Daphnia*. Freshwater Biol. 47:1377-1390.

Pick, F. R., D. R. S. Lean, and C. Nalewajko. 1984. Nutrient status of metalimnetic phytoplankton peaks. Limnol. Oceanogr. **29**:960-971.

Ptacnik, R., A. G. Solimini, T. Andersen, T. Tamminen, P. Brettum, L. Lepistö, E. Willén, and S. Rekolainen. 2008. Diversity predicts stability and resource use efficiency in natural phytoplankton communities. Proc. Natl. Acad. Sci. USA **105**:5134-5138.

Ralston, D. K., D. J. McGillicuddy, and D. W. Townsend. 2007. Asynchronous vertical migration and bimodal distribution of motile phytoplankton. J. Plankton Res. **29**:803-821.

Raven, J. A. 1997. Phagotrophy in phototrophs. Limnol. Oceanogr. **42**:198-205.

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.

Striebel, M., G. Spörl, and H. Stibor. 2008. Light-induced changes of plankton growth and stoichiometry: Experiments with natural phytoplankton communities. Limnol. Oceanogr. **53**:513-522.

Tilman D. 1982. Resource competition and community structure. Princeton University Press, Princeton, New Jersey, USA.

Urabe, J., M. Kyle, W. Makino, T. Yoshida, T. Andersen, and J. J. Elser. 2002. Reduced light increases herbivore production due to stoichiometric effects of light/nutrient balance. Ecology **83**:619-627.

Urabe, J., and R. W. Sterner. 1996. Regulation of herbivore growth by the balance of light and nutrients. Proc. Natl. Acad. Sci. USA **93**:8465-8469.

Vadstein, O. 2000. Heterotrophic, planktonic bacteria and cycling of phosphorus - Phosphorus requirements, competitive ability, and food web interactions. Advances in Microbial Ecology **16**:115-167.

Wall, D., and F. Briand. 1979. Response of lake phytoplankton communities to *in situ* manipulations of light intensity and colour. J. Plankton Res. **1**:103-112.

Wetzel R. G., and G. E. Likens. 2003. Limnological Analyses., 3 edition. Springer-Verlag, New York

Table 1:

Results from two-way ANOVAs for biomass carbon content (POC), biomass carbon-to-phosphorus ratios (C:P) and specific net primary production (sNPP).

Dependent variable	Source of variation					
	Light		Mobility		Interaction	
POC	$F_{4,123}$=16.6	p <0.001	$F_{1,123}$=32.1	p<0.001	$F_{4,123}$=3.0	p=0.021
C:P	$F_{4,123}$=11.4	p <0.001	$F_{1,123}$=7.6	P=0.007	$F_{4,123}$=0.5	p=0.761
sNPP	$F_{4,65}$=41.2	p <0.001	$F_{1,65}$=27.9	P<0.001	$F_{4,65}$=3.2	p=0.018

Table 2:

Photosynthetic parameters (±SE) estimated from photosynthesis–light intensity curves, as described in the Method Section, of different mobile and non-mobile phytoplankton species and mean values (±SE) for mobile and non-mobile species: $Light_{comp}$ [µmol photons m^{-2} s^{-1}], Respiration [µg C mg $C_{initial}^{-1}$ h^{-1}], P_{max} [µg C mg $C_{initial}^{-1}$ h^{-1}], k_s [µmol photons m^{-2} s^{-1}] . Additionally, statistical data (r^2, p) of the regressions are shown.

	Chlamydomonas sp.	*Phacotus* sp.	*Carteria* sp.	Mobile species	*Scenedesmus* sp.	*Tetraedron* sp.	Non-mobile species
$Light_{comp}$	12.94	34.03	10.77	17.47	6.60	5.11	6.04
Respiration	-18.05 (1.47)	-21.43 (2.17)	-5.29 (4.21)	-14.84 (2.35)	-19.55 (6.34)	-9.40 (2.59)	-14.56 (10.52)
P_{max}	197.98 (6.36)	52.43 (2.82)	24.29 (4.47)	86.75 (5.81)	171.64 (8.43)	110.25 (3.74)	140.85 (14.52)
k_s	129.04 (12.48)	49.23 (14.04)	38.64 (37.10)	84.66 (22.47)	51.32 (13.03)	54.86 (9.48)	52.36 (28.03)
Resp / P_{max}	0.09	0.41	0.22	0.17	0.11	0.09	0.10
r^2	0.99	0.97	0.7	0.99	0.98	0.99	0.98
p	<0.0001	<0.0001	0.0006	0.0035	<0.0001	<0.0001	0.0164

Figure Legends

Figure 1:

Biomass data, determined as particulate organic carbon (at the end of the experiment—day 14) from non-mobile (triangles; A: *Golenkinia brevispicula*; B: *Monoraphidium* sp.; C: *Scenedesmus* sp.; D: *Staurastrum tetracerum*; E: *Tetraedron minimum*) and mobile (circles; F: *Carteria* sp.; G: *Chlamydomonas* sp.; H: *Haematococcus pluvialis*; I: *Phacotus lenticularis*) species related to light availability. J: Mean values from mobile (circles) and non-mobile (triangles) species are displayed with standard errors; note different y-axis scaling.

Figure 2:

Biomass carbon-to-phosphorous ratios (at the end of the experiment—day 14) from non-mobile (A-E; triangles, labeling as in Fig. 1) and mobile (F-I; circles, labeling as in Fig. 1) species related to light availability. J: Mean values from mobile (circles) and non-mobile (triangles) species are displayed with standard errors; note different y-axis scaling.

Figure 3:

Specific net primary production (µg carbon assimilation per mg initial biomass (POC) per hour) measured from monocultures of mobile (circles; A: *Carteria* sp.; B: *Chlamydomonas* sp.; C: *Phacotus lenticularis*) and non-mobile (triangles; D: *Scenedesmus* sp.; E: *Tetraedron minimum*) species and related to light availability. F: Mean values from mobile (circles) and non-mobile (triangles) species are displayed with standard errors.

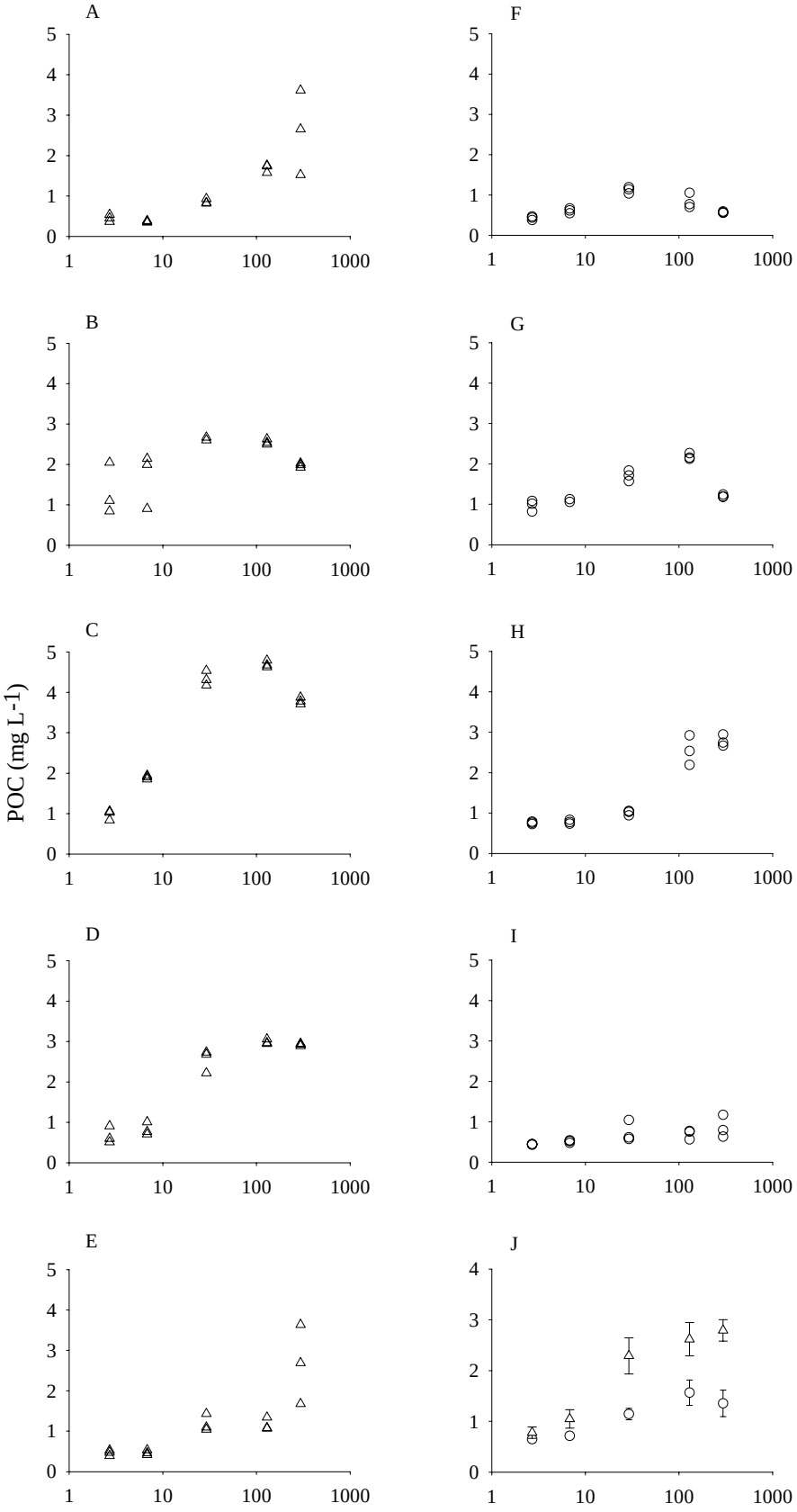


Fig. 1

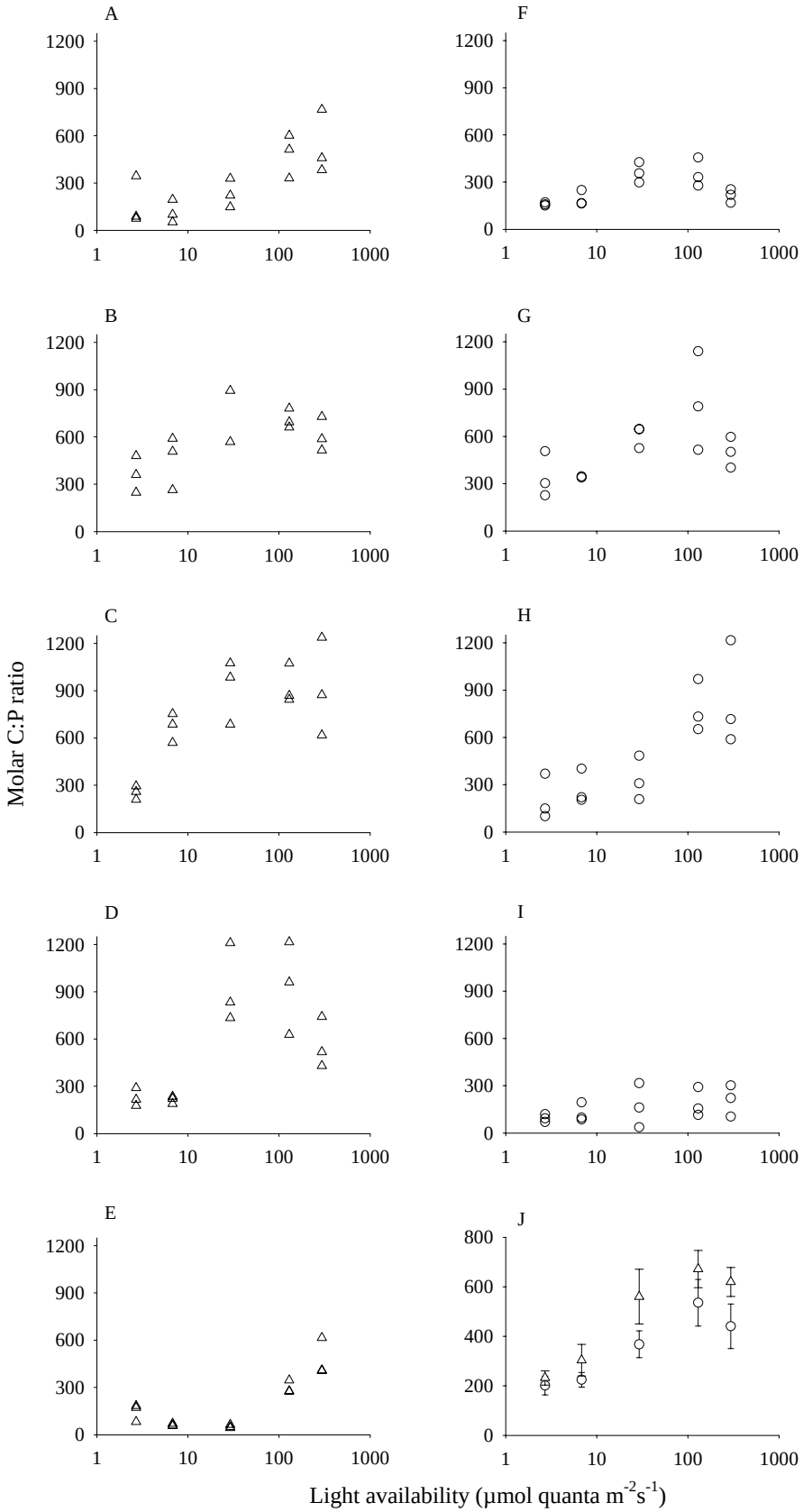

A
F
B
G
C
H
D
I
E
J
Molar C:P ratio
Light availability (µmol quanta m-2s-1)

Fig. 2

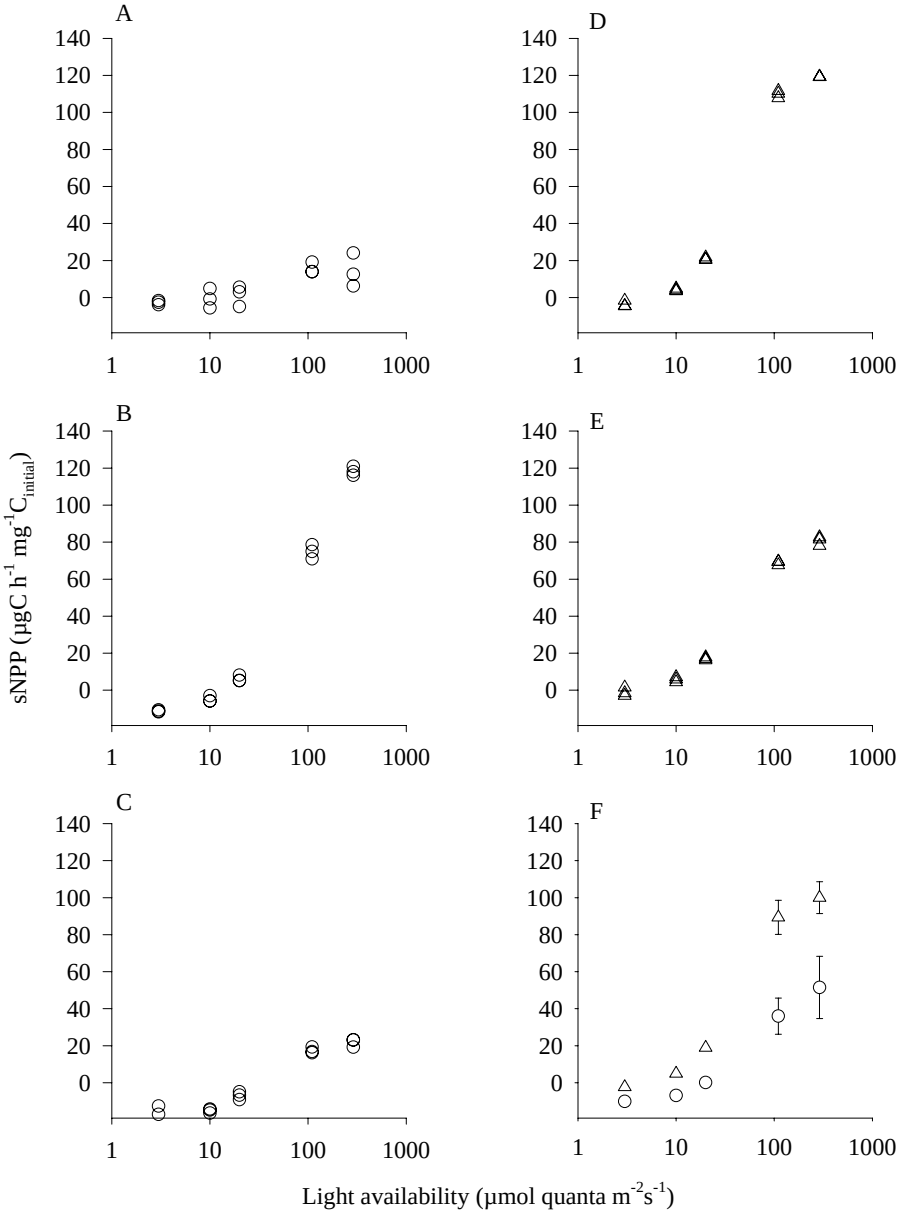

A
B
C
D
E
F
sNPP (µgC h-1 mg-1Cinitial)
Light availability (µmol quanta m-2s-1)
0
20
40
60
80
100
120
140
1
10
100
1000

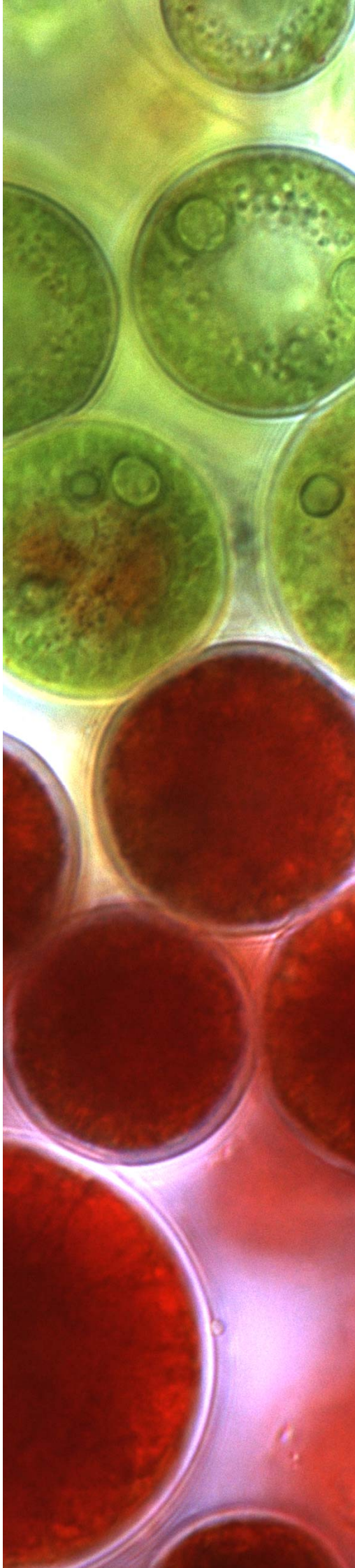
Fig. 3

PAPER 5

COMBINING DIALYSIS AND DILUTION TECHNIQUES TO ESTIMATE GROSS GROWTH RATE OF PHYTOPLANKTON AND GRAZING BY MICRO- AND MESOZOOPLANKTON *IN SITU*

H. STIBOR, A. GELZLEICHTNER, F. HANTZSCHE, U. SOMMER, M. STRIEBEL, O. VADSTEIN, Y. OLSEN. 2006.

ARCHIV FÜR HYDROBIOLOGIE 167 (1-4): 403-419.



Abstract

Measurements of *in situ* phytoplankton growth and grazing by zooplankton normally involve different techniques. We show that a single dilution experiment based on dialysis bags can be used to do these estimates *in situ*. Dialysis bags allow an estimate of the *in situ* phytoplankton gross growth rate whereas the dilution gradient allows a simultaneous estimate of microzooplankton grazing. The comparison of the phytoplankton net growth rate outside dialysis bags and the estimated apparent growth rate of phytoplankton in undiluted samples within dialysis bags allows estimating additional loss processes such as mesozooplankton grazing. The method is especially useful in mesocosms experiments.

Introduction

Phytoplankton dynamic is controlled by the balance between growth and mortality. Phytoplankton production is considered to be controlled by the rate of nutrient supply or light, and final abundances and net growth rates are considered to be determined by predation pressure, by nutrient supply or by both. This simple framework has been routinely used to explain pelagic food web dynamics in experiments and empirical analysis of databases of phytoplankton. To study such mechanisms of pelagic food web dynamics in detail, mesocosm experiments have become an increasingly important tool in plankton research. Additionally, the understanding of those mechanisms structuring pelagic food webs demands for estimates of rates and fluxes within the food web beside biomass estimates of the different food web compartments. The question of how close the growth rates of the phytoplankton are to maximal phytoplankton growth rates in a certain environment is important to estimate to what extent bottom-up vs. top-down factors are acting on a phytoplankton population. Several techniques can be used to study factors influencing *in situ* growth dynamics of phytoplankton. Most methods involve incubation of samples in closed bottles. The use of bottle incubations has, however, several drawbacks. The primary disadvantage lies in the chemical isolation of the incubation bottles from the surrounding water. Depending on the experimental treatment (grazer removal, dilution) nutrient ratios, supply rate and demand might strongly differ between incubation bottles and *in situ* conditions and between bottles with different treatments (Furnas 1982). Nutrient addition at concentrations saturating gross phytoplankton growth rates has been used as a way out of the dilemma. The addition of nutrients can guarantee identical gross growth rates in bottles but the resulting gross growth rates could be different from *in situ* conditions, if nutrients are limiting. The incubation of natural phytoplankton communities enclosed within dialysis bags suspended *in situ* is one of the most reliable approaches to estimate the *in situ* growth rates of marine phytoplankton (Furnas 1990). This technique has been successfully used to

estimate *in situ* growth rates of phytoplankton (e. g., Sakshaug 1977, Sakshaug & Jensen 1978, Mura & Agusti 1996). The advantage of dialysis bag experiments is that they allow the maintenance of chemical exchange between the enclosed population and the surrounding medium, and also that they allow an estimation of growth rates for a wide range of taxa (Furnas 1990). The major disadvantages of the estimation of algal growth rates using incubation in dialysis bags are the relatively long time required relative to other techniques (e. g. tracer incorporation) and the possibility that grazers are included. This is especially important for microzooplankton (largely nanoflagellates, ciliates and very small immature stages of metazoan grazers) which cannot be separated from phytoplankton by screening. Microzooplankton grazing can be an important source of algal mortality so that inclusion of micrograzers would strongly influence the estimation of growth rates. Moreover, grazer activity can contribute nutrients that may enhance algal growth on time – scales of days to weeks.

Three approaches have been used to study the effect of micro-grazers on prey: a) following the population dynamics of both groups during a grazing period, b) tracing labeled prey in a water sample and c) experimental reduction of grazing pressure by dilution and measuring the growth of the prey at the different dilutions. Advantages and drawbacks of these methods have been described in detail by several authors (e. g Landry 1994, Sherr & Sherr 1994, Vaque et al. 1994). Whereas the first approach has been extensively explored to follow predator – prey dynamics and the second approach has been most often employed as a tool to quantify bacteriovory, the third approach proved to be fruitful when the grazing pressure on phototrophic organism is studied.

Established methods to estimate microzooplankton grazing impact on natural communities of marine phytoplankton are dilution experiments (Landry & Hassett 1982). Dilution reduces encounter rates between phytoplankton and microzooplankton. Natural assemblages of phytoplankton and grazers are diluted with filtered seawater in a dilution series. The microzooplankton grazing rate is estimated as the slope of a regression of apparent

phytoplankton growth rate in the various dilutions against dilution factor. The approach relies on three assumptions (Landry & Hasset 1982):

1. Individual phytoplankton growth is not directly affected by the presence or absence of phytoplankton *per se*.

2. The probability of a phytoplankton cell being consumed is a direct function of the rate of encounter of consumers with prey cells. The model assumes that the specific grazing rate does not change implying that predators are not food saturated and predators do not increase their feeding activity at low food concentrations.

3. Phytoplankton growth is exponential.

Dilution experiments are now a standard protocol for the estimation of microzooplankton herbivory (Burkill et al. 1993, Landry 1993). However, incubations of the dilution series are normally done in bottles and have therefore the above described disadvantages (for a detailed discussion of dilution techniques and nutrient dynamics see Andersen et al. 1991). Here, we describe how the combination of the dilution technique to estimate microzooplankton grazing with the incubation of the dilution treatments in dialysis bags *in situ* can be used to estimate gross growth rates of phytoplankton together with microzooplankton and mesozooplankton grazing rates in mesocosms experiments. Dialysis bags have already been used to incubate dilution samples *in situ* by Landry & Hasset (1982) to test whether the amount of nutrients added to natural phytoplankton communities enclosed in bottles aboard a research vessel yielded similar growth responses as *in situ* incubations.

However, the combination of dialysis and dilution techniques has to our knowledge never been used regularly to incubate dilution series *in situ*. With the increasing use of mesocosm techniques in plankton research, dilution experiments in dialysis bags could become a useful tool to investigate growth and grazing dynamics of phytoplankton communities. We demonstrate in the following the use of the method in two marine pelagic mesocosms experiments.

Material and methods

Marine mesocosm experiments

We carried out several series of marine dilution experiments during two large mesocosm experiments in the bay of Hopavågen, central Norway. For the first experiment we moored 10 mesocosms made from transparent polyethylene tubes to floating stands. The volume of each bag was approximately $5m^3$, with a diameter of 0.9m and a total depth of 6.5 m, consisting of a 6m straight tube and a sealed, conical bottom. We filled the mesocosms on the evening before the start of the experiments by lifting them from 7m depth to the surface and enclosing the natural phytoplankton and zooplankton community. Zooplankton consisted mainly of calanoid copepods of the species *Temora longicornis, Centropages* sp., *Pseudocalanus elongates* and *Acartia longiremis*.

More than half of the biomass was copepods of the genera *Temora* and *Pseudocalanus*, which were fairly equal in biomass. The water columns in the bags did not stratify and were well mixed by wave action. We added nutrients to the mesocosms on the evening of day 1 and each of the evenings thereafter. The nutrient addition was comparable to the natural load of the system (Vadstein et al. 2004). Nutrients were added in an atomic ratio of 16:16:1 for Si:N:P. Si was added as silicate, P as phosphate and N as nitrate and ammonia (1:1) The daily doses of added P was 0.5 µg P l^{-1} d^{-1}. We created gradients of predation pressure on copepods by adding different numbers of ctenophores (0, 5, 10, 20, 40 *Bolinopsis infundibulum*) to different mesocosms.

All treatments were in duplicate. Ctenophores were carefully collected by net hauls with plastic bags mounted on the end of the net. Before the ctenophores were added we emptied the mesocosms from ctenophores by using a net with 1cm mesh width and a diameter of 0.9 m. The treatments with 10 ctenophores per enclosure resembled the natural density of *Bolinopsis* in the bay of Hopavågen at the start of our experiment. Ctenophore numbers in the bags were adjusted two times per week to keep the initial gradient during the experiment.

After 4 weeks, we exposed dilution experiments in dialysis bags within the 10 mesocosms (1.5 m water depth) to investigate whether our experimental manipulation of the ctenophore top predator had a cascading influence on micro- and mesozooplankton grazing rates and phytoplankton gross growth rates (see a detailed description below). Mesocosm walls were exposed to wave action and the dialysis bags were therefore incubated in a well mixed water column.

The second experiment was established to investigate the influence of silicate on trophic cascades within the same marine system. 12 mesocosms such as described above were moored in the bay of Hopavågen and fertilized with different amounts of silicate. More details about the second mesocosm experiment are described elsewhere (Sommer et al. 2005). We performed dilution experiments in dialysis bags to estimate the effect of our experimental manipulation on phytoplankton gross growth rates, and micro- and mesozooplankton grazing three weeks after the start of the experiments. We enclosed natural phytoplankton from the mesocosms within dialysis bags along a dilution gradient and suspended the bags *in situ* in 1.5 m water depth (see a detailed description below).

General methods for both experiments

Dilution experiments

Bags with a volume of 250 ml were built with dialysis membrane tubes with a molecular weight cut-off of 6000. This allowed diffusion of molecules smaller than proteins which equilibrilate rapidly with ambient water (<4h, Mura et al. 1996; <8h, Striebel, unpubl. results). Dialysis tubes were hydrated by soaking them in deionised water for 12 h prior to use. Dialysis cultures consisted of depth integrated samples from well mixed enclosures. Samples were taken with a tube sampler and filtered through a 200 µm mesh to exclude macrozooplankton. The original sample was diluted with GF/F filtered water from the same water body in 5 steps. The share unfiltered water was 12.5 %, 25 %, 50 %, 75% and 87.5 %. One or two replicate dialysis bags were prepared for each dilution step. Samples were

incubated for 48 hours and this incubation period resulted in a clear and measurable growth response of phytoplankton in all experiments. Changes in the phytoplankton abundance in the mesocosms in samples taken at the beginning and the end of the incubation period were assumed to represent the net rates of population change (Toth 1980, Furnas 1990, Mura et al. 1996).

After incubation, dialysis tubes were opened and sub samples (50ml) were filtered onto GF/F filters and, following methanol extraction, analyzed for chlorophyll-*a* (a common tracer for dilution experiments, Böttjer & Morales 2005) using a Turner design fluorometer (Strickland & Parsons 1972). Additionally 100 ml sub-samples were fixed with Lugol's iodine and counted according to Utermöhl's inverted microscope technique (Utermöhl 1958). If possible, 400 individuals per category were counted which gives 95% confidence limits of ± 10%, if cells are randomly distributed (Lund et al. 1958). Thereby we want to demonstrate the ability of the technique to follow growth and grazing of single algal species and groups. More examples about the use of this technique to follow growth and grazing dynamics of single algal species and groups can be found in Sommer et al. 2005.

Net growth rates (r; in d-1) in the mesocosms, epilimnion and in the dialysis bags were calculated as:

$$r = (\ln N_2 - \ln N_1)/(t_2-t_1) \quad (1)$$

where N1 and N2 correspond to the initial and final phytoplankton concentrations, t1 and t2 to the initial and final incubation times, respectively. As a measure of total phytoplankton concentration we used chlorophyll *a* concentrations (in $\mu g\ l^{-1}$). In order to determine net growth rates of individual algal, species algae biovolume ($\mu m^3\ l^{-1}$) was used.

Grazing rates by microzooplankton (γ_{micro}) were calculated by linear regression (y = a +bx) of r in the dialysis bags on the share of unfiltered water (x) where y = r, a (intercept) = μ and b (slope) = - γ_{micro} giving Eq. 2:

$$r = \mu - \gamma_{micro}.x \quad (2)$$

μ reflects the gross growth rate under the *in situ* light and nutrient conditions, without grazers present.

Eq. 2 can be transformed to give Eq.3:

$$\gamma_{micro} = (r - \mu)/x \quad (3)$$

Grazing rates by mesozooplankton (copepods; γ_{cop}) were calculated as the difference between net phytoplankton growth rates calculated from Eq. 2 for x = 1 (r_1) and r in the mesocosms (r_{meso})

$$\gamma_{cop} = r_1 - r_{meso} \quad (4)$$

Here we assume that the light at the depth of incubation of the dialysis bags approximately equals the average light intensity of the mixed layer in the mesocosms. Furthermore, we also assume that, in the mesocosms, losses through sedimentation were negligible or that other losses were not different between the mesocosms and the dialysis bags. The calculated γcop for control mesocosms without copepods can serve as a check for the realism of these assumptions (see Sommer et al. 2005 for a test of this assumption).

Zooplankton sampling

Mesozooplankton samples were collected with a 200 μm net and counted with a Leitz M3 dissecting microscope. Samples for determination of ciliate biomass were taken with a 2m long Ramberg tube sampler. Integrated composite water samples were taken to represent the whole mesocosms from 0 to 6m depth. Samples were settled in 50 ml Utermöhl chambers and counted in an inverted microscope. Normally >200 cells were counted per sample, which should give a coefficient of variation of <7%.

Results

Experiment 1

Our manipulation of the ctenophore abundances in the different enclosures influenced a trophic cascade down to phytoplankton (Gelzleichter 2002). Increasing ctenophore numbers resulted in decreasing copepod abundances (Fig. 1). Decreasing copepod abundances resulted in increasing ciliate abundances (Fig. 2). Copepod numbers at the time of the dilution experiments (after four weeks) were between 2 and 11 copepods l^{-1} and ciliate abundances were between 27 and 58 ciliates ml^{-1} (Table 1). These abundances are within the natural range in the bay of Hopavågen (Vadstein et al. 2004). Copepods showed a negative correlation with large phytoplankton (diatoms) whereas ciliates had a negative impact on small phytoplankton (Gelzleichter 2002). In the different dilution series apparent growth rates of phytoplankton were highest in dilute waters and decreased with the proportion of unfiltered water (Fig. 3). The estimated gross growth rates of phytoplankton (intercept, μ) were similar between treatments (Table 1, Fig. 4). However, the slopes of the regression of apparent phytoplankton growth rate to dilution (i. e. γ_{micro}) differed and showed a significant linear relation to ciliate abundance (Table 1, Fig. 5).

Microzooplankton grazing was between 0.65 day^{-1} at low ciliate abundances and 0.95 day^{-1} at the highest ciliate abundances. Enclosures with small numbers of ctenophores showed larger stocks of mesozooplankton (Fig. 1) which in turn suppressed micrograzers like ciliates (Fig. 2). Additionally, larger stocks of mesozooplankton resulted in larger mesozooplankton grazing rates (γ_{cop}) on phytoplankton (Fig. 6). Our mesozooplankton grazing estimates ranged from 0.3 day^{-1} at high copepod abundances (10–12 l^{-1}) to around 0 day^{-1} at densities below 2 copepods l^{-1}. The results of our dilution experiments in dialysis bags fitted well to the general results of this and similar mesocosm experiments (Gelzleichter 2002, Hantzsche 2002, Stibor et al. 2004, Vadstein et al. 2004).

Experiment 2

Fig. 7 shows two examples of phytoplankton gross growth rates and micro- and mesozooplankton grazing rates from the second mesocosm experiment. The figures are based on cell count data, demonstrating the use of the method to estimate growth parameters of individual algal groups. Both examples are taken from the same mesocosm, which had been stocked with a natural density of copepods (approximately 20 l^{-1}). The apparent growth rate of nanoflagellates had a significantly negative slope on the share of undiluted water (b=–0.98; s. e. ±0.103; p<0.01; Fig. 7, upper panel). This indicates that nanoflagellates were strongly grazed by microzooplankton. There was almost no difference between the apparent growth rate predicted for an undiluted dialysis bag (no copepod grazing) and the growth rate in the mesocosm (–0.09 d^{-1}), thus indicating no significant grazing by copepods (Fig. 7). In the case of the large diatom *R. hebetata*, the slope of the regression was insignificant (b=0.09; s. e. ±0.056; p=0.541) indicating no grazing by microzooplankton. There was a conspicuous difference between the apparent growth rate for an undiluted dialysis bag and the apparent growth rate in the mesocosm (0.54 d^{-1}). This difference can be taken as an estimate of the strong copepod grazing rate of *R. hebetata* in the mesocosm. Among the species sufficiently abundant for counting, no single species was grazed upon by microzooplankton and copepods >200 µm at the same time (Sommer et al. 2005).

Discussion

The combination of dilution and dialysis techniques allowed us to estimate the *in situ* phytoplankton gross growth rate and the impact of micro-and mesozooplankton on phytoplankton communities and algal species with a single and easy to perform experiment. The combination of these two methods may overcome some of the problems associated with methods estimating apparent phytoplankton growth rates and microzooplankton grazing in bottle experiments as it includes less artificial manipulations. The usually slower growth rates of phytoplankton in bottles may be due to a variety of causes, including nutrient limitation and differences between phytoplankton growth in bottles and more open environments have been already discussed in detail (Furnas 1982).

The combination of dilution and dialysis methods may also give estimates of mesozooplankton grazing in mesocosms experiments where control mesocosms without mesozooplankton can be established to control for additional losses of phytoplankton beyond micro- and mesozooplankton grazing. Techniques to estimate mesozooplankton grazing *in situ* are normally including tracers such as radioactive isotopes. However, it is not always possible to use radioactive tracer methods in the field. Additionally, radioactive tracer methods cannot as easily give simultaneous grazing rates for individual algal groups or species. Our results of the marine mesocosms experiments clearly show that the above described method was able to yield ecological meaningful estimates of phytoplankton gross growth rates and micro- and mesozooplankton grazing. In experiment 1, ctenophores were influencing the abundance of meso- and microzooplankton via trophic cascade effects (Figs 1 and 2). These trophic cascade effects were also visible in the grazing impact of ciliates and copepods on phytoplankton which we estimated by dilution experiments incubate in dialysis bags. (Figs 5 and 6). The phytoplankton gross growth rates which were quantified within the same dilution experiments were similar between the treatments (Fig. 4). We expected this, as all treatments received the same amount of light and nutrients.

The estimates of meso- and microzooplankton grazing rates on different sized algae during experiment 2 were in concordance with estimates received by alternative methods and previous knowledge about size selective grazing in the marine pelagic zone (Sommer & Stibor 2002). According to our dilution experiments in dialysis bags large diatoms were not eaten by microzooplankton but by copepods, whereas small phytoplankton species were eaten by microzooplankton but not by copepods (Fig. 7; Sommer et al. 2005). Alternative methods to obtain such simultaneous estimates of community- and group- or species-specific phytoplankton gross growth rates and meso- and microzooplankton grazing rates in a mesocosms experiment would normally involve a variety of different techniques.

There are limitations associated with the majority of methods estimating growth and grazing parameters in environmental microbiology. A common problem in dilution experiments is that grazers can grow or die during the incubation and that these processes and per-capita feeding rate of grazers are different between undiluted and diluted samples, resulting in uncertainties in measured grazing rates (Gallegos 1989, Evans & Paranjape 1992, Dolan et al. 2000). However, these problems can be met by examining grazer populations during the experiments to assess possible artifacts in grazing rate estimates. Hence, counting the individual algal species in the dilution series by Utermöhl- or other related techniques would allow estimating micrograzer abundances within the same sample.

Whereas we concentrated on microzooplankton grazing influencing the growth response of phytoplankton within the dialysis bags one should have in mind that also a variety of other factors are of importance. The species composition (inherent growth potential), the standing crop in the bags (onset of diffusion limitation), external nutrient concentrations, temperature and irradiance will as well influence phytoplankton growth responses within the dialysis bags and a comparison of such experiments must consider these factors. The effective turnover time for nutrients in dialysis tubes will be dependent upon the type of dialysis tubing used and the shape of the dialysis bags. The surface to volume ratio of the bags and the degree of mixing around and within the bags will influence the diffusion dynamics across the dialysis

membrane. However, detailed studies showed that the half life time for water in comparable dialysis bags were approximately 3 hours (Furnas 1982) and measurements of nutrient dynamics showed that equilibrium with ambient water is reached between 4 and 8 hours (Mura et al. 1996). Limitations of dilution methods are mostly defined by the assumptions on which the method is based and which are described in the introduction. Limitations will mainly evolve from shifts in possible density dependent nutrient competition between phytoplankton due to dilution and a deviation from a linear grazing impact of microzooplankton on phytoplankton. This could result from different microzooplankton population dynamics within the dilution treatments in the dialysis bags (Dolan et al. 2000). The question arises how large deviations from the assumptions can be before estimates of phytoplankton growth and grazing parameters become seriously incorrect. It will take some time before dilution related differences in phytoplankton competition or microzooplankton growth dynamics affect the apparent phytoplankton growth rates in the different dilution treatments. However, the shapes of the growth response of phytoplankton to dilution can be used to assess whether the assumptions of the method are sufficiently met. Non linear growth responses of phytoplankton to dilution will indicate that the assumptions are not valid in that specific case. Our examples demonstrate that the incubation of dilution experiments within dialysis bags in our mesocosms experiments led to linear growth responses of the phytoplankton in all treatments. It seems that the dilution experiments were performed for a short enough period before dilution related differences in competition between phytoplankton and/or microzooplankton population dynamics resulted in non linear growth responses of phytoplankton to dilution. We have shown that the combination of dilution and dialysis techniques can be used to quantify simultaneously how grazer abundance, selective susceptibility to different grazers and nutrient supply will act on phytoplankton communities. Thereby, this method allows an estimate to which extend bottom-up and top-down forces act on phytoplankton dynamics *in situ.* The method is especially useful in mesocosms experiments where the exposition of dialysis bags is normally without problems and control

mesocosms without mesozooplankton can easily be installed to separate between mesozooplankton grazing and other losses such as sedimentation. Therefore, the incubation of dilution experiments within dialysis bags may be an additional useful method to estimate important top-down and bottom-up fluxes within pelagic communities.

References

Andersen, T., Schartau, A. K. L. & Paasche, E. (1991): Quantifying external and internal nitrogen and phosphorus pools, as well as nitrogen and phosphorus supplied through remineralization, in coastal marine plankton by means of a dilution technique. – Mar. Ecol. Progr. Ser. **69:** 67–80.

Böttjer, D. & Morales, C. E. (2005): Microzooplankton grazing in a coastal embayment off Concepcion, Chile during non-upwelling conditions. – J. Plankton Res. **27:** 383–391.

Burkill P. H., Edwards, E. S., Landry, M., Paranjape, M., Reckermann, M., Sieracki, M. E., Sleigh, M. A., Stoecker, D., K. & Verity, P. (1993): Microzooplankton herbivory. – In: Ducklow, H. W. (ed.): Revised JGOFS protocols. Intergovernmental Oceanographic Commision, Paris, pp.185–191.

Dolan, J. R., Gallegos, C. L. & Moigis, A. (2000): Dilution effects on microzooplankton in dilution grazing experiments. – Mar. Ecol. Progr. Ser. **200:** 127–139.

Evans, G. T. & Paranjape, M. A. (1992): Precision of estimates of phytoplankton growth and microzooplankton grazing when functional responses of grazers may be nonlinear. – Mar. Ecol. Progr. Ser. **80:** 285–290.

Furnas, M. J. (1982): An evaluation of two diffusion culture techniques for estimating phytoplankton growth rates *in situ*. – Mar. Biol. **70:** 63–72.

Furnas, M. J. (1990): In situ growth rates of marine phytoplankton: approaches to measurement, community and species growth rates. – J. Plankton Res. **12:** 1117–1151.

Gallegos, C. L. (1989): Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics. – Mar. Ecol. Progr. Ser. **57:** 23–33.

Gelzleichter, A. (2002): Experimentelle Untersuchungen zum Einfluss von Rippenquallen auf marine Nahrungsnetze: Effekte auf das Phytoplankton. – Diploma-thesis, University of Munich.

Hantzsche, F. (2002): Experimentelle Untersuchungen zum Einfluss von Rippenquallen auf marine Nahrungsnetze: Effekte auf das Zooplankton. – Diploma-thesis, University of Munich.

Landry, M. R. (1993): Estimating rates of growth and grazing mortality of phytoplankton by the dilution method. – In: Kemp, P. F., Sherr, B. & Cole, J. J. (eds): Handbook of methods in aquatic microbial ecology. – Lewis Publishers, Ann Arbor, Michigan, pp.715–772.

Landry, M. R. (1994): Methods and controls for measuring the grazing impact of planktonic protests. –Mar. Microb. Food Webs **8:** 37–57.

Landry, M. R. & Hassett, R. P. (1982): Estimating the grazing impact of marine microzooplankton. – Mar. Biol. **67:** 283–288.

Lund, J. W. G, Kipling, C. & LeCren, E. D. (1958): The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. – Hydrobiologia **11:** 143–170.

Mura, M. P. & Agusti, S. (1996): Growth rates of diatoms from coastal Antarctic waters estimated by in situ dialysis incubation. – Mar. Ecol. Progr. Ser. **144:** 237–245.

Mura, M. P., Agusti, S., del Giorgio, P. A., Gasol, J. M., Vaque, D. & Duarte, C. M. (1996): Loss-controlled phytoplankton production in nutrient-poor litoral waters of the NW Mediterranean: *in situ* experimental evidence. – Mar. Ecol. Progr. Ser. **130:** 213–219.

Sakshaug, E. (1977): Limiting nutrients and maximum growth rates for diatoms in Narragansett Bay. – J. exp. Mar. Biol. Ecol. **28:** 109–123.

Sakshaug, E. & Jensen, A. (1978): The use of cage cultures in studies of the biochemistry and ecology of marine phytoplankton. – Oceanogr. Mar. Biol. Ann. Rev. **16:** 81–106.

Sherr, E. B. & Sherr, B. F. (1994): Bakterivorie and herbivory: Key roles of phagotrophic protists in pelagic food webs. – Microb. Ecol. **28:** 223–235.

Sommer, U., Hansen, T., Blum, O., Holzner, N., Vadstein, O. & Stibor, H. (2005): Copepod and microzooplankton grazing in mesocosms fertilised with different Si : N ratios: no overlap between food spectra and Si:N influence on zooplankton trophic level. – Oecologia **142:** 274–283.

Sommer, U. & Stibor, H. (2002): Copepoda – Cladocera – Tunicata: The role of three major mesozooplankton groups in pelagic food webs. – Eco. Res. **17:** 161–174.

Stibor, H., Vadstein, O., Diehl, S., Gelzleichter, A., Hansen, T., Katechakis, A., Lippert, B., Løseth, K., Peters, C., Roederer, W., Sandow, M., Sundt-Hansen, L. & Olsen, Y. (2004): Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. – Ecol. Lett. **7:** 321–328.

Strickland, J. D. H. & Parsons, T. R. (1972): A practical handbook of seawater analysis, 2nd Ed. – Bull. Fish. Res. Bd. Can. **167**.

Toth, L. G. (1980): The use of dialysing sacks in estimation of production of bacterioplankton and phytoplankton. – Arch. Hydrobiol. **89:** 474–482.

Utermöhl, H. (1958): Zur Vervollkommnung der quantitativen Phytoplankton-Methodik.– Mitt. Int. Verein. Limnol. **9:** 263–272.

Vadstein, O., Stibor, H., Lippert, B., Løseth, K., Roederer, W., Sundt-Hansen, L. & Olsen,Y. (2004): Moderate increase in the biomass of omnivorous copepods may release grazing control of planktonic algae. – Mar. Ecol. Progr. Ser. **270:** 199–207.

Vaque, D., Gasol, J. M. & Marrase, C. (1994): Grazing rates on bacteria: the significance of methodology and ecological factors. – Mar. Ecol. Progr. Ser. **109:** 263–274.

Table 1:

Copepod abundance, ciliate abundance, phytoplankton gross growth rates and microzooplankton and mesozooplankton grazing in the different ctenophore treatments after 4 weeks of the experiment. Gross growth rates and microzooplankton grazing were estimated from linear regressions of apparent phytoplankton growth rates on dilution (Fig. 3), standard errors of these estimates are given in parentheses. A and B indicate the two replicate mesocosms per ctenophore treatment.

Treatment [ctenophores enclosure^{-1}]	Copepods [l^{-1}]	Ciliates [ml^{-1}]	Phytoplankton gross growth rate [day^{-1}]	Mikrozooplankton grazing [day^{-1}]	Mesozooplankton grazing [day^{-1}]
0 A	10.1	34.6	1.01 (0.10)	0.91 (0.18)	0.36
0 B	11.2	27.0	0.89 (0.12)	0.53 (0.20)	0.25
5 A	9.8	27.7	0.59 (0.06)	0.62 (0.11)	0.34
5 B	6.2	39.0	0.94 (0.13)	0.70 (0.23)	0.28
10 A	4.2	29.5	0.78 (0.09)	0.63 (0.16)	0.05
10 B	3.4	36.0	1.06 (0.09)	0.88 (0.15)	0.26
20 A	4.2	39.0	0.86 (0.08)	0.89 (0.16)	–0.06
20 B	5.4	48.0	0.86 (0.12)	1.04 (0.21)	0.07
40 A	2.4	54.0	1.13 (0.11)	0.92 (0.19)	–0.05
40 B	2	58.0	0.75 (0.10)	0.93 (0.18)	–0.06

Figure legends

Figure 1: Copepod abundance as a function of initial ctenophore abundance in the mesocosms. Linear regression analysis gave: y = 8.63-0.18x; r^2 = 0.62; p< 0.01. Dotted lines represent 95% confidence intervals.

Figure 2: Ciliate abundance as a function of copepod abundance in the mesocosms. Linear regression analysis gave: y = 52.9-2.32x; r^2 = 0.51; p< 0.05. Dotted lines represent 95% confidence intervals.

Figure 3: Apparent growth rate of phytoplankton (circles) in dilution series in mesocosms with different ctenophore densities (0 – 40 individuals per mesocosms). Triangles indicate apparent growth rates in mesocosms (r_{meso}). The difference between the regression line and r_{meso} is an estimate of mesozooplankton (copepod) grazing (γ_{cop}). Open and filled symbols represent the two replicate mesocosms per ctenophore treatment.

Figure 4: Gross growth rate of phytoplankton in the different ctenophore treatments. Linear regression analysis gave: y = 0.87+0x; r^2 = 0.01; p = 0.73. Dotted lines represent 95% confidence intervals.

Figure 5: Microzooplankton grazing as a function of ciliate abundance. Linear regression gave: y = 0.34+0.01x; r^2 = 0.58; p = 0.01. Dotted lines represent 95% confidence intervals.

Figure 6: Mesozooplankton grazing as a function of copepod abundance. Linear regression gave: y = -0.1+0.04x; r^2 = 0.66; p< 0.01. Dotted lines represent 95% confidence intervals.

Figure 7: Apparent growth rates of unidentified nanoflagellates and of *Rhizosolenia hebetata*

(black circles) in the dilution series in a copepod containing mesocosm. Triangles indicate apparent growth rates in mesocosm (r_{meso}). The difference between the regression line and r_{meso} is an estimate of mesozooplankton (copepod) grazing (γ_{cop}). Dotted lines represent 95% confidence intervals.

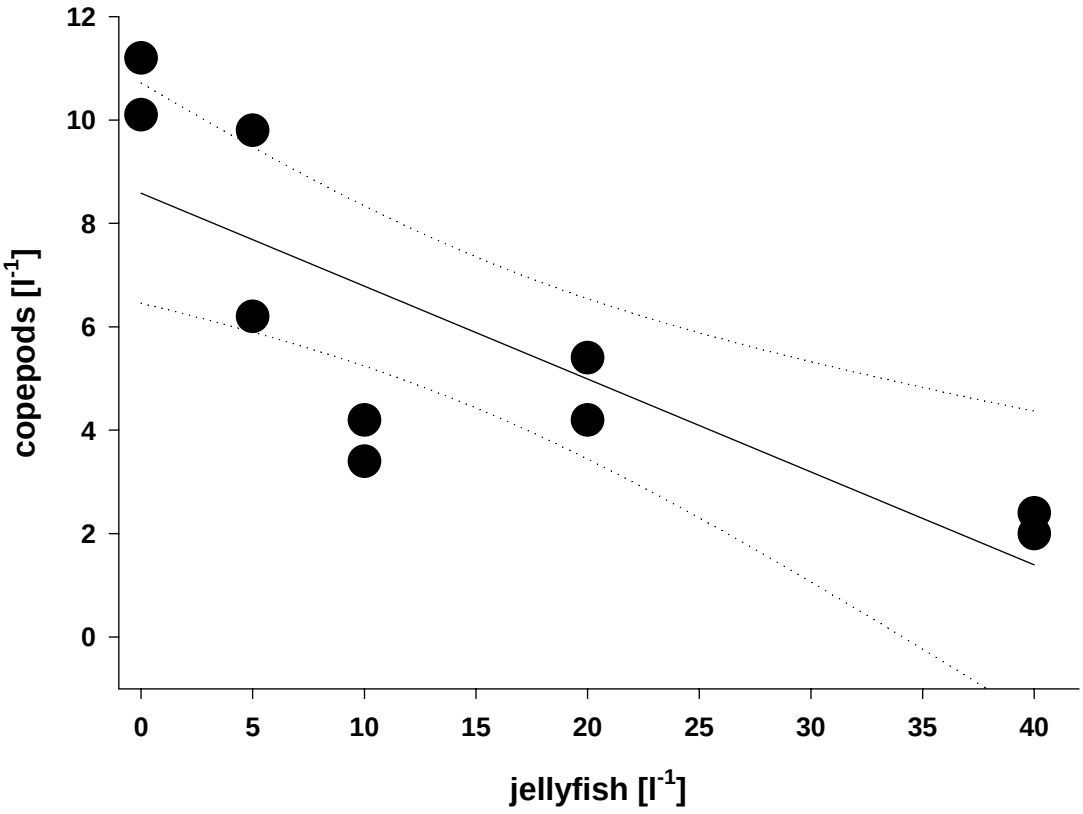


Figure 1

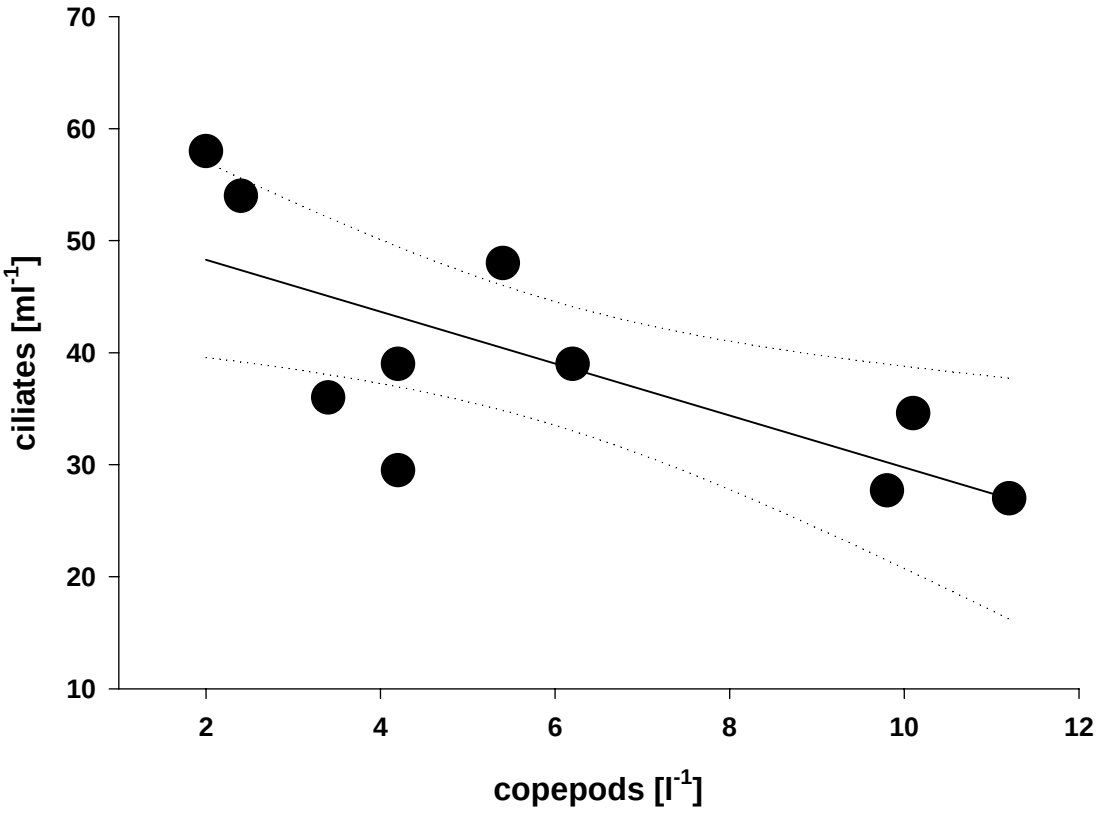


Figure 2

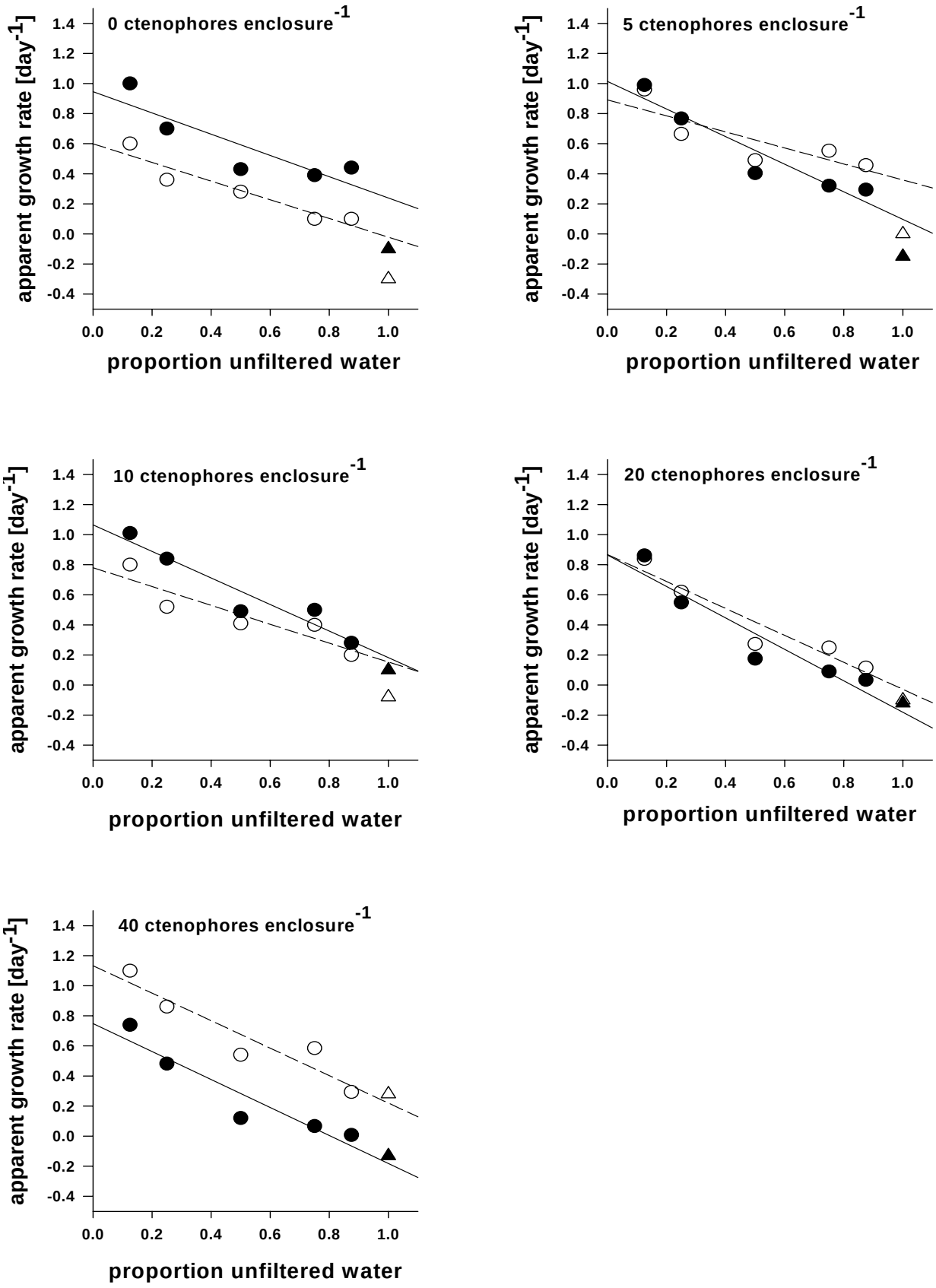

0 ctenophores enclosure-1
5 ctenophores enclosure-1
10 ctenophores enclosure-1
20 ctenophores enclosure-1
40 ctenophores enclosure-1
apparent growth rate [day-1]
proportion unfiltered water
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
0.0
0.2
0.4
0.6
0.8
1.0

Figure 3

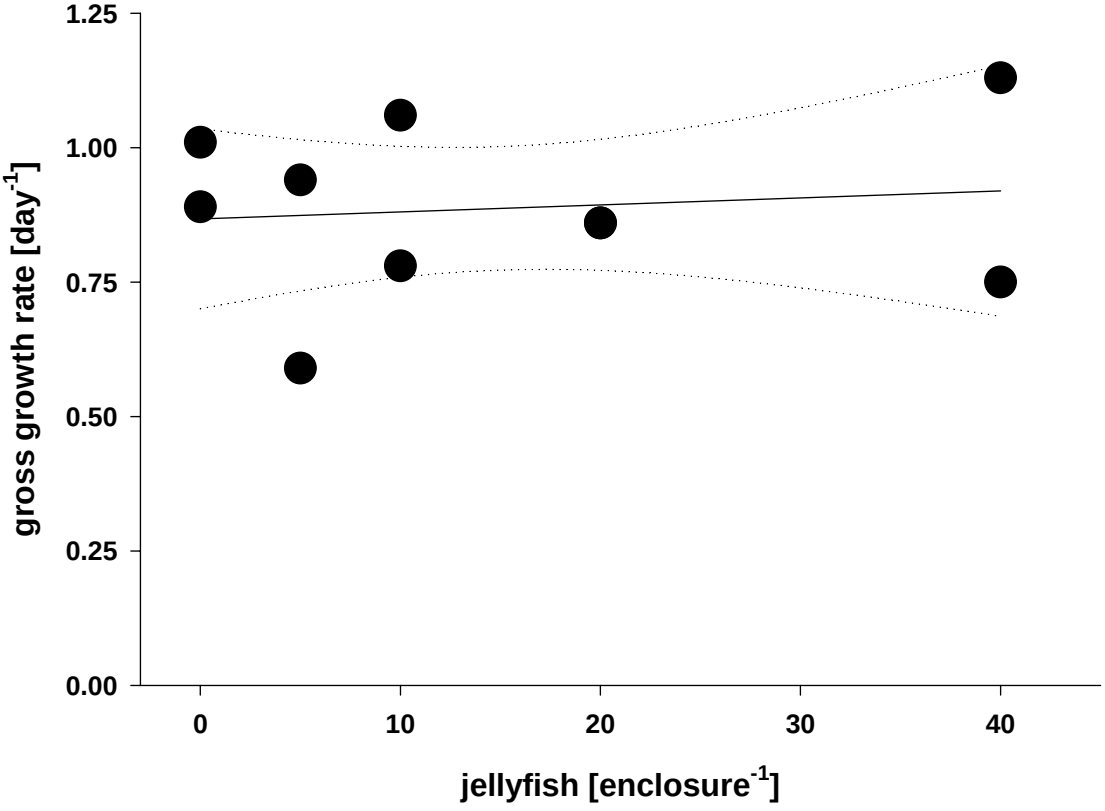

1.25
1.00
0.75
0.50
0.25
0.00
gross growth rate [day-1]
0
10
20
30
40
jellyfish [enclosure-1]

Figure 4

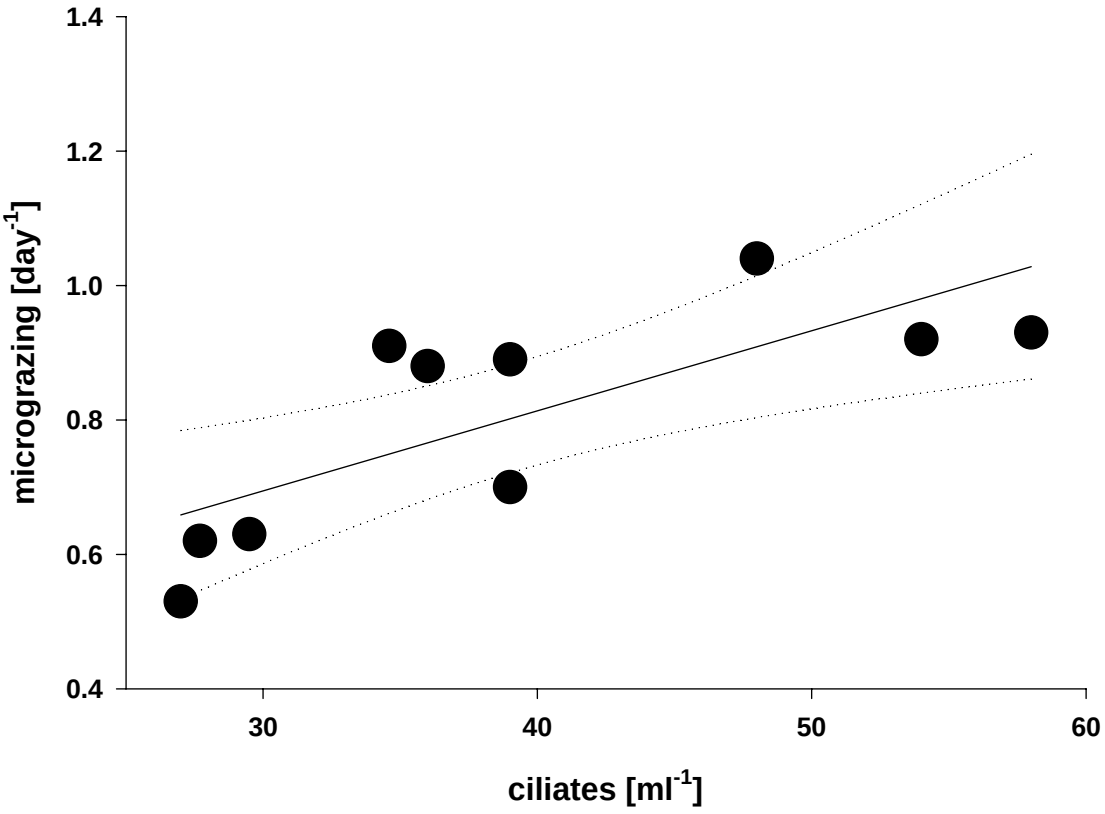


Figure 5

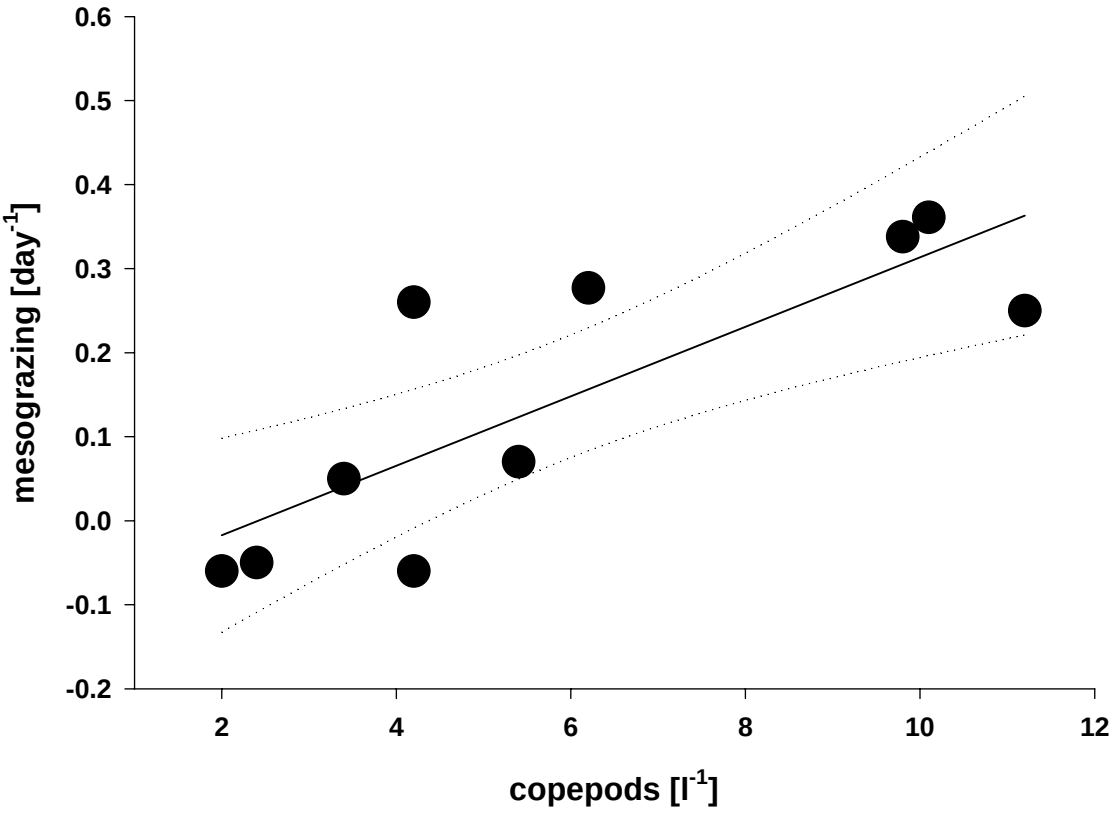

0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
mesograzing [day-1]
2
4
6
8
10
12
copepods [l-1]

Figure 6

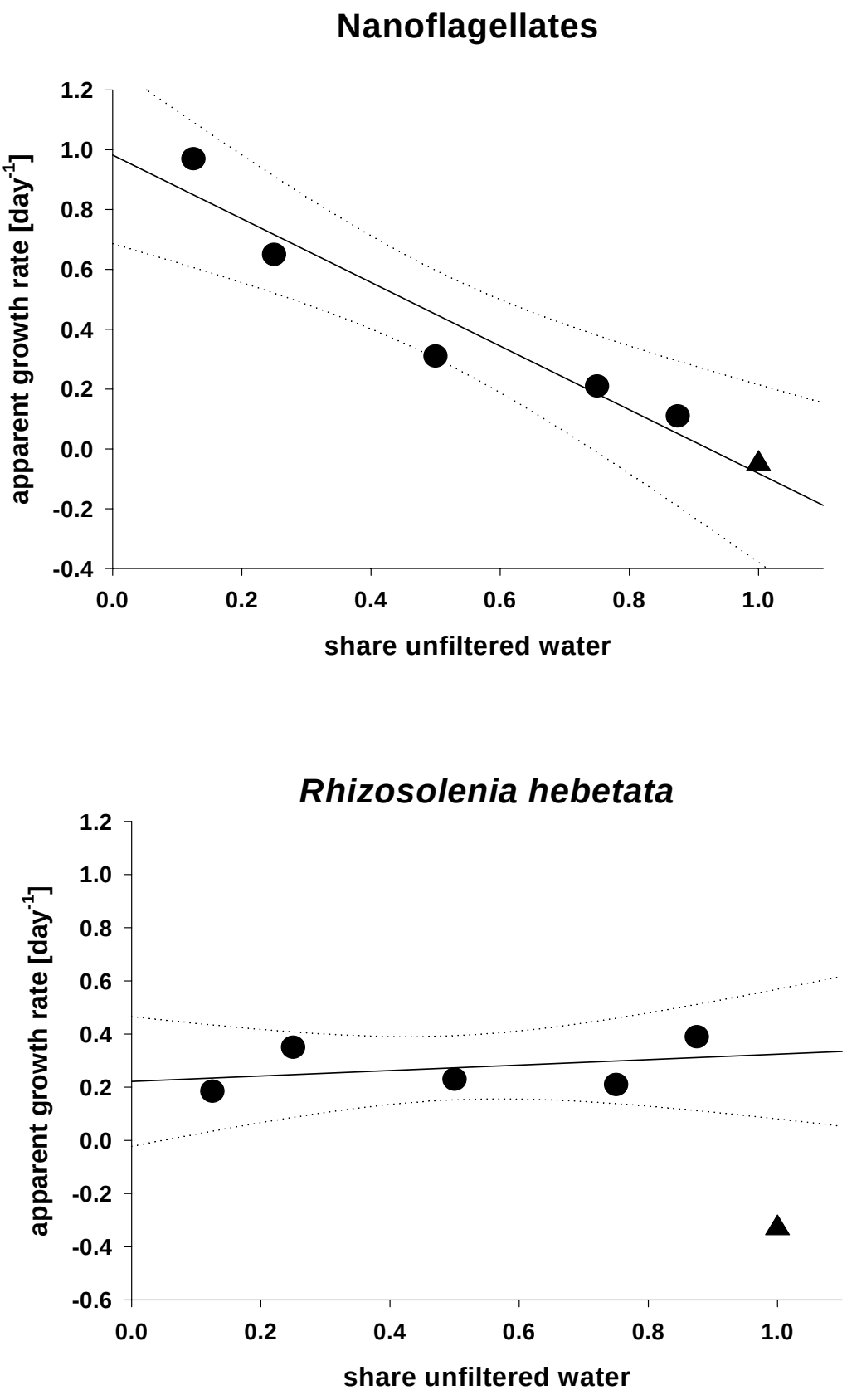

Nanoflagellates
apparent growth rate [day-1]
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
0.0
0.2
0.4
0.6
0.8
1.0
share unfiltered water
Rhizosolenia hebetata
apparent growth rate [day-1]
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
0.0
0.2
0.4
0.6
0.8
1.0
share unfiltered water

Figure 7

3. GENERAL DISCUSSION AND OUTLOOK

In my thesis I investigated the importance of light, nutrients and diversity for phytoplankton dynamics. I considered these interactions from different ecological perspectives, including ecophysiology, population and community dynamics and behavioural aspects.

EFFECTS OF DIVERSITY ON PHYTOPLANKTON RESOURCE UPTAKE AND GROWTH

A recent analysis of more than 3000 phytoplankton samples from freshwater and brackish habitats showed that species richness is the best predictor for resource use efficiency (= phototrophic carbon assimilation per unit of limiting nutrient) of phytoplankton communities (Ptacnik et al. 2008). This result parallels similar findings in terrestrial systems, where a positive relationship between plant biodiversity and primary production has been observed in numerous experimental studies (Balvanera et al. 2006; Cardinale et al. 2006; Hector et al. 2007). A limitation of the aquatic study by Ptacnik et al. (2008) and of essentially all terrestrial studies is that the mechanisms explaining this positive relationship are usually unknown, and therefore, must be addressed *ad hoc*. A favored, but debated, hypothesis suggests that species differ in their capacities to use available resources, leading to complementary (and thus more complete) resource use by more diverse producer communities. If this was true, it should be possible to *a priori* identify traits that give unique niches and functions to individual producer species.

The main accomplishment of the research described in my first paper is that we have *a priori* identified easily measurable, complementary traits in the resource use of different phytoplankton species. Furthermore we provide compelling evidence suggesting that this resource use complementarity is responsible for the positive relationships between biodiversity, production and resource use efficiency in 76 natural and assembled phytoplankton communities. We argue that taxon-specific differences in by means of various

photosynthetically active pigments provides a biochemical mechanism of resource use complementarity among phototrophic microorganisms, enabling more diverse communities to harvest the light spectrum more completely. In line with this, more diverse phytoplankton communities showed higher pigment diversity, higher biomass-specific light absorbance, and higher rates of primary production and biomass accrual. These relationships were found in both highly controlled, assembled phytoplankton communities in the laboratory and in natural lake communities with shared evolutionary histories. Until now, most studies dealing with the relationship between productivity and species diversity, regarded productivity to determine the diversity of species (Huston and DeAngelis 1994) expecting a unimodal relationship between productivity and species diversity. Indeed, in systems with little productivity, such as pure water rock pools, only few or even no species exists (Dodson 1987) and also in highly productive lakes, such as sewage lagoons, only few species can be found (Ganapati 1940). Lakes with productivities ranging between these extreme conditions generally show the highest species richness (Dodson 1992; Dodson et al. 2000). However, in contrast, to discuss diversity as a result of productivity, one can also consider productivity to be depended on diversity. Our results support the idea that the complementary use of resources, facilitation between species and the higher probability to include very productive species in highly diverse communities can result in a diversity dependent productivity of phytoplankton communities. Additionally, our field data fit very well into a recent metaanalysis of phytoplankton communities resulting in a positive link between phytoplankton carbon dynamics and diversity (Ptacnik et al. 2008). Our experiments show evidence that complementarity in the use of light (mediated by functional diversity) is obviously the mechanism underlying this correlation.

EFFECTS OF DIVERSITY ON PHYTOPLANKTON RESOURCE UPTAKE AND BIOMASS COMPOSITION (STOICHIOMETRY)

We have shown that highly diverse phytoplankton communities had higher pigment diversity, higher biomass-specific light absorbance, higher primary production and higher biomass carbon accrual. Data from European and American lakes (Dickman et al. 2006; Striebel et al. 2008) showed that phytoplankton biodiversity influences not only carbon production but also phytoplankton stoichiometry (biomass carbon to phosphorus ratios). However, these studies lack an explanation of this correlation and this gap lead us to further experimental investigations of phytoplankton biodiversity-biomass stoichiometry relationships. Our experiments included assembled and natural phytoplankton communities and showed that biodiversity influenced carbon assimilation and nutrient uptake in different ways. This resulted in diversity dependent shifts of phytoplankton biomass stoichiometry. Effects of diversity on phytoplankton stoichiometry can influence the food quality of phytoplankton communities for herbivorous zooplankton and can thereby have an impact on pelagic food web dynamics.

THE SUPPLY OF LIGHT AND NUTRIENTS AND ITS CONSEQUENCES FOR PHYTOPLANKTON-ZOOPLANKTON INTERACTIONS

The impact of light and nutrients on phytoplankton and the assessment of algal food quality for zooplankton (light-nutrient hypothesis) were nearly exclusively tested in small-scale laboratory experiments. To my knowledge, the first approach to investigate how applicable the ‘light-nutrient hypothesis’ is to describe the influence of light and nutrients on natural phytoplankton communities and on herbivores feeding these phytoplankton communities, was a field experiment from Urabe et al. (2002b). The authors investigated the response of a plankton community from an oligotrophic lake to the factorial manipulation of light (shading)

and nutrients (phosphorus-enrichment) in field mesocosms. These manipulations had major effects on seston carbon to nutrient stoichiometry and on zooplankton growth over the time course of four weeks during the experiment and most importantly, the results were consistent with the predictions of the ‘light-nutrient hypothesis'.

However, the short-term response of an algal community to an experimental manipulation may be constrained by its initial characteristics, such as species richness and taxonomic composition. Therefore, we conducted a field experiment (Paper 3) with different natural phytoplankton communities from six lakes chosen along a gradient of total phosphorus concentration. Instead of supplying a plankton community with nutrients (which restricts the response of the community to the specifications of the original species pool), we used plankton communities from oligotrophic, mesotrophic and eutrophic lakes to investigate the nutrient dependent changes in the stoichiometry of plankton communities in response to light manipulations. By using different natural algal communities, we furthermore assured realistic species combinations and algal communities with shared evolutionary histories as experimental systems. The 'light-nutrient hypothesis' predicts a unimodal relation between light intensity and *Daphnia* growth over a broad range of the light-nutrient supply space. Such a unimodal response can only be captured with a gradient design. To test the light-nutrient hypothesis in a rigorous way we used an experimental gradient of light intensities (five steps) instead of only shaded and unshaded treatments. Our results show that both, light dependent changes in biomass quantity and the stoichiometry of natural phytoplankton communities, can affect zooplankton growth. Finally, our experiment provides evidence that the relations of light-nutrient interactions described by the 'light-nutrient hypothesis' may also occur under field conditions with different natural phytoplankton communities.

COSTS OF BEHAVIOURAL STRATEGIES FOR PHYTOPLANKTON RESOURCES UPTAKE

The most important resources for phytoplankton growth are light and nutrients. While nutrients can be either distributed homogeneously along the water column (if mixing of the water column is sufficient) or accumulate at deeper water layers (during stratification periods), light always decreases exponentially with depth. Most phytoplankton species have specific densities somewhat higher than the surrounding medium and may permanently sink into deeper layers where light levels are insufficient to sustain photosynthesis (Reynolds 1984; Kirk 1994). Mobile phytoplankton species are able to conduct periodic migrations and thereby are able to choose their position in the water column. Dial vertical migration of marine and freshwater dinoflagellates has been the subject of numerous field and laboratory investigations showing that light and nutrients influence the migration (Eppley et al. 1968; Seliger et al. 1970; Harris et al. 1979; Cullen and Horrigan 1981). Mobile phytoplankton species are able to change their position to optimize the availability of light and nutrients while non-mobile species have to cope with spatially separated resources. We therefore hypothesised that non-mobile taxa have to be more plastic in their carbon to nutrient stoichiometry. To investigate this hypothesis we did experiments with mobile and non-mobile green algae and exposed them to light gradients (Paper 4). In agreement with our hypothesis we observed higher biomass production and carbon to phosphorus ratios of non-mobile species compared to mobile species. We quantified costs of mobility in terms of lower resource use efficiency and higher energy demands to balance basal metabolisms of mobile phytoplankton species. This is in agreement with earlier studies, showing that the relationship between respiration and maximal production of mobile species is worse than those of non-mobile species (Cushing 1989). To cover the higher demands for energy and nutrients, mobile species might have to access alternative nutrient sources such as bacteria. Mixotrophic nutrition might be a possibility for mobile species to increase their nutrient uptake. First experiments with mobile, mixotroph phytoplankton species indeed showed that

mixotrophic nutrition is a possibility to keep biomass phosphorus level constant and high (Katechakis et al. 2005).

NEW METHODS TO ESTIMATE GROWTH AND MORTALITY OF PHYTOPLANKTON COMMUNITIES

To estimate phytoplankton growth and loss rates (mainly grazing by micro- and mesozooplankton), *in situ*, different techniques were developed within the last centuries. Disadvantages of these techniques were their often complicated enforcement and the neccessarity to use potential harmful substances such as radioactive tracers. Dilution experiments, to investigate the impact of microzooplankton grazing on phytoplankton, are normally conducted in closed bottles with addition of limiting nutrients. This makes this technique not reliable in low-nutrient systems and not practicable for *in situ* measurements (Andersen et al. 1991).

We present a modification of the dilution method and used dialysis bags to estimate growth and loss rates of phytoplankton instead of non permeable glass bottles (Paper 5). Dialysis membranes possess the advantage to be permeable for nutrients and thereby allow an *in situ* estimation of phytoplankton gross growth rates. Dialysis bags also allow simultaneously the estimation of microzooplankton grazing by dilution of plankton communities.

Additionally, to the marine experiments presented in Paper 5, I conducted detailed combined dilution-dialysis experiments in a variety of freshwater systems. The marine and freshwater experiments showed that the incubation of dilution experiments with dialysis bags are a straightforward and very useful method to simultaneously estimate phytoplankton growth and loss rates. A series of recent publications exploring phytoplankton dynamics in response to environmental perturbations (such as nutrient manipulations and climate changes) show that our combined dilution-dialysis method is already successfully used to quantify phytoplankton growth dynamics (Sommer et al. 2005; Aberle et al. 2007).

OUTLOOK

LIGHT AS A MULTITUDE OF RESOURCES

Phytoplankton harvest light with photosynthetic active pigments and different groups of phytoplankton are defined and characterized through differences in their pigmental composition. The specific combination of different pigments determines which part of the light spectrum can be used for photosynthesis by individual phytoplankton species (Stomp et al. 2004). I am only just performing first experiments with phytoplankton communities where I manipulate the available light spectrum in its spectral composition. First results indicate that offering a multitude of different wavelengths of the light spectrum maintain phytoplankton communities highly diverse whereas offering only a small range of the light spectrum results in less diverse communities. Differential utilization of the light gradient indicates a potential for partitioning this resource, thus alleviating competition. In studies by Stomp et al. (2004; 2007) the authors demonstrated niche separation of cyanobacteria in using the resource light. Different pigment coloration allows cyanobacteria to use different parts of the light spectrum for photosynthesis.

The experiments from Stomp et al. (2004; 2007) are restricted to cyanobacteria, but my experiments extend these findings to phytoplankton communities containing different phytoplankton groups and show that light should be considered as a multitude of resources for phytoplankton. Niche differentiation along this multitude of resources can partly explain the paradox of the plankton, asking why so many species can coexist in the nearly unstructured pelagic environment. Further research should test experimentally whether the concept of niche differentiation along spectral light gradients is useful to explain the diversity of natural phytoplankton communities.

STRUGGLING FOR LIGHT AND NUTRIENTS: MIXOTROPHY AS STRATEGY TO OPTIMIZE RESOURCE UPTAKE

Mobility has been considered to be a strategy to optimize light and nutrient uptake. However, mixotrophic phytoplankton species are also able to bridge gaps between light or nutrient limitations by ingesting particles or organic substances to endure situations of nutrient or energy (carbon) limitations. It seems possible that within a lot of phytoplankton species mobility and mixotrophy are linked. A detailed investigation of mobile species is therefore probably incomplete without a simultaneous investigation of the mixotrophic potential of these species.

One approach could be to investigate the abundance of mobile and/or mixotrophic phytoplankton species within lakes of different trophic status and to observe if their abundance depends on the concentration and spatial distribution of nutrients in lakes. Further investigations could include field experiments exposing natural phytoplankton communities to gradients of light and nutrient availabilities to estimate whether the abundance of mixotrophic and mobile phytoplankton species change along a gradient of nutrient or light. Furthermore, such an experiment could show if mixotrophy is an adaptive behaviour that can be rapidly regulated by environmental fluctuations.

Finally, long time data sets from Lake Brunnensee could be analysed by correlating the abundance of mixotrophic and mobile phytoplankton species with environmental factors and thereby support potential experimental findings with empirical field data.

THE IMPACT OF LIGHT AND NUTRIENTS ON PHYTOPLANKTON: CONSEQUENCES FOR HERBIVOROUS ZOOPLANKTON

Phytoplankton that supports good growth and reproduction of herbivorous are termed “high quality”. Whether or not a particular phytoplankton species is considered to be high food

quality depends on the edibility of the species for a particular herbivore and on its chemical content. The edibility of phytoplankton is a function of ingestibility (defined by size and shape and digestibility (defined by assimilation efficiency). The assimilation efficiency of *Daphnia* for phytoplankton carbon is probably considerably more variable than the assimilation efficiency for phytoplankton biomass phosphorus because phytoplankton cells show a higher flexibility in the structural variations of carbon rich cell structures (Lampert and Sommer 2007). Phosphorus limitation of *Daphnia* growth should occur at higher phytoplankton carbon to phosphorus ratios if much of phytoplankton carbon is located in cell structures that *Daphnia* cannot assimilate with high efficiency (such as cellulose containing cell walls) (VanDonk et al. 1997).

Therefore, besides estimating phytoplankton biomass carbon to phosphorus ratios, it would be an interesting idea to additionally determine the fraction of phytoplankton carbon that can not be assimilated by a specific herbivorous zooplankton species. Carbon to phosphorus ratios are definitely most valuable as a component measure of phytoplankton food quality once the suitability of the phytoplankton carbon fraction for zooplankton assimilation has been characterized.

Research on the importance of resources for phytoplankton growth has a long and fruitful history. Since the pioneering ideas of Hutchinson (1965), generations of plankton ecologists have been investigating the effects of resources on plankton dynamics. However, many of the interesting research questions still remain unanswered. Additionally, answering one research question opens a multitude of new ones. The development of new techniques allowing novel insights into plankton dynamics, we should expect new concepts and breakthroughs on how resources interact with plankton.

4. REFERENCES

Aberle, N., K. Lengfellner, and U. Sommer. 2007. Spring bloom succession, grazing impact and herbivore selectivity of ciliate communities in response to winter warming. Oecologia **150**: 668-681.

Andersen, T., J. J. Elser, and D. O. Hessen. 2004. Stoichiometry and population dynamics. Ecology Letters **7**: 884-900.

Andersen, T., and D. O. Hessen. 1991. Carbon, nitrogen, and phosphorus-content of fresh water zooplankton. Limnology and Oceanography **36**: 807-814.

Andersen, T., A. K. L. Schartau, and E. Paasche. 1991. Quantifying external and internal nitrogen and phosphorus pools, as well as nitrogen and phosphorus supplied through remineralization, in coastal marine plankton by means of a dilution technique. Marine Ecology Progress Series **69**: 67-80.

Balvanera, P., A. B. Pfisterer, N. Buchmann, J. S. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters **9**: 1146-1156.

Bergmann, T., G. Fahnenstiel, S. Lohrenz, D. Millie, and O. Schofield. 2004. Impacts of a recurrent resuspension event and variable phytoplankton community composition on remote sensing reflectance. Journal of Geophysical Research-Oceans **109**: C10S15.

Brett, M. T., D. C. Müller-Navarra, and S. K. Park. 2000. Empirical analysis of the effect of phosphorus limitation on algal food quality for freshwater zooplankton. Limnology and Oceanography **45**: 1564-1575.

Capriulo, G. M., and E. J. Carpenter. 1980. Grazing by 35 to 202 μm micro-zooplankton in long-island sound. Marine Biology **56**: 319-326.

Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature **443**: 989-992.

Cloern, J. E., C. Grenz, and L. VidergarLucas. 1995. An empirical model of the phytoplankton chlorophyll:carbon ratio - The conversion factor between productivity and growth rate. Limnology and Oceanography **40**: 1313-1321.

Crawford, D. W. 1992. Metabolic cost of mobility in planktonic protists - theoretical considerations on size scaling and swimming speed. Microbial Ecology **24**: 1-10.

Cullen, J. J., and S. G. Horrigan. 1981. Effects of nitrate on the diurnal vertical migration, carbon to nitrogen ratio, and the photosynthetic capacity of the dinoflagellate *Gymnodinium splendens*. Marine Biology **62**: 81-89.

Cushing, D. H. 1989. A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. Journal of Plankton Research **11**: 1-13.

DeMott, W. R., and B. J. Pape. 2004. Stoichiometrie in an ecological context: testing for links between *Daphnia* P-content, growth rate and habitat preference. Oecologia **142**: 20-27.

DeMott, W. R., and A. J. Tessier. 2002. Stoichiometric constraints vs. algal defenses: Testing mechanisms of zooplankton food limitation. Ecology **83**: 3426-3433.

Dickman, E. M., M. J. Vanni, and M. J. Horgan. 2006. Interactive effects of light and nutrients on phytoplankton stoichiometry. Oecologia **149**: 676-689.

Diehl, S. 2007. Paradoxes of enrichment: Effects of increased light versus nutrient supply on pelagic producer-grazer systems. American Naturalist **169**: E173-E191.

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

Dodson, S. 1992. Predicting crustacean zooplankton species richness. Limnology and Oceanography **37**: 848-856.

Dodson, S. I. 1987. Animal assemblages in temporary desert rock pools: aspects of the ecology of *Dasyhelea sublettei* (Diptera: Ceratopogonidae). Journal of the North American Benthological Society **6**: 65-71.

Dodson, S. I., S. E. Arnott, and K. L. Cottingham. 2000. The relationship in lake communities between primary productivity and species richness. Ecology **81**: 2662-2679.

Elser, J. J., D. R. Dobberfuhl, N. A. MacKay, and J. H. Schampel. 1996. Organism size, life history, and N:P stoichiometry. Bioscience **46**: 674-684.

Elser, J. J., W. F. Fagan, R. F. Denno, D. R. Dobberfuhl, A. Folarin, A. Huberty, S. Interlandi, S. S. Kilham, E. McCauley, K. L. Schulz, E. H. Siemann, and R. W. Sterner. 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature **408**: 578-580.

Eppley, R. W., O. Holm-Harisen, and J.D. Strickland. 1968. Some observations on vertical migration of dinoflagellates. Journal of Phycology **4**: 333-340.

Falkowski, P., and J. A. Raven. 2007. Aquatic Photosynthesis. Princton University Press.

Falkowski, P. G. 1994. The role of phytoplankton phytosynthesis in global biogeochemical cycles. Photosynthesis Research **39**: 235-258.

Falkowski, P. G., and J. Laroche. 1991. Acclimation to spectral irradiance in algae. Journal of Phycology **27**: 8-14.

Field, C. B., M. J. Behrenfeld, J. T. Randerson, and P. Falkowski. 1998. Primary production of the biosphere: Integrating terrestrial and oceanic components. Science **281**: 237-240.

Fox, J. W. 2005. Interpreting the 'selection effect' of biodiversity on ecosystem functioning. Ecology Letters **8**: 846-856.

Ganapati, S. V. 1940. The ecology of a temple tank containing a permanent bloom of *Microcystis aeruginosa*. Journal of the Bombay Natural History Society **17**: 65-77.

Gaston, K. J. 1996. Biodiversity: a biology of numbers and difference. Blackwell Science, Oxford.

Geider, R. J. 1987. Light and temperature-dependence of the carbon to chlorophyll-a ratio in microalgae and cyanobacteria – implications for physiology and growth of phytoplankton. New Phytologist **106**: 1-34.

Geider, R. J. 1993. Quantitative phytoplankton physiology - implications for primary production and phytoplankton growth. ICES marine science symposia. **197**: 52-62.

Geider, R. J., H. L. MacIntyre, and T. M. Kana. 1997. Dynamic model of phytoplankton growth and acclimation: Responses of the balanced growth rate and the chlorophyll a:carbon ratio to light, nutrient-limitation and temperature. Marine Ecology Progress Series **148**: 187-200.

Graham, L. E., Wilcox, and L.W. 2000. Algae. Prentice Hall, Upper Saddler River.

Harper, J. L., and D. L. Hawksworth. 1994. Biodiversity: measurement and estimation. Philosophical Transactions of the Royal Society of London B **345**: 5-12.

Harris, G. P., S. I. Heaney, and J. F. Talling. 1979. Physiological and environmental constraints in the ecology of the planktonic dinoflagellates *Ceratium hirundinella*. Freshwater Biology **9**: 413-428.

Hector, A., J. Joshi, M. Scherer-Lorenzen, B. Schmid et al. 2007. Biodiversity and ecosystem functioning: reconciling the results of experimental and observational studies. Functional Ecology **21**: 998-1002.

Hector, A., B. Schmid, C. Beierkuhnlein et al. 1999. Plant diversity and productivity experiments in European grasslands. Science **286**: 1123-1127.

Hessen, D. O. 1992. Nutrient element limitation of zooplankton production. American Naturalist **140**: 799-814.

Hessen, D. O., P. J. Faerovig, and T. Andersen. 2002. Light, nutrients, and P : C ratios in algae: Grazer performance related to food quality and quantity. Ecology **83**: 1886-1898.

Hooper, D. U., and J. S. Dukes. 2004. Overyielding among plant functional groups in a long-term experiment. Ecology Letters **7** : 95-105.

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

Huston, M. A., and D. L. DeAngelis. 1994. Competition and coexistence - the effects of resource transport and supply rates. American Naturalist **144**: 954-977.

Hutchinson, G. E. 1965. The Ecological Theater and the Evolutionary Play. Yale University Press, New Haven.

Jiang, L., Z. Pu, and D. R. Nemergut. 2008. On the importance of the negative selection effect for the relationship between biodiversity and ecosystem functioning. Oikos **117**: 488-493.

Jones, R. I. 1993. Phytoplankton migrations: Patterns, processes and profits. Archiv für Hydrobiologie Beiheft Ergebnisse der Limnologie **39**: 67-77.

Katechakis, A., T. Haseneder, R. Kling, and H. Stibor. 2005. Mixotrophic versus photoautotrophic specialist algae as food for zooplankton: The light: nutrient hypothesis might not hold for mixotrophs. Limnology and Oceanography **50**: 1290-1299.

Kirk, J. T. O. 1994. Light & Photosynthesis in Aquatic Ecosystems, Second ed. Cambridge University Press, Cambridge.

Knapp, C. W., F. deNoyelles, D. W. Graham, and S. Bergin. 2003. Physical and chemical conditions surrounding the diurnal vertical migration of *Cryptomonas* spp. (Cryptophyceae) in a seasonally stratified midwestern reservior (USA). Journal of Phycology **39**: 855-861.

Lalli, C. M., and T. R. Parsons. 1997. Biological oceanography, Second ed. Butterworth-Heinemann, Oxford.

Lampert, W., and U. Sommer. 2007. Limnoecology, Second ed. Oxford University Press Inc., Oxford.

Landry, M. R., and R. P. Hassett. 1982. Estimating the grazing impact of marine micro-zooplankton. Marine Biology **67**: 283-288.

Langdon, C. 1988. On the causes of interspecific differences in the growth irradiance relationship for phytoplankton. A general-review. Journal of Plankton Research **10**: 1291-1312.

Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature **413**: 72-76.

Main, T. M., D. R. Dobberfuhl, and J. J. Elser. 1997. N : P stoichiometry and ontogeny of crustacean zooplankton: A test of the growth rate hypothesis. Limnology and Oceanography **42**: 1474-1478.

Pick, F. R., D. R. S. Lean, and C. Nalewajko. 1984. Nutrient status of metalimnetic phytoplankton peaks. Limnology and Oceanography **29**: 960-971.

Ptacnik, R., A. G. Solimini, T. Andersen, T. Tamminen, P. Brettum, L. Lepistö, E. Willén, and S. Rekolainen. 2008. Diversity predicts stability and resource use efficiency in natural phytoplankton communities. Proceedings of the National Academy of Sciences of the United States of America **105**: 5134-5138.

Ralston, D. K., D. J. McGillicuddy, and D. W. Townsend. 2007. Asynchronous vertical migration and bimodal distribution of motile phytoplankton. Journal of Plankton Research **29**: 803-821.

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

Reynolds, C. S. 2006. Ecology of Phytoplankton. Cambridge University Press, Cambridge.

Seliger, H. H., Carpente.JH, M. Loftus, and W. D. McElroy. 1970. Mechanisms for accumulation of high concentrations of dinoflagellates in a bioluminescent bay. Limnology and Oceanography **15**: 234-&.

Sommer, U., T. Hansen, O. Blum, N. Holzner, O. Vadstein, and H. Stibor. 2005. Copepod and microzooplankton grazing in mesocosms fertilised with different Si : N ratios: no overlap between food spectra and Si : N influence on zooplankton trophic level. Oecologia **142**: 274-283.

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. American Naturalist **150**: 663-684.

Stomp, M., J. Huisman, F. de Jongh, A. J. Veraart, D. Gerla, M. Rijkeboer, B. W. Ibelings, U. I. A. Wollenzien, and L. J. Stal. 2004. Adaptive divergence in pigment composition promotes phytoplankton biodiversity. Nature **432**: 104-107.

Stomp, M., J. Huisman, L. Voros, F. R. Pick, M. Laamanen, T. Haverkamp, and L. J. Stal. 2007. Colourful coexistence of red and green picocyanobacteria in lakes and seas. Ecology Letters **10**: 290-298.

Striebel, M., G. Spörl, and H. Stibor. 2008. Light-induced changes of plankton growth and stoichiometry: Experiments with natural phytoplankton communities. Limnology and Oceanography **53**: 513-522.

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

Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. Science **294**: 843-845.

Tilman, D., P. B. Reich, and J. M. H. Knops. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature **441**: 629-632.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature **379**: 718-720.

Urabe, J., J. J. Elser, M. Kyle, T. Yoshida, T. Sekino, and Z. Kawabata. 2002a. Herbivorous animals can mitigate unfavourable ratios of energy and material supplies by enhancing nutrient recycling. Ecology Letters **5**: 177-185.

Urabe, J., M. Kyle, W. Makino, T. Yoshida, T. Andersen, and J. J. Elser. 2002b. Reduced light increases herbivore production due to stoichiometric effects of light/nutrient balance. Ecology **83**: 619-627.

Urabe, J., and R. W. Sterner. 1996. Regulation of herbivore growth by the balance of light and nutrients. Proceedings of the National Academy of Sciences of the United States of America **93**: 8465-8469.

Urabe, J., and Y. Watanabe. 1992. Possibility of N-limitation or P-limitation for planktonic cladocerans: an experimental test. Limnology and Oceanography **37**: 244-251.

VanDonk, E., M. Lurling, D. O. Hessen, and G. M. Lokhorst. 1997. Altered cell wall morphology in nutrient-deficient phytoplankton and its impact on grazers. Limnology and Oceanography **42**: 357-364.

Wall, D., and F. Briand. 1979. Response of lake phytoplankton communities to *in situ* manipulations of light intensity and colour. Journal of Plankton Research **1**: 103-112.

5. PERSONAL NOTES

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September 1985 - July 1989	Grundschule Ulm-Böfingen
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ACADEMIC EDUCATION

September 1998 - August 2000	Basic study period in biology at University Ulm
August 2000 - July 2004	Main study period in biology at Ludwig-Maximilians-Universität Munich. Main subject: Neurobiology, minor subjects: Ecology, Zoology and Conservation Biology (Wildbiologie) Diploma thesis: “The influence of mixing depth on phytoplankton-*Daphnia* interactions with focus on the phytoplankton” (in German)
July 2004	Diploma in Biology
July 2004 - October 2005	Research assistant at Ludwig-Maximilians-Universität Munich during AQUASHIFT (DFG founded project); Teaching assistant in various courses in ecology: Plankton ecology, Experimental plankton ecology, Basal course Ecology and Evolutionary Biology
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PUBLICATIONS

Stibor, H., A. Gelzleichter, F. Hantzsche, U. Sommer, **M. Striebel**, O. Vadstein, and Y. Olsen 2006. Combining dialysis and dilution techniques to estimate gross growth rate of phytoplankton and grazing by micro- and mesozooplankton in situ. Archiv für Hydrobiologie 167: 403-419.

Berger, S. A., S. Diehl, H. Stibor, G. Trommer, M. Ruhenstroth, A. Wild, A. Weigert, C. G. Jäger, and **M. Striebel**. 2007. Water temperature and mixing depth affect timing and magnitude of events during spring succession of the plankton. Oecologia 150: 643-654.

Striebel, M., G. Spörl, H. Stibor. 2008. Light induced changes of plankton growth and stoichiometry: Experiments with natural phytoplankton communities. 2008. Limnology and Oceanography 53(2): 513-522.

Striebel, M., S. Behl, S. Diehl, H. Stibor. Colorful niches link biodiversity to carbon dynamics in pelagic ecosystems. Submitted to Ecology Letters.

Striebel, M., S. Behl, H. Stibor. The coupling of biodiversity and productivity in phytoplankton communities: Consequences for biomass stoichiometry. Submitted to Ecology.

Striebel, M., S. Bartholmé, R. Zernecke, C. Steinlein, S. Diehl and H. Stibor. Carbon sequestration and stoichiometry of mobile and non-mobile green algae. Manuscript prepared for submission to Limnology and Oceanography.

PRESENTATIONS

ASLO summer meeting 2005, Santiago de Compostela.

Poster: Light-nutrient ratios and phytoplankton-zooplankton dynamics in lakes of different trophic status;

ASLO poster award.

DGL Jahrestagung 2006, Dresden.

Talk: Licht-Nährstoff Verhältnisse und Phytoplankton-Zooplankton Dynamiken in verschieden nährstoffreichen Seen.

SIL Congress 2007, Montreal.

Talk: The effect of diversity on light mediated changes in phytoplankton production and stoichiometry: A laboratory experiment.

CLIMAX Master Class 2007, Amsterdam.

Talk: Light induced changes of plankton growth and stoichiometry: Experiments with natural phytoplankton communities.

Biodiversity Research – Safeguarding the Future 2008, Bonn.

Poster: The effect of diversity on light mediated changes in phytoplankton production and stoichiometry: A laboratory experiment.

6. ACKNOWLEDGEMENTS

I want to thank:

- First of all PD Dr. Herwig Stibor for his infinite support during my work.

- Prof. Dr. Sebastian Diehl for his support and his helpful comments on my manuscripts.

- Especially Gertrud Spörl, Stephan Behl, Veronika Gründl, Julia Zeininger, Inga Schirmer-Kortstock, and Rebekka Zernecke who conducted several experiments with me during their diploma theses.

- Margit Feissel, Angelika Wild, and Achim Weigert for help during my field and laboratory experiments.

- Stephan Behl, Christian Matauschek, Michael Effenberger, Mia Stockenreiter, Florian Haupt, and Christoph Jäger, Silvia Bartholmé, Sabine Wollrab and Martin Steinböck.

- Everybody in the Aquatic Ecology Group.

- Mechthild Kredler.

Especially I want to thank Dr. Elke Reichwaldt for her encouragement during this work!

Special thanks to Christoph Mangold for his support during this work!

7. DECLARATION

DIESE PROMOTION WURDE IM SINNE §12 DER PROMOTIONSORDNUNG VON PD DR. HERWIG STIBOR BETREUT. ICH ERKLÄRE HIERMIT, DASS DIE DISSERTATION KEINER ANDEREN PRÜFUNGSKOMMISSION VORGELEGT WORDEN IST UND DASS ICH MICH NICHT ANDERWEITIG EINER DOKTORPRÜFUNG OHNE ERFOLG UNTERZOGEN HABE.

EHRENWÖRTLICHE VERSICHERUNG

ICH VERSICHERE HIERMIT, DASS DIE VORGELEGTE DISSERTATION VON MIR SELBSTÄNDIG, OHNE UNERLAUBTE HILFE ANGEFERTIGT WURDE.

MÜNCHEN, DEN 31. JULI 2008

MAREN STRIEBEL

BEITRAG DER KOAUTOREN UND EIGENER BEITRAG:

PAPER 1:

Stephan Behl war im Rahmen seiner Diplomarbeit an der Durchführung und Auswertung (Auszählen der Phytoplanktonproben) der Experimente beteiligt. Sebastian Diehl half bei der Fertigstellung des Manuskripts.

PAPER 2:

Stephan Behl war im Rahmen seiner Diplomarbeit an der Durchführung und Auswertung (Auszählen der Phytoplanktonproben) der Experimente beteiligt.

PAPER 3:

Gertrud Spörl war im Rahmen ihrer Diplomarbeit an der Durchführung und Auswertung (Auszählen der Phytoplanktonproben) der Experimente beteiligt.

PAPER 4:

Silvia Barthomé war an der Konzipierung und der Durchführung der Versuche sowie beim Schreiben des Manuskripts beteiligt. Rebekka Zernecke war im Rahmen ihrer Diplomarbeit an der Durchführung und Auswertung der Experimente beteiligt. Christina Steinlein war an der Durchführung der Experimente beteiligt. Sebastian Diehl war an der Auswertung der Ergebnisse beteiligt.

PAPER 5:

Ich war an der Konzeption, Durchführung und Auswertung der Experimente sowie am Verfassen des Manuskripts beteiligt.

Herwig Stibor unterstützte mich bei der Konzipierung der Versuche, war an der Diskussion der Ergebnisse beteiligt und half beim Anfertigen der Manuskripte 1 bis 4.