Plankton vertical migrations
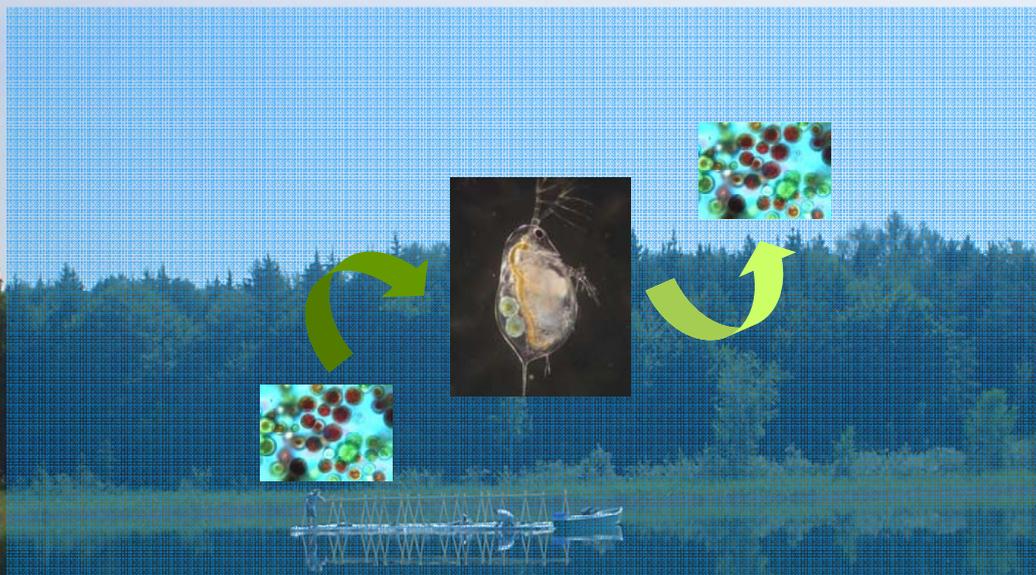
Implications for the pelagic ecosystem

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Abstract

Habitat selection is an important behavior of many organisms. The direction and strength of this behavior is often characterized as a result of a trade off between predator avoidance and obtaining resources. A characteristic example of this trade off may be seen in organisms in the pelagic ecosystem in the form of vertical migrations. Diel vertical migration (DVM) is a predator avoidance behavior of many zooplankton species, which is marked by a significant shift in the vertical distribution of the zooplankton where night time is spent in the epilimnion and day time in the hypolimnion While the causes of DVM and its ecophysiological consequences for the zooplankton are well studied, little is known about the consequences of DVM for the pelagic food ecosystem. Vertical migrations are not only restricted to zooplankton but are often exhibited by phytoplankton species, which respond to vertical gradients of light and nutrient availability. Many phytoplankton species cope with light and nutrient gradients by changing their position in the water column through active movement or buoyancy adjustment. The costs and consequences of this phytoplankton behavior are hardly studied.

In my thesis, I studied the consequences of zooplankton DVM for the pelagic food web and the consequences of phytoplankton vertical migrations on individual growth and biomass composition through both field and laboratory experiments.

I, Upward phosphorus transport by *Daphnia* DVM

During stagnation periods of the water column, physical upward transport processes are very unlikely and nutrients become scarce in the photic zone of many lakes. DVM of zooplankton could be a mechanism of nutrient repletion in the epilimnion. I experimentally examined the upward transport of phosphorus by *Daphnia* DVM. Results revealed that *Daphnia* DVM caused an upward nutrient transport. The amount of phosphorus transported and released by *Daphnia* in my study was within a biologically meaningful range: five percent of the estimated daily maximum phosphorus uptake of the phytoplankton community in the epilimnion. Therefore, nutrient transport by *Daphnia* DVM could be a significant mechanism in fuelling primary production in the phosphorus limited epilimnion.

II, *Daphnia* DVM: implications beyond zooplankton

DVM creates a temporal and spatial predator-free niche for the phytoplankton, and theoretical models predict that parts of the phytoplankton community could use this niche. I experimentally investigated the influence of *Daphnia* DVM on the phytoplankton community of an oligotrophic lake in field mesocosms. My results suggest that *Daphnia* DVM had significant effects on quantitative and qualitative characteristics of the phytoplankton community. Phytoplankton biomass was higher in "no DVM" treatments. DVM also increased diversity in the phytoplankton community. The analyses showed that the gelatinous green algae *Planktosphaeria gelatinosa* was the main species influencing phytoplankton dynamics in the experiment, and therefore the effects of *Daphnia* DVM were highly species specific.

III, Initial size structure of natural phytoplankton communities determines the response to *Daphnia* DVM

Previous studies have shown that the direction and strength of phytoplankton responses to zooplankton DVM most likely depends on the size of the phytoplankton species. To examine the influence of DVM on different sized phytoplankton communities, I manipulated the size distribution of a natural phytoplankton community *a priori* in field mesocosms. The results reveal that DVM oppositely affected the two different phytoplankton communities. A comparison of "DVM" and "no DVM" treatments showed that nutrient availability and total phytoplankton biovolume was higher in "no DVM" treatments of phytoplankton communities consisting mainly of small algae, whereas it was higher in "DVM" treatments of phytoplankton communities with a wide size spectrum of algae. It seemed that two different mechanisms on how DVM can influence the phytoplankton community were at work. In communities of mainly small algae nutrient recycling was important, seemed to be important, whereas in communities with a wide size spectrum of algae the refuge effect played the dominant role.

IV, Carbon sequestration and stoichiometry of motile and non-motile green algae

The ability to move actively should entail costs in terms of increased energy expenditure and the provision of specific cell structures for movement. In a laboratory experiment, I studied whether motile, flagellated and non-motile phytoplankton taxa differ with respect to their energetic costs, phosphorus requirements, and structural carbon requirements. The results show that flagellated taxa had higher respiration rates and higher light requirements for growth than non-motile taxa. Accordingly, both short-term photosynthetic rates and long-term biomass accrual were lower for flagellated than for non-motile taxa. My results point at significant costs of motility, which may explain why flagellated taxa are often outcompeted by non-motile taxa in turbulently mixed environments, where active motility is of little use. The data in this study also suggest that motility alone may not be sufficient to explain the lower C: P ratios of flagellates.

In summary, my results show that migrating phytoplankton and zooplankton species can act as a vector transporting energy, organic matter and ecological interaction. The complex consequences for the pelagic ecosystem are thereby determined by the organisms´ activity and characterized by their life history.

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Preface

The open-water zone of lakes and oceans is known as the pelagic zone. The organisms of the pelagic ecosystem are traditionally divided in two communities: the plankton and the nekton communities, which are distinguished by their ability to swim. Plankton are suspended in the water column and passively transported by water movement, whereas nektonic organisms are swimmers, actively determining their position in the pelagic realm.

Plankton are subdivided in different functional levels: phytoplankton, bacterioplankton and zooplankton. The phytoplankton, as primary producers, consists of algae and cyanobacteria, or blue-green algae, ranging in size from 0.5 µm to 1 mm. The bacterioplankton have the most diverse trophic positions and are usually smaller than 1 µm. Bacterioplanctonic species can be decomposers and chemolithoautotroph primary producers in aerobic water zones or photolithoautotroph primary producers in anaerobic zones. Zooplankton are consumers and are made up mainly of protozoa, rotifera, cnidaria, thaliacea and crustacea with sizes ranging from a few micrometers up to 1 cm and even far above for jellyfish. In the zooplankton, different trophic levels exist: herbivory, bacteriovory and zooplanktivory. In addition to these three main groups, the plankton includes fungi, which can be decomposers or parasites, and planktonic viruses with mostly unknown ecological roles. The nekton is made up mainly of fish species, which may be either planktivores (usually zooplanktivores) or piscivores.

This strict, traditional view of plankton and nekton is not justified if one considers the ability for active swimming by many planktonic organisms, such as flagellates and many of the crustacean species. Most have the ability to move and migrate and to position themselves within the water column to a certain degree.

The reasons to migrate within the water column are manifold. Planktonic species can position themselves to optimize the uptake of resources. In phytoplankton light or mineral nutrients, which are normally not evenly distributed within the water column, can cause repositioning. Due to sedimentation and remineralization processes, light

attenuates exponentially with depth, and nutrient concentrations often increase with depth.

Planktonic species can also migrate to avoid predation. Within the pelagic environment few structures exist that can be used for hiding; however, zooplankton can swim down to deeper waters where light is low and darkness provides cover. One of the most conspicuous features of zooplankton is the marked vertical migration of these small animals over large distances on a daily basis. This so called diel vertical migration (DVM) occurs in a wide range of both freshwater and marine zooplankton taxa and could represents the largest animal migration in terms of biomass in the world. In the case of phytoplankton, vertical migrations can surely be seen as the largest plant migration in terms of biomass.

In this thesis, I focus on the effects of zooplankton DVM on the ecological dynamics of the pelagic zone in freshwater ecosystems and additionally on the individual physiological consequences of migrating phytoplankton species.

1   Vertical migrations – the history of its research

1.1   Zooplankton

The first published observation about vertical migration behavior of zooplankton in freshwater ecosystems was published in the late 19th century (Weismann 1877, Forel 1877). It is not surprisingly that since the first descriptions of the vertical migration phenomenon in zooplankton, there has been extensive research on the adaptive significance and consequences for the wider ecosystem (e.g. Forel 1878, Hardy and Gunther 1935, Cushing 1951, Pearre 2003). The daily movement of the zooplankton was first studied in Lake Constance nearly one and a half century ago by Weismann (1877). At the end of the 19th and the beginning of the 20th century, several studies regarding zooplankton vertical migration in different lakes and ponds across Europe were published (Pavesi 1882, Francé 1884, Blanc 1898, Steuer 1901, Lozeron 1902). These studies established the ubiquity of the phenomenon in freshwater systems, and since then a large amount of studies dealing with zooplankton DVM have been conducted.

Due to the fact that DVM was best observed in deep, unproductive and thus transparent lakes, Lozeron (1902) compared lakes with different transparency levels. He noted that the amplitude of the migration behavior is larger in transparent lakes than in less transparent lakes. Kikuchi (1930) could show very clearly that the depth of the largest population (in his case the genus *Diaphanosoma*) depends on the transparency of the water column. As the light level in the water column decreases with increasing water depth and decreasing transparency, light was considered a controlling mechanism of migration behavior. During the whole 20th century, various authors could show that a light-mediated circadian rhythm underlies many cases of vertical migration of zooplankton (Dice 1914, Siebeck 1960, Ringelberg et al. 1967, Loose 1993).

Most evidence indicates that changes in light intensity trigger diurnal vertical migration (Enright and Hamner 1967). Ringelberg et al. (1967) demonstrated that migration stops when light changes more slowly than the threshold value, that is, when light change is slower than the eye. Movement itself can vary considerably depending on the size and

shape of the lake. Important distinguishing factors are underwater light conditions, season, predation pressure, predator presence, and age and sex of migrating species.

The most common migration behavior of zooplankton species is the upward migration from deeper waters to upper water strata at dusk and return to deeper strata at dawn. This behavior results in a maximum number of migrating individuals in the upper water strata somewhere between sunset and sunrise and is called nocturnal migration. Twilight migration, an unusual migration behavior, results in two surface maxima, one at dusk and one at dawn. Finally, the reverse migration is characterized by a surface maximum during daytime. However, variations in vertical migration, also within one of these three groups are great and the amplitude of migrations varies strongly during the year. The reasons for this are manifold. Some main aspects are a decrease of oxygen in deeper water layers during the course of the year and a change in light conditions resulting from seasonal changes in turbidity. Furthermore, changes in predation pressure may also be of importance.

The widespread occurrence of DVM in lakes and marine waters performed by many zooplankton taxa suggests that it has an adaptive value, and research has long focused on the ultimate cause of DVM. At first it seems unlikely that all zooplankton organisms in freshwater and marine environments are driven by the same ecological needs; however all grazing zooplankton species have comparable costs and benefits resulting from DVM. Migrating individuals spend the night in warmer, food-rich shallow water and the day in deeper, colder water where the quantity of food tends to be lower. The time spent in the deeper water is disadvantageous in terms of growth and reproduction, because low temperature slows down individual growth and egg development (Bottrell 1975, Reichwaldt et al. 2005). These two factors lower the reproduction rate, which shows that there is a strong selection pressure to stay in warmer and food richer upper layers. Therefore, migrating genotypes of a zooplankton population should be outcompeted by non-migrating ones very quickly, but this is not the case (Stich and Lampert 1981).

Since the early studies about zooplankton DVM, many theories about reasons and consequences of DVM for migrating zooplankton species have been stated. They can be summarized in two main categories. The first category deals with the fact that metabolic disadvantages experienced by migrating zooplankton species may be lower than previously assumed and that changing conditions during migration may even be of

advantage for the zooplankton due to a more efficient use of energy. The second category assumes that avoiding shallow water during the day reduces light dependent mortality. The hypothesis for the first category assumes that there is a metabolic advantage of switching between feeding at high temperatures and staying and growing at lower temperatures. All experimental tests of this hypothesis based on bioenergetics have concluded that it is energetically better to stay in shallow water (summarized in Lampert 1993). For this reason, the hypothesis belonging to the second category - stating increased fitness by reduced mortality as an advantage of DVM - seemed more likely.

Kozhov (1963) postulated a very convincing hypothesis: DVM is a strategy of zooplankton to avoid optical orientated predators, which are mainly fish. This "predator avoidance" hypothesis states, that zooplankton stay in darker water during daytime and migrate up to shallower water only during the night, thereby using the darkness as protection. This resulted in three predictions:

1. Zooplankton migrates up in the evening and down in the morning.
2. DVM should mainly occur in zooplankton that are visible to fish
3. The amplitude of the migration should be influenced by the activity and abundance of fish

These predictions cleary state that several general conditions have to been fulfilled to induce DVM. As mentioned above, light is the primary controlling (proximate) factor inducing and regulating the amplitude of DVM (Ringelberg 1991, 1993). The general controlling, evolutionary (ultimate) factor is the presence of fish (Zaret and Suffern 1976, Stich and Lampert 1981). Ringelberg et al. (1991) could show that zooplankton only migrate when fish are present. It was also shown that fish release kairomones, or predator released chemicals that could benefit the receiver, which can be detected by zooplankton and influence the migration behavior (Dawidowicz et al. 1990, Loose et al. 1993). Additionally, as shown in experiments, a reduction of fish abundance clearly resulted in a cessation of DVM behavior (Dini and Carpenter 1988).

The results of the studies in the past one and a half centuries show that DVM can be characterized as a synchronized vertical migration upward at dusk and downwards at dawn. These migrations occur mainly among zooplankton species, which are easily

visible to fish in both marine and freshwater ecosystems. The amplitude of the vertical migration varies with water turbidity and presence and activity of fish. The controlling factors are changes in light intensity and kairomones released by fish.

As the general mechanisms of how DVM is induced and controlled were clarified, further studies focused on the effects of the migration behavior on the migrating zooplankton itself. Costs and benefits of DVM, created by changing food and temperature conditions between shallow and deep water and the dependency of amplitude of the migration due to external factors, were highly studied und discussed (Orcutt and Porter 1983, Stich and Lampert 1984, Lampert et al. 1988, Loose and Dawidowicz 1994, Lass et al. 2000, Pearre 2003, Reichwaldt et al. 2005).

Even though the causes and consequences for the migrating zooplankton individuals are well studied and understood nowadays, the consequences of DVM for the pelagic ecosystem are not yet studied in detail. This mismatch between individual and ecosystem approaches of DVM studies can be seen in a current textbook about DVM (Ringelberg 2010), entitled as "…the first critical discussion of the literature in 100 years of research…" that also lacks at least one chapter about the consequences of zooplankton DVM on the pelagic ecosystem.

There are only a small number of empirical studies (Reichwaldt et al. 2004, Reichwaldt and Stibor 2005) and modeling studies (Lampert 1987, Petzoldt et al. 2009) dealing with the consequences of DVM on the pelagic ecosystem. Considering the global amount of zooplankton biomass involved in performing DVM, it seems clear that DVM should have tremendous consequences for the pelagic ecosystem and its food web structures.

1.2 Phytoplankton

Migrations are not restricted to zooplankton. Indeed, phytoplankton species from different algal groups can show distinct migration patterns. The earliest published observations of migrations of phytoplankton species appeared about a century later than those of zooplankton; e.g. *Ceratium* sp. (dinoflagellate) Gran (1919), *Gonyostomum semen* (dinoflagellate) Cowles and Brambel (1936) and *Coscinodiscus bouvet* (diatom) Hardy and Gunther (1935). DVM of motile phytoplankton does not normally span the amplitudes characteristic of zooplankton but can extend to an

amplitude of up to 10 m for freshwater species (Berman and Rodhe 1971) and up to 20 m for marine species (Eppley et al. 1968, Blasco 1978).

Three types of migration patterns have been reported. Flagellates can be concentrated in upper water by day and in deeper strata by night (Sommer and Gliwicz 1986). Flagellates can be concentrated in the upper water during the day and disperse in the whole water column during the night (Sommer 1982). A slight descent during the period of maximum irradiance may be superimposed on both patterns. A reverse migration pattern (concentration near the bottom during daytime, dispersal during night) was reported only for the mountainous Finstertaler Lake (Tilzer 1973). The type of the migrational behavior of phytoplankton species may change during the year (Frempong 1984).

There are various reasons for the migration behavior of phytoplankton. For example, the grazing pressure by zooplankton could force the algae to migrate. Certain algal species such as motile flagellates can move downward during darkness to avoid the predation, which can be seen as a survival adaptation to the increased grazing pressure during the night. Migrating phytoplankton species can also absorb nutrients in the dark in deep water (Gran, 1919, Fogg and Walsby 1971, Villareal and Lipschultz, 1995) in addition to light and CO_2 (Fogg and Walsby 1971, Paerl and Ustach 1982) during the day in shallow water, so that resource uptake may be considered to govern both directions of movement. Raven and Richardson (1984) estimated that at least for dinophytes, this strategy could be energetically very advantageous, and it is now often considered to be the primary adaptive reason for vertical migrations in photosynthetic organisms (Arvola et al. 1991, Salonen and Rosenberg 2000).

Despite the considerable experimentation and speculation over the years, neither the proximate nor the ultimate controlling factors of this phytoplankton behavior are fully understood yet (Bormans et al. 1999). The migration behavior of algae has additional causes, such as optimization of nutrient and light uptake in the vertical gradient. Contrary to zooplankton, the costs of active movement in phytoplankton are nearly not investigated. Estimates of the costs of mobility in terms of growth and biomass composition are rare (e.g. Raven and Richardson 1984) and the influence of these parameters on carbon and phosphorus dynamics of mobile algae species are not yet studied.

2 Zooplankton diel vertical migration – consequences for the pelagic ecosystem

2.1 Reduced grazing

2.1.1 Discontinuous grazing

Numerous studies have shown that mesozooplankton grazing in marine and freshwater ecosystems can affect the phytoplankton community composition and the total phytoplankton biomass (Sarnelle 1992, Sommer et al. 2001, Sommer and Stibor 2002, Stibor et al. 2004a, Smith et al. 2010). Therefore, the predator induced DVM behavior by the zooplankton should influence the grazing pressure and the temporal and spatial grazing pattern in the epi- and hypolimnion and therewith phytoplankton population dynamics in those habitats.

This is a classic example of a "trait mediated effect". Trait mediated effects emerge from the influence of a predator not by direct trophic interactions accompanied by mortality (predation) but from indirect interaction such as behavioral changes of the prey during escape responses. These indirect interactions also affect the resource of the prey. In the case of DVM, the predator (fish) would not only influence the phytoplankton community directly by a reduction of its prey (the zooplankton) but also indirectly by the induced migration behavior of the zooplankton.

The fact that DVM of zooplankton influence pelagic food webs was recognized early (Lampert 1987, 1992). However, despite the general interest in theoretical ecology and in experimental studies of "trait mediated interactions" (Peacor and Werner 2001, Trussell et al. 2003, Schmitz et al. 2004) and the general importance of phytoplankton-zooplankton interactions for pelagic food webs, nearly no empirical data (except Reichwaldt et al. 2004, Reichwaldt and Stibor 2005) exists.

One of the most important points in studying the effects of DVM on the pelagic food web structure is the temporal and local refuge for all edible algal species in the phytoplankton community of shallow water created by the downward migration behavior of the zooplankton. This refuge can affect the growth of the phytoplankton

community (Petipa and Makarova 1969) by creating discontinuous predator-caused mortality during the day, which follows with the theory that prey organisms with high growth rates should be able to use this refuge better than slower growing ones.

As a result, fast growing algal species could be fostered disproportionate and shift competition between algal species with different growth rates. A basic theoretical model (Lampert 1987) (Fig. 1) implies that discontinuous grazing could also lead to a higher phytoplankton biomass, compared to similar systems with continuous grazing, even if total grazing of zooplankton under discontinuous and continuous conditions is similar during the observation period. The fostering effect of discontinuous grazing increases with increasing phytoplankton growth rate.
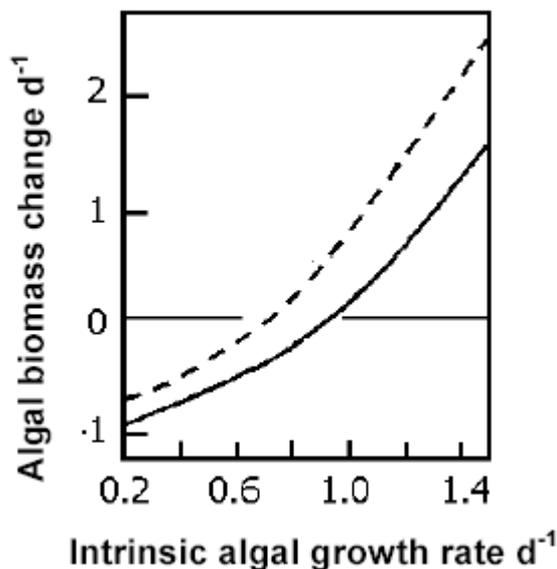

Fig. 1: Model calculation of the effect of different diel grazing patterns of zooplankton on edible phytoplankton net production. In both patterns, the same total algal biomass is consumed by the zooplankton per day. The lower line estimates algal biomass change if grazing is continuously (no DVM). The upper dashed line estimates algal biomass change if grazing takes place only during the night (DVM). The area between the two lines indicates the difference in the relative change of algal biomass for the two grazing patterns. In this example, the grazed algal biomass is equal to the unaffected primary production per day (Lampert 1987).

These theoretical expectations could be confirmed in first laboratory studies. In treatments where the total amount of grazed algae was not different under a continuous and discontinuous grazing regime, Reichwaldt et al. (2004) could show a higher daily algae growth under discontinuous grazing. A second result of this study was the strong influence of the grazing regime on the competition between the algae. In algal communities with different species, highly competitive algae under continuous grazing regime proved to be bad competitors under discontinuous grazing regime.

2.1.2 Temperature effects

Another important aspect of how DVM can influence the phytoplankton community is the temperature difference between the upper and lower water layers, which is the normal case in stratified lakes of temperate regions (Lampert and Sommer 2007). Zooplankton experience a lower temperature in deeper water layers, which can drastically reduce the growth rate of the zooplankton community (Bottrell 1975, Loose and Dawidowicz 1994, Reichwaldt and Stibor 2005). Zooplankton population abundance can for this reason be lower, which would result in a lower grazing pressure on the phytoplankton community. The lower density of migrating compared to non-migrating zooplankton populations is certainly only the case if one leaves out predation as a potential mortality factor, because if predation is present, a non-migrating population would also have lower growth due to this predation (Stich and Lampert 1981). However, until now little is known about the influence of a fluctuating temperature regime experienced by zooplankton during their migration and the resulting consequences for the pelagic ecosystem. (Note that such temperature effects of DVM were not part of my study but of a previous one, Reichwaldt and Stibor 2005).

This section mentioned aspects of how changes in zooplankton grazing patterns - created by DVM - can influence pelagic ecosystems are rarely studied in field studies. Initial experiments by Reichwaldt and Stibor (2005) indicate the importance of the above mentioned effects on zooplankton-phytoplankton interactions under DVM conditions. They were able to show that DVM of zooplankton could enhance the biomass of the phytoplankton community in the epilimnion and that it can have strong impacts on the composition of the phytoplankton community by fostering small edible algae.

2.2 Nutrient dynamics

In the pelagic ecosystem of a lake, vertical gradients play an important role. In most lakes abiotic parameters such as light, temperature and oxygen concentration decrease with increasing water depth. Additionally, a vertical nutrient gradient exists in nearly every lake. This gradient is caused by the continuous sedimentation of organisms due to gravity and also the demineralization of organisms. Nutrients thereby get lost in upper water layers and concentrated in deeper water layers. In lakes of temperate regions, full circulation of the water column (which mostly happens twice a year in spring and fall) redistributes the nutrients in the whole water column.

However, during stagnant periods of the water column, physical upward transport processes are very unlikely. Therefore, due to the high nutrient demand of primary production, which nearly exclusively takes place in the epilimnion, nutrients are depleted in upper water layers resulting in a strong shortage of nutrients. Even dissolved phosphorus and its biologically relevant form, orthophosphate, which is the most important growth-limiting nutrient in most lakes, is often reduced below the detection limit (for a detailed description of the succession processes in lakes of temperate regions, see Sommer et al. 1986). This depletion of nutrients, therefore, has a strong influence on the often nutrient limited bacterio- and phytoplankton communities in the epilimnion.

DVM of zooplankton could be an upward nutrient transport process. The daily migration behavior of zooplankton provides more nutrients for the phytoplankton community in upper water layers but only if zooplankton have enough food in deeper water layers (Kitchell et al. 1979, Dini et al. 1987, Winder et al. 2003). Seston in deeper water layers often have a high quality as food because the nutrient concentrations are often high in deeper water layers. Additionally, light intensity is low in deeper water layers but often sufficient for low primary production. Phytoplankton in deeper water layers are therefore often characterized by a high nutrient (e.g. phosphorus) uptake and low light-dependent carbon assimilation, which results in a low carbon: phosphorus (C: P) ratio of their biomass (Sterner et al. 1997).

Zooplankton organisms exhibit a lower C: P ratio than algae, and their C: P ratio is not as variable as those of algae; therefore algae with a lower C: P ratio are supposed to be a better food than algae with a high C: P ratio (Urabe and Sterner 1996, Boersma 2000, Becker and Boersma 2003). Additionally, protozoa in deep water layers are also high quality food for zooplankton. For examples copepods, as omnivores, use protozoa as an additionally food source (Zöllner et al. 2003, Stibor et al. 2004b).The conditions found in the upper water layers are often contrary to the conditions found in deep water layers. In surface waters, light availability is high, but nutrient availability, especially phosphorus, is low during periods of stratification. Therefore, algae in the epilimnion often exhibit high C: P ratios and can be seen as low quality food (Urabe and Sterner 1996, Sterner and Schwalbach 2001).

The possible mechanism for upward nutrient transport can be as follows: zooplankton graze on potentially nutrient-rich seston (Winder et al. 2003) during the day in the

hypolimnion, migrate up to the epilimnion in the evening and excrete nutrients in upper water layers (Sterner and Schwalbach 2001), causing a nutrient transport between the epi- and hypolimnion. The quality and quantity of the transported nutrients would depend on the amount and the nutrient content of the food in the hypolimnion, as well as on the metabolic rate of the zooplankton, which is strongly temperature-dependent (Orcutt and Porter 1984). Clearly zooplankton also cause a downward nutrient transport due to their downward migration in the morning. Therefore, whether DVM causes a net nutrient transport to upper or deeper water layers depends on the total amount of food, the quality of food in epi- and hypolimnion, the food demands of the zooplankton, the temperature, and the length of stay by zooplankton in epi- and hypolimnion.

Additionally, the DVM coupled nutrient transport could change the nutrient composition of algae in the epilimnion and lower their C: P ratio, which can increase the quantity and improve the nutritional quality of algae as for food for zooplankton. For this reason, a loop seems possible; the change in seston quality and quantity can influence zooplankton dynamics and therewith the entire pelagic trophic cascade.

3   Phytoplankton vertical migration – consequences for the phytoplankton

Vertical migrations in stratified water columns are not only performed by zooplankton. Light and nutrients are essential components needed for primary production in pelagic ecosystems; the availability of both strongly varies in the water column. Therefore, phytoplankton species often experience contrary vertical gradients of light and nutrients (Olli 1999). Many phytoplankton species cope with this problem by changing their position in the water column through active movement or buoyancy adjustment. These periodic vertical migrations allow motile algae to access deeper nutrient-rich waters and to adjust for optimal irradiance.

Active movements are reflected in higher metabolic rates and higher light requirements for growth. The uptake of nutrients and the rate of light-dependent carbon fixation of phytoplankton are not tightly coupled, and the carbon to nutrient ratio in phytoplankton biomass is often very variable (Sterner et al. 1997, Striebel et al. 2008). The C: P ratio can vary 20-fold as a result of varying light and phosphorus availability (Urabe and Sterner 1996). Therefore, it seems obvious that actively motile and non-motile phytoplankton species should differ in flexibility and range of their biomass C: P ratios.

Motile taxa, which perform periodical vertical migrations between illuminated, upper water layers and nutrient rich, deep water layers, exhibit a more balanced ratio of carbon fixation to phosphorus uptake, compared to non-motile taxa. Additionally, motile taxa respire more carbon and need more phosphorus, due to higher metabolic rates demanded by active movement reflected in lower C: P ratios. Finally, many non-motile taxa of green algae possess cell walls with high carbon compounds, which increase their C: P ratios compared to motile taxa. Studies investigating the different C: P demands and biomass composition of motile and non-motile taxa are until now missing.

In conclusion, the ability to move actively changes the biomass composition of motile species and entails costs in terms of increased energy expenditure and in specific cell structures needed for movement. However, the consequences and costs are not yet known.

4   Hypotheses

This thesis focuses on the consequences of migrating zooplankton and phytoplankton species for pelagic ecosystem composition and dynamics. The following scientific issues are addressed:

1. Modern ecological stoichiometry points towards the fact that herbivores can be important vectors of nutrients. Dependent on the biochemical composition of their food, herbivores can be sources or sinks of important, potentially growth-limiting nutrients, such as phosphorus or nitrogen. Zooplankton DVM therefore could be an important mechanism of how nutrients from nutrient rich deep waters can be transported to normally nutrient poor surface waters. Up to now an estimate of the magnitude and the biological relevance of a zooplankton DVM-mediated nutrient transport for phytoplankton community growth is missing.

2. DVM of zooplankton may also strongly influence the phytoplankton community by migration pattern dependent grazing. Zooplankton DVM creates a spatial and temporal refuge for phytoplankton species in allowing a period of reduced mortality due to less grazing by zooplankton during the day in upper water layers. Theoretical models suggest that especially fast-growing algae could use such a refuge for growth, which would enhance phytoplankton biomass during periods of zooplankton DVM.

3. Body size plays an important role in all ecological interactions. Previous studies made clear that individual size of phytoplankton species determines to a large extent their response to zooplankton DVM. Different size structured phytoplankton communities, as caused by yearly plankton succession, could show a different reaction to zooplankton DVM. Field experiments investigating the effects of zooplankton DVM on phytoplankton community dynamics normally allow only *post hoc* reasoning about mechanisms on how zooplankton DVM can influence phytoplankton performance. To answer whether phytoplankton community structure has an influence on the direction and strength of zooplankton DVM mediated effects, one has to experimentally manipulate phytoplankton community structure *a priori*. Only this will allow investigations of how community structure interacts with zooplankton DVM under identical environmental conditions.

4. In pelagic ecosystems vertical migration is not only restricted to zooplankton; a variety of phytoplankton species also show diurnal vertical migration behavior. One possible reason for this is that algae can thereby position themselves in optimal light and nutrient conditions within the water column. Light is decreasing with depth, whereas nutrients are normally increasing. Migrating into deeper waters can increase nutrient uptake, whereas upwards migration ensures optimal light uptake during day. Such behavior could have consequences for the carbon to nutrient ratio of migrating phytoplankton species with additionally consequences for pelagic nutrient dynamics and phytoplankton-zooplankton interactions.

The above described open research fields motivated me to investigate the following four hypotheses.

I.   DVM of zooplankton cause a measurable and biologically relevant upward nutrient transport between hypolimnion and epilimnion.

II.   DVM of zooplankton influence the growth and biomass of the phytoplankton community by creating a discontinuous grazing regime.

III.   DVM of zooplankton have different effects on phytoplankton communities with different size structures.

IV.   Vertical migration of motile phytoplankton influences the biochemical composition of phytoplankton communities, because motile species have higher nutrient demands compared to non-motile species.

5   Publications

The above mentioned hypotheses were experimentally tested using field and laboratory experiments. The resulting four studies are presented in detail in the following papers.

5.1   Upward phosphorus transport by *Daphnia* diel vertical migration

Haupt, F., Stockenreiter, M., Reichwaldt, E. S., Baumgartner, M., Lampert, W., Boersma, M. and Stibor, H. (2010)

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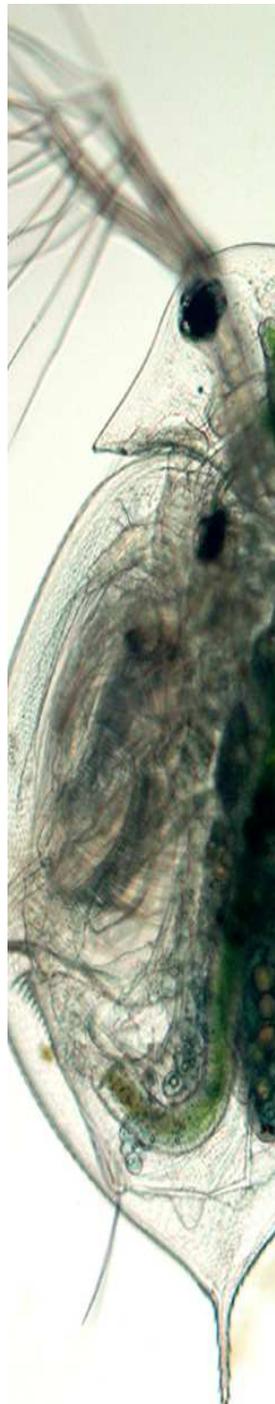
Upward phosphorus transport by *Daphnia* diel vertical migration

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Abstract

In many lakes, zooplankton show a distinct diel vertical migration (DVM) behavior, especially during periods of stratification. Excretion products of these zooplankton could potentially cause an upward nutrient transport and consequent nutrient enrichment for phytoplankton in the epilimnion. We quantified the upward transport of phosphorus by the cladoceran *Daphnia* DVM experimentally by adding a radioactive tracer (^{33}P) to the hypolimnion of large indoor mesocosms and measuring tracer accumulation in the epilimnion over time. During the daytime, when all *Daphnia* were found in the hypolimnion, no phosphorus transport from the hypolimnion into the epilimnion took place. As soon as the *Daphnia* started their upward migration, around dusk, we observed a continuous increase in phosphorus concentration in the epilimnion. The amount of phosphorus transported was in a biologically meaningful range. Our results strongly suggest that *Daphnia* vertical migration presents a continuous nutrient supply for the epilimnion.

Diel vertical migration (DVM) of zooplankton is one of the world's largest synchronized movements of animals (Hays 2003). It is a daily habitat shift of zooplankton from deeper water regions during the day to surface waters at night. DVM is a well-investigated phenomenon and both the ultimate and proximate reasons for this behavior have been elucidated (Ringelberg 1991; Lampert 1993; Loose et al. 1993). Previous DVM research has focused primarily on the ecophysiological consequences for the migrating zooplankton (Loose and Dawidowicz 1994; Reichwaldt et al. 2005), since environmental factors such as temperature or feeding conditions vary in different water layers. In contrast, there have been very few studies on the consequences of DVM for the dynamics of the pelagic ecosystem as a whole (Reichwaldt and Stibor 2005; Haupt et al. 2009). This is surprising since, considering the ecosystem-scale amount of biomass involved in DVM, it is likely that DVM also influences ecosystem dynamics.

Implications of DVM for ecosystem dynamics can be manifold. DVM can influence phytoplankton dynamics by altering grazing patterns, by influencing zooplankton population growth (and thereby grazing pressure on phytoplankton), and by nutrient redistribution due to excretion of migrating zooplankton. DVM results in a discontinuous grazing pattern of zooplankton on phytoplankton in upper pelagic layers during the daytime. Theoretical models and some experimental evidence suggest that such a temporal refuge for algae can promote growth of certain algal species (Lampert et al. 1988; Reichwaldt et al. 2005), and actually increase the diversity of the phytoplankton community (Haupt et al. 2009).

DVM also results in a lower growth rate of migrating zooplankton populations (Loose and Dawidowicz 1994), as animals stay in the colder hypolimnion during the daytime. This DVM directly decreases the grazing pressure on phytoplankton communities when lower zooplankton growth rates result in lower population densities. DVM may also result in nutrient transport and redistribution between deeper and upper water layers. This transport may affect the availability of limiting nutrients, such as phosphorus, and thereby influences the temporal and spatial growth dynamics of primary producers.

The interacting mechanisms by which DVM influences phytoplankton dynamics cannot be separated in nature. For example, grazing and release of nutrients are tightly coupled processes. However, to fully understand the consequences of DVM on phytoplankton dynamics, separate estimates are needed for the different mechanisms by which DVM influences pelagic food webs. Hence, detailed studies examining the effects of DVM on nutrients are needed. Although Lampert and Grey (2003) showed that *Daphnia* could transport nitrogen from the hypolimnion into the epilimnion, their study did not include DVM conditions.

Because of the permanent gravity-induced downward nutrient flux caused by sinking organisms and particles, nutrients tend to concentrate in deeper water layers, especially during periods of stratification, thus depleting the epilimnion of nutrients. Hence, especially during those periods, DVM could be a daily source of nutrients for phytoplankton growth. Physical diffusion processes in the water column can hardly compensate these losses because vertical eddy diffusivities are normally two or three orders of magnitude smaller than horizontal ones (Spigel and Imberger 1987). Hence, biological upward nutrient trans-

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port mediated by migrating zooplankton from deeper water layers may be the only regular internal supply during summer stratification when nutrients, especially phosphorus, are severely limiting in upper water layers (Sommer et al. 1986).

Recent evidence showing that zooplankton release phosphorus continuously (DeMott et al. 1998; Anderson et al. 2005; Boersma and Wiltshire 2006) contradicted the traditional view that suggested that excretion of limiting nutrient should approach zero under severe nutrient limitation (Olsen et al. 1986). A physiological explanation for a continuous release is that animals will generally not approach 100% element assimilation efficiency (DeMott et al. 1998), and do not have perfect retention capacity during excretion. Furthermore, there will be some metabolic phosphorus demand, which also results in a net loss (Anderson et al. 2005).

Migrating zooplankton transport nutrients upward as they migrate up at dusk, and transport material downward when they migrate down at dawn. To our knowledge, no direct measurements exist of nutrients transported by *Daphnia* DVM. Dini et al. (1987) estimated the amount of phosphorus transported downward by *Daphnia* DVM to be half of the upward transport. However, the ecological effects of the downward transport are completely different from the effects of upward transport. Downward transport is accompanied by the large gravity-driven sedimentation of particles, and phosphorus is not limiting in deeper water layers. Although upper layers of pelagic ecosystems are often nutrient but not light limited, deeper water layers are usually light limited but have sufficient nutrients. Thus, the input of nutrients into deeper water layers will not enhance primary production. Additionally, the downward migration of zooplankton during DVM also removes phytoplankton from the upper layers and thereby additionally reduces nutrient competition in the upper layers. In contrast, the upward nutrient transport into the nutrient-limited upper layers, where most primary production generally occurs, will immediately result in nutrient uptake by phytoplankton.

The above-mentioned arguments show that the ecosystem-scale consequences of upward and downward nutrient fluxes by DVM are very different. Irrespective of the size of the downward flux, an upward flux will always have direct consequences for phytoplankton primary production. The greater the nutrient limitation of the phytoplankton community in the epilimnion, the larger the effects of upward nutrient import will be. Until now, nutrient transport into upper water layers by migrating zooplankton has not been quantified. Measurements are difficult to perform because it is impossible to distinguish phosphorus originating from upper and lower water layers in natural systems, and also because the amounts of phosphorus involved during migration may be too small to be technically analyzed. The detection limit using photometric methods is far above phosphorus levels that could be important for biological dynamics such as primary production or bacterial growth (Vadstein 2000). The use of radioactive tracers provides an elegant solution to overcome both hindrances.
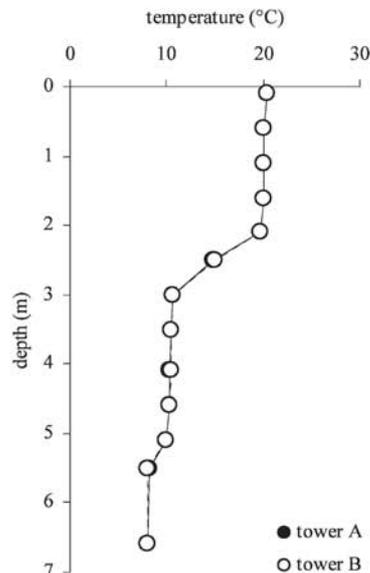

Fig. 1. Vertical temperature gradient in the two plankton towers. The two profiles are nearly identical and the symbols overlay one another.

Methods

We investigated the upward flux of phosphorus using the radioactive phosphorus tracer ^{33}P during *Daphnia* DVM in two large indoor mesocosms, the so-called "plankton towers" at the Max Planck Institute for Limnology in Plön, Germany, which have since been removed. The plankton towers were 11.5 m high, had an inner diameter of 0.85 m, and were used in a variety of studies investigating plankton dynamics in controlled large-scale indoor systems. The towers were described in detail elsewhere (Lampert and Loose 1992). We filled the towers with 10 μm of filtered epilimnetic water from mesotrophic Lake Schöhsee, Germany. The experiment was carried out in August 2006, the period of the year when the epilimnion of Lake Schöhsee is largely nutrient depleted. After filling, water was thermally stratified. Temperatures during the experiment were 20°C in the epilimnion (0 to 2.1 m) and 10°C in the hypolimnion (3.0 to 5.1 m), with a steep thermocline between the layers. The maximum hypolimnion depth of 5.1 m was chosen because earlier experiments in the plankton towers showed that *Daphnia* would not easily migrate down the entire plankton tower depth (Reichwaldt 2008). To avoid mixing of the algae into the lower layer of the towers (5.5 to 11.5 m), we adjusted the temperature in this layer to 8°C. Figure 1 shows the temperature profiles in the epi- and hypolimnion in both towers. The light : dark cycle was 12 : 12 h.

After thermal stratification, 0.6 mg C L^{-1} of the chlorophyte *Scenedesmus obliquus* was added into the epilimnion and 0.2 mg C L^{-1} added into the hypolimnion (Fig. 2). *Scenedesmus obliquus* concentrations were deter-
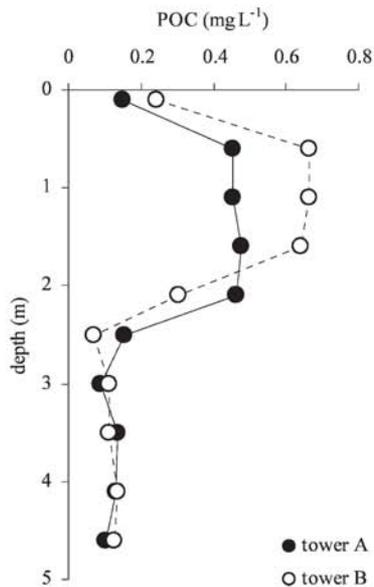
Fig. 2.   Vertical gradient of particulate organic carbon (POC) concentrations in the two plankton towers.

mined by measuring fluorescence using a FluroProbe (bbe Moldaenke) and subsequent conversion to particulate organic carbon using a previously established calibration curve. *Scenedesmus obliquus* was cultured in a batch system with Z/4 medium (Zehnder and Gorham 1960) under continuous light conditions. Thereafter, 10,000 to 15,000 *Daphnia magna* were put into each tower. *Daphnia* originated from cultures that had been cultivated at the Max Planck Institute for Limnology for several years, and this clone is known to perform DVM (Loose and Dawidowicz 1994). They were reared in 200-liter containers with 10-μm-filtered water from Lake Schöhsee and *S. obliquus* (> 1 mg C L^{-1}). To induce DVM behavior of *Daphnia*, each tower was stocked with three fish (*Leuciscus idus*) in a cage. The cage was located within the epilimnion of each tower at a depth of 0.5 m and was not moved during the experiment. To estimate *Daphnia* density, vertical hauls with a small plankton net (0.25-m diameter, 150-μm mesh size) were performed in the epilimnion from 2.1 m depth to the surface twice during the light cycle and four times during the dark cycle. We preserved the samples in 4% sucrose–formaldehyde solution (Haney and Hall 1973) and counted all individuals under a dissecting microscope.

To quantify the transport of phosphorus from the hypolimnion into the epilimnion, we incubated 400 mL of a dense culture of *S. obliquus* with the radioactive phosphorus isotope ^{33}P (total activity 185 MBq; specific activity 92.5 TBq mmol^{-1}). We added 100 mL of an unlabeled orthophosphate solution (50 μmol L^{-1} P) to the culture to ensure uniform uptake of radioactive phosphorus by *S. obliquus*. After 24 h of incubation, labeled algae were centrifuged and resuspended in Z/4 medium. At the

beginning of the light period, the labeled cultures were split into equal amounts and added to the hypolimnion of the two towers by injecting them via ports (for a detailed description of the ports, *see* Lampert and Loose 1992) at a depth of 4.1 m.

To estimate the transported and released total phosphorus amount, we measured the total phosphorus concentration in the hypolimnion of both towers and related them to radioactive counts. Water samples from the hypolimnion of both towers were taken 3 h after adding the radioactive tracer algae, at which time labeled algae were homogenously distributed. Water samples were taken via ports at 4.1-m depth and filtered through 250-μm gauze to exclude *Daphnia*. Total phosphorus concentrations were measured using standard methods (Wetzel and Likens 1991). Total phosphorus in the hypolimnion was 11.5 μg L^{-1} in tower A and 13 μg L^{-1} in tower B. For radioactivity measurements, 4 mL of the samples were transferred into scintillation vials and 12 mL of scintillation cocktail (Ultima Gold, Packard) were added. The samples were immediately analyzed with a scintillation counter (Packard Tricarb 2900). The addition of the radioactive tracer algae resulted in 10,223 disintegrations per minute (dpm) mL^{-1} on average in tower A, and 20,654 dpm mL^{-1} in tower B within the respective hypolimnion. Therefore, 1 dpm accounted for 1.12 pg P L^{-1} in tower A and 0.63 pg P L^{-1} in tower B.

After adding the radioactive labeled algae, the epilimnion of both towers was sampled continuously (twice during the light phase, once at the start of the dark phase, and three times during the dark phase) for radioactive phosphorus. Water samples were again filtered through a 250-μm plankton net to exclude *Daphnia* and 4 mL of each sample were transferred into scintillation vials to analyze radioactivity as above. Additionally, unpreserved *Daphnia* samples, sampled as described above at the start of the dark cycle in the epilimnion, were used to estimate the amount of labeled algae ingested by *Daphnia* in the hypolimnion. We transferred the *Daphnia* into scintillation vials and added 4 mL of a tissue solubilizer (Soluene-350, Packard). After 24 h, we added 12 mL of scintillation cocktail (Hi-Ionic-Fluor, Packard) and analyzed radioactivity immediately with a scintillation counter (Packard Tricarb 2900).

Results

Daphnia density in the epilimnion during the daytime was 0.02 $\pm$ 0.00 individuals L^{-1} (mean $\pm$ SE). Immediately after switching the lights off, *Daphnia* started to migrate into the warmer and food-richer epilimnion. We found 6.14 $\pm$ 1.02 individuals L^{-1} (mean $\pm$ SE) within the epilimnion during the night. A one-way repeated-measure ANOVA calculated with data from both towers revealed no significant differences in *Daphnia* density in the epilimnion between the time of the dark phase: $F_{1,3} = 0.13$; $p = 0.75$. Hence, the change in light intensity between day and night, together with the presence of fish, caused a strong DVM behavior in *Daphnia*, which was already shown in earlier experiments (Loose et al. 1993).

No radioactive phosphorus was found in the epilimnion during the light period, when *Daphnia* almost exclusively

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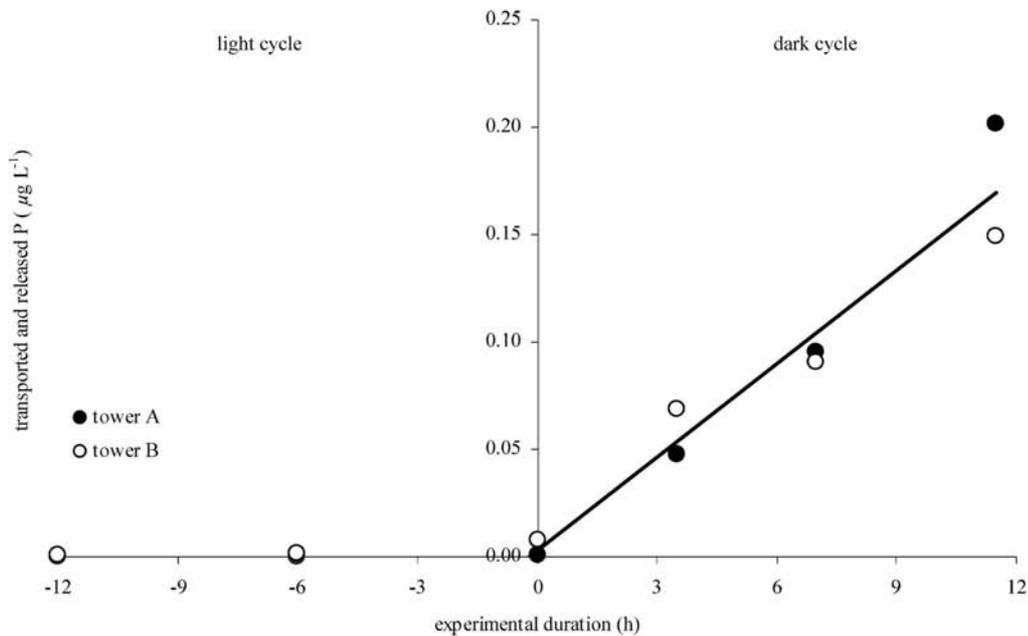

Fig. 3. Phosphorus transported and released from the hypolimnion into the epilimnion by *Daphnia* DVM, shown as changes in phosphorus concentration in the epilimnion in each tower along experimental time (point -12 represents the start of the light cycle and addition of labeled algae to the hypolimnion, point 0 represents the start of the dark cycle and onset of upward migration behavior by *Daphnia*). The line represents the regression between transported and released phosphorus in the epilimnion and time for the dark cycle ($y = 0.015x + 0.003$; $R^2 = 0.94$; $p < 0.001$). Regression was calculated using data from both towers.

remained in the hypolimnion. Immediately after the start of the dark period, *Daphnia* migrated upward and ^{33}P concentrations increased in the epilimnion (Fig. 3). The ^{33}P content of *Daphnia* in the epilimnion shortly after the start of the dark phase accounted for an uptake of 47.4 ± 4.6 ng P *Daphnia*$^{-1}$ (mean $\pm$ SE) within the hypolimnion during the 12-h light cycle.

The increase of phosphorus within the epilimnion can be described by a linear function of transported phosphorus vs. time in both towers; the linear regressions were significant (tower A: $y = 0.017x - 0.009$; $R^2 = 0.98$; $F_{1,2} = 84.46$, $p = 0.012$; slope: $t_{(2)} = 9.19$, $p = 0.01$; intercept: $t_{(2)} = 0.66$, $p = 0.58$. Tower B: $y = 0.012x + 0.015$; $R^2 = 0.97$; $F_{1,2} = 75.69$, $p = 0.013$; slope: $t_{(2)} = 8.70$, $p = 0.01$; intercept: $t_{(2)} = 1.57$, $p = 0.26$). An analysis of covariance revealed no statistical difference between the towers at the 5% level (slopes: $F_{1,4} = 5.79$; $p = 0.07$; intercepts: $F_{1,5} = 0.28$; $p = 0.62$.) Therefore, we used data from both towers to calculate a combined regression between phosphorus release in the epilimnion and time (Fig. 3): $y = 0.015x + 0.003$; $R^2 = 0.94$; $F_{1,6} = 91.30$, $p < 0.001$; slope: $t_{(6)} = 9.56$, $p < 0.001$; intercept: $t_{(6)} = 0.29$, $p = 0.79$. On average, the total *Daphnia* community transported and released 15 ng P L^{-1} h^{-1} from the hypolimnion into the epilimnion. This resulted in an overall 180 ng P L^{-1} transported in the 12-h dark phase.

We estimated the released phosphorus as a total phosphorus fraction. Boersma and Wiltshire (2006) showed

that *Daphnia* sp. release phosphorus in two fractions: 80% as dissolved phosphorus and 20% as particulate phosphorus. Only the dissolved phosphorus fraction can be used immediately by the phytoplankton community. Hence, in our experiment the amount of transported and released dissolved phosphorus by *Daphnia* from the hypo- into the epilimnion can be estimated as 12 ng L^{-1} h^{-1} and 144 ng L^{-1}, respectively, during the 12-h dark phase.

Discussion

Phytoplankton compete for released phosphorus within the epilimnion. Phosphorus uptake rates for *Scenedesmus* sp. depend on dissolved phosphorus concentration within the pelagic environment (Rhee 1973). Rhee (1973) suggested a phosphorus uptake range for *Scenedesmus* sp. under laboratory conditions between 1×10^{-11} and 3×10^{-11} μmol cell^{-1} min^{-1}. Applying this value to our *S. obliquus* population (6000 cells mL^{-1}), we can calculate a maximum uptake rate of 110 ng P L^{-1} h^{-1} in our experiment. This value is in good agreement with one of the few estimates of phosphorus uptake in a natural bacterial and phytoplankton community in a eutrophic lake, which suggested a maximum total biologically reactive phosphorus uptake of 120 ng L^{-1} h^{-1} (Lean 1973). The amount of phosphorus transported and released by migrating *Daphnia* in our experiment was at least 10% of the maximum uptake rates given by Rhee (1973) and Lean (1973). A recent study of

phosphorus dynamics in a stratified eutrophic lake revealed a mean daily uptake rate of the primary producers in the epilimnion of 7.83 mg P m^{-2} d^{-1} (Kamarainen et al. 2009). The transported and released phosphorus in our experiment accounted for 0.36 mg m^{-2} in the 12-h dark cycle, and thus the transported phosphorous was about 5% of the daily uptake rate estimated by Kamarainen et al. (2009). Both of the above examples show that the transported and released phosphorus in our experimental pelagic system is certainly biologically relevant and is probably one of the mechanisms fueling primary production in the highly phosphorous-limited epilimnion.

Although the upward flux of phosphorus by zooplankton DVM may be low compared with other processes influencing phosphorus dynamics in the epilimnion, it is a regular nutrient supply during periods of stratification. The phosphorus transported by DVM may comprise a substantial phosphorus source for primary production. During periods of stratification, a constant gravity-driven flux of phosphorus from upper water layers to the sediments often results in limited phytoplankton growth. In this case, new production of phytoplankton biomass relies on direct input of phosphorus from terrestrial sources and nutrient recycling within the epilimnion. Phosphorus concentration is often extremely low within the epilimnion of stratified lakes such that any new input of phosphorus is immediately translated into an increase in primary production. Therefore, even if the nutrient balance resulting from nutrient transport by DVM for the epilimnion is negative, a daily transport of phosphorus into the epilimnion will have consequences for phytoplankton growth. Our results indicate that even at natural *Daphnia* densities (approximately 6 *Daphnia* L^{-1} in our experiment), this transport is within a biologically relevant range.

The phenomenon described here can be considered of real ecological value given that in deeper lakes, during summer stratification, a distinct epilimnion exists with concurrent low exchange rates with deeper water layers and therefore an increasing nutrient depletion in the epilimnion caused by sinking losses. A relatively low mixing depth of the epilimnion would increase the nutrient depletion due to a high light availability for the phytoplankton community, resulting in a higher primary production rate requiring more nutrients (Sommer et al. 1986). The upward-transported nutrients should hence be mainly of interest in oligo- and mesotrophic lakes with a nutrient-rich hypolimnion. Whether *Daphnia* DVM causes a net up- or downward nutrient transport depends on the quantity and quality of food in upper and deeper water layers and the duration spent in these layers. Most likely, the upward transport would be most relevant in lakes with a chlorophyl maximum, ample food, and sufficient amount of phosphorous in deeper water (Lampert and Grey 2003; Winder et al. 2003).

DVM is not confined to freshwater systems. Most marine pelagic environments are also affected by DVM of zooplankton (Hays 2003). Longhurst and Harrison (1989) suggested that DVM is a critical component of the "biological pump" that draws organic carbon and atmospheric CO_2 into the ocean. The present results suggest that zooplankton DVM may also play an important role in the redistribution of other nutrients, such as nitrogen. Further experimental analyses on the role of nutrient transport by migrating marine zooplankton are necessary to fully elucidate the importance of the world's largest synchronized movement of biomass on global carbon and nutrient dynamics.

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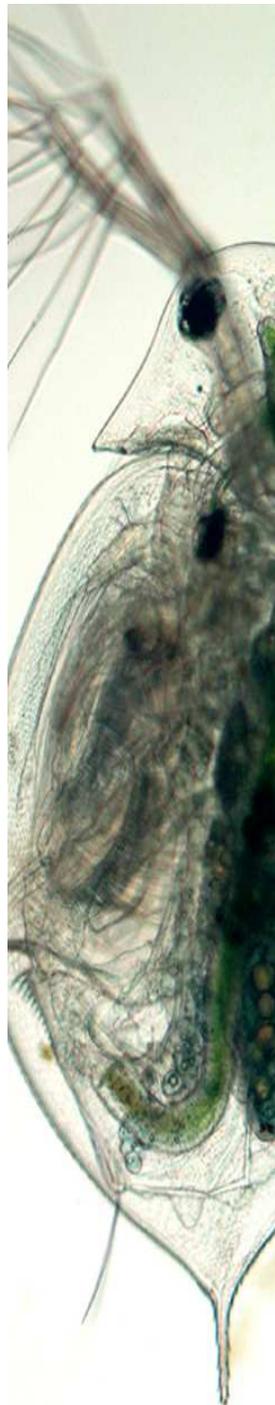
5.2 *Daphnia* diel vertical migration: implications beyond zooplankton

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Daphnia diel vertical migration: implications beyond zooplankton

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Diel vertical migration (DVM) is a common behaviour of many pelagic zooplankton species. While the causes (mostly predator avoidance) and ecophysiological consequences of DVM for zooplankton have been well studied, little is known about the consequences of DVM for the pelagic food web. DVM creates a temporal and spatial grazer-free niche for edible phytoplankton, and theoretical models predict that parts of the phytoplankton community should use this niche. Conceivably, DVM could also cause nutrient transport between separated water layers. We experimentally investigated the influence of DVM of the zooplankton species, Daphnia hyalina, on the nutrient and phytoplankton dynamics of an oligotrophic lake. We used 10-m deep field enclosures with a 4-m deep, well-mixed surface layer. The enclosures contained either migrating or non-migrating Daphnia populations; temperature was kept nearly constant across the entire enclosure depth. Our results show that DVM had significant quantitative and qualitative effects on the phytoplankton community. There was no measurable net nutrient transport between hypolimnion and epilimnion. The gelatinous green alga Planktosphaeria gelatinosa, was the dominant algal species in our experiment. Its abundance decreased in DVM treatments, and thus also influenced the total biomass and diversity of phytoplankton communities.

INTRODUCTION

Trophic cascades, reflecting the indirect impact of carnivores on plants through effects on herbivores, are important in food web dynamics, linking predators to lower trophic levels. Trophic cascades occur in a wide variety of ecosystems, and there has been much debate about their nature, strength and overall importance (Strong, 1992; Polis *et al.*, 2000; Shurin *et al.*, 2002). Most descriptions of trophic cascades focus on numerical decreases in herbivore populations due to direct removal by predators. Lower herbivore abundance relieves predation pressure on plants; thus, carnivores can have positive indirect effects on plants. These mechanisms are called density-mediated indirect effects (Abrams, 1995; Werner and Peacor, 2003). However, prey animals are normally not oblivious to the risk of predation and often respond to the presence of predators through changes in behaviour. For example, prey may hide or seek other habitats, behavioural changes that diminish foraging activities and thereby lessen herbivore impacts on plants (Abrams, 1984, 1995; Werner and Peacor, 2003). These indirect effects of carnivores on plants derive from individual traits of herbivores and are called trait-mediated indirect interactions. Recent analyses suggest that the trait-mediated impact of predators on plants may often be larger than density-mediated effects (Schmitz *et al.*, 2004).

Since the discovery of the importance of trait-mediated effects, numerous examples have demonstrated their relevance in a variety of ecosystems (summarized in Schmitz *et al.*, 2004). One of the most important escape responses of aquatic herbivores is the vertical migration of zooplankton (Hays, 2003). Zooplankton in lakes and in the sea often exhibit a significant shift in their vertical distribution, spending the night primarily in upper water

layers, and migrating down at dawn to spend the daytime in deeper, and hence darker and colder, water layers (Lampert, 1989). Many studies on this diel vertical migration (DVM) behaviour have focused on the reasons for the daily migration, and have clearly established predator (mainly fish) avoidance as the ultimate reason for DVM (Gliwicz, 1986); proximate reasons include changes in light intensity around dawn and dusk (Ringelberg, 1991) and the presence of a chemical trigger substance (kairomone) released by fish (Loose *et al.*, 1993).

The escape responses used by zooplankton to avoid their planktivorous predators may have different consequences for phytoplankton communities:

(i) Vertical migration behaviour creates a refuge for phytoplankton during daytime. Theoretical considerations (Lampert, 1987) assume that algal species can profit from a discontinuous grazing regime if they can use the grazer-free time for growth, and recent studies (Reichwaldt *et al.*, 2004; Reichwaldt and Stibor, 2005) indeed support these assumptions. How much an individual algal species profits from DVM depends on the growth rate of the species: faster-growing species should benefit more from DVM than slower growers.

(ii) Migration behaviour should reduce the growth of the zooplankton population (Loose and Dawidowicz, 1994). The darker, deeper water layers of the pelagic water column into which zooplankton migrate are usually associated with lower temperatures and diminished food availability (Lampert, 1989). Both factors will limit the growth of zooplankton populations and thereby lessen their grazing impact on phytoplankton.

(iii) Migration behaviour can cause nutrient transport between nutrient-rich deeper waters to the often nutrient-limited upper layers of the water column. Phytoplankton in surface waters often experience strong nutrient limitations during stratified periods, and concentrations of the limiting nutrient are often near the detection limit (Sommer *et al.*, 1986). An upward transport of nutrients from deeper, nutrient-rich waters by migrating zooplankton would then immediately result in a positive growth response of phytoplankton.

Despite the fact that phytoplankton are very important to global carbon dynamics (Geider *et al.*, 2001), and the behavioural response of zooplankton to predators might result in several mechanisms leading to trait-mediated effects on the phytoplankton community, the consequences of DVM for plankton dynamics have received astonishingly little detailed study. A reason for this may

be the difficulties in inducing and regulating migration behaviour in controlled experiments. Although predation is considered to be one of the most important causes of DVM (Zaret and Suffern, 1976), attempts to establish a predatory dynamic by stocking experimental setups with fish is associated with uncertainties caused by indirect effects on the phytoplankton community resulting from nutrients excreted by enclosed fish (Schindler, 1992; Vanni and Layne, 1997; Attayde and Hansson, 1999). In practice, it is also not possible to induce DVM behaviour using kairomones alone because too little is known about the kairomones and their molecular structures.

In this study, we investigated the refuge effect of DVM on the phytoplankton community and the consequences of DVM for nutrient dynamics in a large mesocosm experiment. It is difficult to assess the consequences of the refuge effect of DVM on phytoplankton because depth and temperature are normally coupled in temperate pelagic environments: migrating zooplankton experience lower temperatures in deeper water layers, leading to slower individual growth. In systems lacking predation, such as our mesocosms, this coupling would also result in decreased zooplankton densities. Thus, the refuge effect of DVM on phytoplankton cannot be examined separately from the temperature effect, which also causes a decrease in zooplankton populations and diminishes grazing. Hence, we used a modification of the experimental setup of Reichwaldt and Stibor (Reichwaldt and Stibor, 2005) to separate refuge effects from temperature effects. This method allows the refuge effect of DVM on a natural phytoplankton community to be examined under field conditions in the absence of significant differences in the temperatures experienced by zooplankton migrating between upper and deeper water layers. We hypothesized that algal densities should increase under experimental conditions that induce DVM of zooplankton.

METHOD

Studies were conducted in an experimental enclosure system deployed in oligotrophic Lake Brunnensee in southern Germany ($47°59'N$, $12°26'E$). This is a small (5.8 ha), deep (18.6 m), hardwater lake that is strongly phosphorus-limited (total P: $0.4\ \mu M\ L^{-1}$) and has high nitrate (NO_3^-: $80\ \mu M\ L^{-1}$) and silicate concentrations (SiO_2: $70\ \mu M\ L^{-1}$) during summer. We investigated the effect of vertically migrating zooplankton on the natural phytoplankton community of this lake by artificially moving *Daphnia* populations up and down using cages. A vertical temperature gradient within the enclosures

was prevented by surrounding all enclosures by a 15-m deep, transparent silage film, which acted as a homogenous tempered water bath; uniform mixing was achieved by intermittently blowing compressed air (5 min on, 20 min off) at a depth of 12 m.

Experimental design

Eighteen cylindrical enclosures (transparent Trikoron bags) were suspended from a raft at a depth of 10 m. Each 0.9-m diameter enclosure was heat-sealed at the bottom and open to the atmosphere. In the enclosures, we mimicked an unmixed 6 m deep hypolimnion and a well-mixed, 4-m deep epilimnion; the latter was produced by intermittently blowing compressed air (3 min on, 40 min off) through PVC tubes at a depth of 4 m. The enclosures were filled with 30 μm filtered epilimnetic lake water. In Lake Brunnensee, a 30-μm mesh size is known to effectively exclude all mesozooplankton, while retaining virtually the entire ambient phytoplankton community (Jäger *et al.*, 2008). The chlorophyll-*a* concentration after filling was 5 μM L^{-1} in every enclosure. Potential effects of the strong phosphorus-limited nature of Lake Brunnensee on phytoplankton growth were compensated by enriching each enclosure with 0.5 μM P L^{-1} as KH_2PO_4.

Each of the 18 enclosures contained a gauze cage with a mesh size of 224 μm, a size that ensured that all daphnids were retained within the cages while allowing free exchange of all phytoplankton species. The cage dimensions were 0.7 m $\times$ 3.5 m, and each cage had a re-sealable gauze cap to allow cage contents to be sampled. The volume of the cages was approximately 50% of the epilimnion. DVM, and thus a discontinuous grazing regime in the epilimnion, was mimicked by moving *Daphnia*-containing cages up and down within the enclosures.

For the *Daphnia* "migration" treatment group, six cages containing daphnids were kept in the epilimnion (top of cage: 0.25 m depth) at night (20:00–08:00 h), and then lowered into the hypolimnion (top of cage: 5.5 m depth) for the daytime hours (08:00 h to 20:00 h). All "migration" treatment cages were moved up and down manually as slowly as possible (maximum speed: 0.05 m s^{-1}). In six other enclosures (*Daphnia* "no migration" treatment group), the *Daphnia*-containing cages were kept permanently in the epilimnion (top of cage: 0.25 m depth). As controls to test whether the migrating cages themselves influenced plankton and nutrient dynamics, we installed three enclosures with migrating empty cages and three enclosures with non-migrating empty cages.

We used a clone of *Daphnia hyalina* that originated from Lake Brunnensee to stock the cages. Daphnids were reared in advance in 30 L buckets with a semi-artificial culture medium in a climate chamber with a constant temperature of 20°C. They were fed *Scenedesmus obliquus* (>1 mg C L^{-1}) every second day, and 50% of their medium was renewed every fifth day. Two days before the beginning of the experiment, all daphnids were transferred to 30-μm-filtered epilimnetic lake water. At the beginning of each experiment, daphnids were released into the cages for "migration" and "no migration" treatment groups at a starting density of 5 individuals L^{-1} (where the value of L represents the entire epilimnion volume), a density that is typical for this species in Lake Brunnensee (H. Stibor, unpublished data).

The experiment started with the stocking of the daphnids on 15 August 2006, and lasted for 4 weeks until 12 September 2006. This period represents an ecologically meaningful experimental time scale for plankton dynamics: it is long enough to allow a numerical response of plankton to the experimental manipulations, but short enough that it should prevent the development of most unwanted side effects associated with longer experimental durations, such as intensive wall growth and strong nutrient depletion due to sedimentation within the mesocosms.

Sampling program

Water temperature was measured weekly in vertical steps of 1 m using a WTW model Lf 191 meter with LT1/T probe (Wissenschaftlich-Technische Werkstätten). Vertical profiles of photosynthetically active radiation (PAR) were measured in all enclosures once using a spherical quantum sensor (LI-139SA, Licor). In the "no migration" treatment groups, where cages were present in the epilimnion throughout the day, light intensities were measured with the cages present in the epilimnion to account for possible shading effects of the cages. In both "migration" and "no migration" conditions, PAR was measured stepwise from the surface to a depth of 5 m and used to calculate the depth-averaged light attenuation coefficient (Diehl *et al.*, 2002) for each enclosure. A *t*-test revealed no significant differences between the "migration" and "no migration" treatments ($t_{(16)} = 1.75$; $P = 0.62$), indicating that "migration" and "no migration" treatments had no effects on shading regimes.

Once a week, water samples were taken from each enclosure outside the cages at a depth of 0.5 m (epilimnion) and 7 m (hypolimnion) using a hand pump. All samples taken before the "migration" treatment cages were lowered in the hypolimnion. The samples were 250-μm filtered and immediately analysed for biological and chemical parameters. Water from each sample was filtered onto pre-combusted and acid-washed glass-fibre

filters (Whatman GF/F) to determine seston carbon concentration as particular organic carbon (POC) (Elemental Analyzer, CE Instruments). Concentrations of dissolved inorganic phosphorus (SRP), particulate phosphorus (PP) and total phosphorus (TP) were measured using standard methods (Wetzel and Likens, 1991). Chlorophyll-*a* concentrations were measured fluorometrically (TD 700, Turner Design). For enumeration and identification of phytoplankton species, subsamples were immediately preserved with acid Lugol's iodine and counted later using an inverted microscope (Utermöhl, 1958). If present, at least 100 individuals of each of the species present were counted and the size of 20 individuals was measured using an image analysis programme (Analysis Pro 3.00, Soft Imaging Software). Where present, gelatinous coverings of phytoplankton species were included in the size measurements. The biomass of phytoplankton species was estimated as biovolume, which was calculated by converting size into biovolume using appropriate geometrical figures (Hillebrand *et al.*, 1999). We counted 52 phytoplankton species belonging to six main groups during the experiment: Diatoms (23 species), Chlorophyta (19 species), Cyanophyta (five species), Chrysophyta (three species), Cryptophyta (one species) and Dinophyta (one species). The three dominant groups in terms of biovolume throughout the experimental period were Diatoms, Chlorophyta and Cyanophyta, which together comprised between 97 and 100% of total biovolume in all treatment groups.

Semi-quantitative zooplankton samples were collected weekly from all cages in the morning before the migrating cages were lowered. After opening cages at the top and mixing with a Secchi disc to distribute the *Daphnia* uniformly, a vertical net haul from the bottom to the top inside the cage (net diameter: 0.25 m; mesh size: 150 μm) was taken. This sampling method allowed direct comparisons between enclosures, although it probably under-sampled actual *Daphnia* densities inside the cages because daphnids staying near the cage bottom are not effectively caught (Haupt, 2004). The samples were preserved in a 4% sucrose-formaldehyde solution (Haney and Hall, 1973) and all individuals were counted under a dissecting microscope.

Data processing

Phytoplankton community diversity was calculated using the Shannon–Wiener index (H):

$$H = -\sum_i^n \frac{B_i}{B_{\text{sum}}} \log_2\left(\frac{B_i}{B_{\text{sum}}}\right), \quad (1)$$

where B_i is the biovolume of algal species i, B_{sum} the biovolume of all algal species and n the number of algal species within an enclosure.

Because we were more interested in the ultimate effects of the different treatments than the time-courses leading to these effects, we only used the last sampling date for the analysis of the algal communities in this study. Moreover, because all models and predictions of the effects of DVM on algal communities apply to the epilimnion, we primarily report data from this layer. We used *t*-tests to determine (i) the DVM effect, comparing the epilimnion data from *Daphnia* "migration" and *Daphnia* "no migration" treatment groups; (ii) cage effects, comparing the epilimnion data from migrating and non-migrating empty cages and (iii) potential mixing of the two water layers due to cage movements in the *Daphnia* "migration" treatment groups, comparing phytoplankton data from the epilimnion and hypolimnion. Data are presented as mean $\pm$ one standard error of the mean (mean $\pm$1 SE). Where appropriate to meet statistical assumptions (Sokal and Rohlf, 1981), data were ln-transformed.

RESULTS

General conditions

The water temperature between the enclosures was constant, averaging $11.2 \pm 0.01°C$ at all depths. There was virtually no vertical temperature gradient; within the enclosures, the temperature difference between depths of 0 and 10 m was only $0.5 \pm 0.04°C$. Seston carbon concentrations were similar within the water columns of each enclosure, with differences between water depths of 0.5 and 7 m never exceeding 0.1 mg C L^{-1}. *Daphnia* densities inside the cages averaged 4.7 ± 0.6 individuals L^{-1}, where the value of L represents the entire epilimnion volume. Due to the low temperatures during our study, *Daphnia* densities did not increase substantially until the end of the experiment. We found no significant differences in *Daphnia* densities between *Daphnia* "migration" and *Daphnia* "no migration" treatment groups ($t_{(10)} = 0.83$, $P = 0.43$). Although control treatments were not stocked with *Daphnia*, it was not possible to fully avoid the growth of some daphnids in these groups. However, *Daphnia* densities in control treatments were always lower than 0.1 individuals L^{-1}.

Control treatments

The *Daphnia*-free control treatment groups were included to test for potential effects on nutrient and phytoplankton dynamics due to cage movement.

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However, *t*-tests revealed no significant differences between migrating and non-migrating control cages for any of the parameters that we measured (i.e. PP: $t_{(4)} = 0.05$, $P = 0.97$; TP: $t_{(4)} = 1.43$, $P = 0.23$; seston carbon content: $t_{(4)} = 0.17$, $P = 0.87$; chlorophyll-*a* concentration: $t_{(4)} = 2.09$, $P = 0.10$; diversity (H) of the phytoplankton community: $t_{(4)} = 1.00$, $P = 0.38$). Additionally, we found no significant effect of migrating cages on the total biovolume of any algal species measured (see Table I, cage effect).

Nutrient dynamics

Dissolved inorganic phosphorus (SRP) was always near or below the detection limit ($0.03 \, \mu\text{M} \, \text{L}^{-1}$) in every treatment; therefore, phosphorus was the limiting nutrient throughout the experiment. In the *Daphnia* "migration" and *Daphnia* "no migration" treatments, respectively, PP values were 0.07 ± 0.004 and $0.08 \pm 0.01 \, \mu\text{M} \, \text{L}^{-1}$, and TP values were 0.22 ± 0.01 and $0.21 \pm 0.01 \, \mu\text{M} \, \text{L}^{-1}$ (Fig. 1 A and B). *t*-Tests showed that these values were not significantly different between experimental groups (PP: $t_{(10)} = 0.96$, $P = 0.36$; TP $t_{(10)} = 0.22$, $P = 0.83$). Dissolved nitrate and silicate were available in non-limiting concentrations throughout the experiment (nitrate $>50 \, \mu\text{M} \, \text{L}^{-1}$; silicate $>30 \, \mu\text{M} \, \text{L}^{-1}$).

Seston carbon content

Seston carbon concentrations were lower in the *Daphnia* "migration" treatment group ($513 \pm 16 \, \mu\text{g POC L}^{-1}$) than in the *Daphnia* "no migration" group ($790 \pm 80 \, \mu\text{g POC L}^{-1}$), a difference that was significant by *t*-test ($t_{(10)} = 2.96$, $P = 0.01$; Fig. 2A).

Phytoplankton concentration and composition

Chlorophyll-*a* concentrations were lower in the *Daphnia* "migration" group ($6.6 \pm 0.4 \, \mu\text{g L}^{-1}$) than in the *Daphnia* "no migration" group ($9.4 \pm 1.1 \, \mu\text{g L}^{-1}$), a difference that was marginally significant by *t*-test ($t_{(10)} = 2.15$, $P = 0.057$; Fig. 2B).

Of the 52 counted phytoplankton species, 13 were present in all treatments at the end of the experiment. These 13 species belonged to three groups, Chlorophyta (four species), Diatoms (eight species) and Cyanophyta (one species), which together contributed, on average, $95.3 \pm 1.8\%$ of total phytoplankton biovolume in all enclosures.

Phytoplankton community composition differed between the *Daphnia* "migration" and *Daphnia* "no migration" treatment groups, resulting in differences in the

Table I: Taxonomy of the reported algal species

Main group	Species	Cage effect (P)	DVM effect (P)	Stratification (P)
Chlorophyta				
	Ankistrodesmus falcatus	0.40	0.86	**<0.001**
	Planktosphaeria gelatinosa	0.42	**0.003**	**<0.001**
	Scenedesmus obliquus	0.36	0.29	**0.009**
	Selenastrum gracile	0.84	0.19	–
Diatoms				
	Achnanthes coarctata	0.76	0.70	**<0.001**
	Achnanthes microcephala	0.07	**<0.001**	**<0.001**
	Cyclotella kützingiana	0.54	0.09	**<0.001**
	Cymbella helvetica	0.09	**0.04**	**<0.001**
	Fragilaria crotonensis	0.24	0.13	**<0.001**
	Navicula cryptocephala	0.86	0.23	–
	Navicula radiosa	0.92	0.77	**<0.001**
	Synedra ulna	0.15	0.08	**0.03**
Cyanophyta				
	Pseudoanabaena schmidlei	0.45	0.12	**<0.001**

Effect of migrating and non-migrating control treatments (cage effect) and effect of *Daphnia* "migration" and *Daphnia* "no migration" treatments (DVM effect) on algal species of the epilimnion. Differences in the abundance of algal species in the epilimnion and hypolimnion (stratification effect) with *Daphnia* "migration" treatment. Significant *P*-values are in bold.

diversity of the phytoplankton community. Phytoplankton community diversity (H) was higher in the *Daphnia* "migration" treatment group (2.9 ± 0.04) than in the *Daphnia* "no migration" group (2.4 ± 0.14), a difference that was highly significant by *t*-test ($t_{(10)} = 3.24$, $P = 0.009$; Fig. 3).

One of the most important species of the phytoplankton community in terms of biovolume was the gelatinous Chlorophyta species, *Planktosphaeria gelatinosa*. Despite its small individual biovolume ($35 \, \mu\text{m}^3$), *P. gelatinosa* contributed the greatest percentage to the total initial phytoplankton community biovolume (25.7 ± 3.9) in all treatments. *Planktosphaeria gelatinosa* abundance at the end of the experiment was lower in the *Daphnia* "migration" treatment group ($5.4 \times 10^8 \pm 1.0 \times 10^8 \, \mu\text{m}^3\text{L}^{-1}$) than in the *Daphnia* "no migration" group ($2.0 \times 10^9 \pm 3.8 \times 10^8 \, \mu\text{m}^3\text{L}^{-1}$), a difference that was highly significant by *t*-test ($t_{(10)} = 3.96$, $P = 0.003$; Fig. 4A). The remaining three Chlorophyta species did not significantly differ between *Daphnia* "migration" and *Daphnia* "no migration" treatment groups (Table I and Fig. 4A).

In addition to the effects of DVM on *P. gelatinosa*, we found significant effects of DVM on the two Diatom
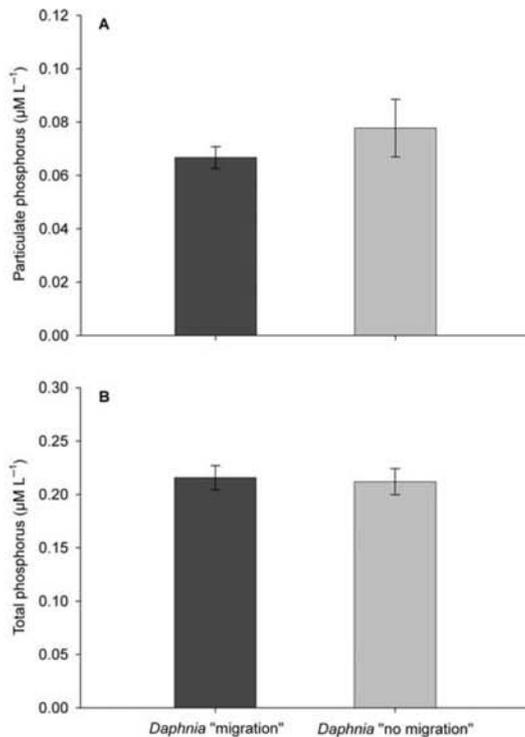
Fig. 1. Mean values of particulate phosphorus (**A**) and total phosphorus (**B**) concentrations in *Daphnia* "migration" and *Daphnia* "no migration" treatment groups in the epilimnion. Error bars represent ± 1 SE.
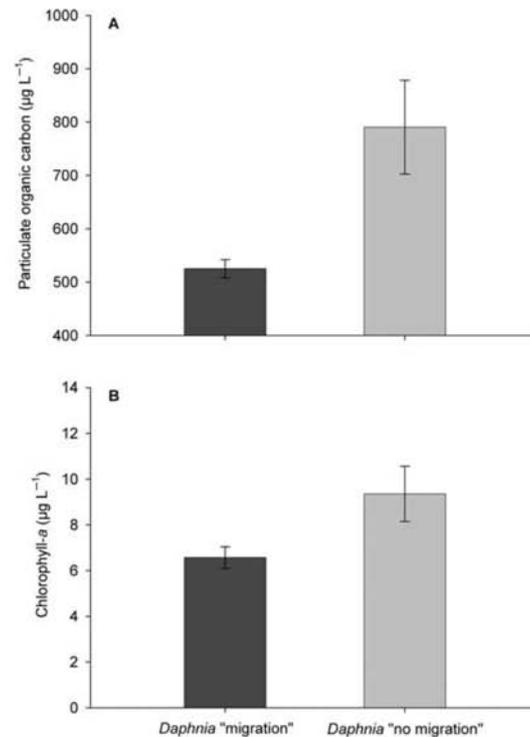

Fig. 2. Mean values of particulate organic carbon (**A**) and chlorophyll-*a* (**B**) concentrations in *Daphnia* "migration" and *Daphnia* "no migration" treatment groups in the epilimnion. Error bars represent ± 1 SE.

species, *Achnanthes microcephala* and *Cymbella helvetica*. In the *Daphnia* "migration" treatment group, the abundances of both *A. microcephala* $(7.0 \times 10^6 \pm 4.6 \times 10^5 \, \mu m^3 \, L^{-1})$ and *C. helvetica* $(8.0 \times 10^7 \pm 6.4 \times 10^6 \, \mu m^3 \, L^{-1})$ were significantly higher than those in the *Daphnia* "no migration" group $(2.5 \times 10^6 \pm 3.4 \times 10^5$ and $5.0 \times 10^7 \pm 7.0 \times 10^6 \, \mu m^3 \, L^{-1}$ for *A. microcephala* and *C. helvetica*, respectively) based on *t*-tests (*A. microcephala* $t_{(10)} = 5.80$, $P < 0.001$; *C. helvetica* $t_{(10)} = 2.41$, $P = 0.04$; Table I and Fig. 4A). *Pseudoanabaena schmidlei*, the only Cyanophyta species present ($>1\%$ of total algal biovolume) in all treatments, showed no significant difference in abundance between the *Daphnia* "migration" and *Daphnia* "no migration" treatment groups (Table I and Fig. 4A).

Hypolimnion algal biomass

Of the 13 phytoplankton species present in all epilimnion treatments, 11 were found in all hypolimnion treatments; only *Selenastrum gracile* and *Navicula cryptocephala* could not be detected in the hypolimnion
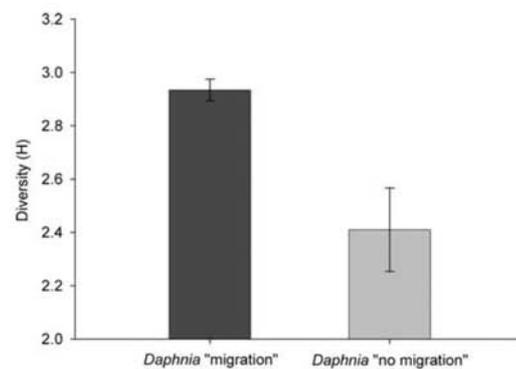

Fig. 3. Mean values of phytoplankton community diversity (H) in *Daphnia* "migration" and *Daphnia* "no migration" treatment groups in the epilimnion. Error bars represent ± 1 SE.

(Fig. 4B). The abundance of all 11 phytoplankton species differed significantly between hypolimnion and epilimnion, indicating the presence of two different water layers (Table I).
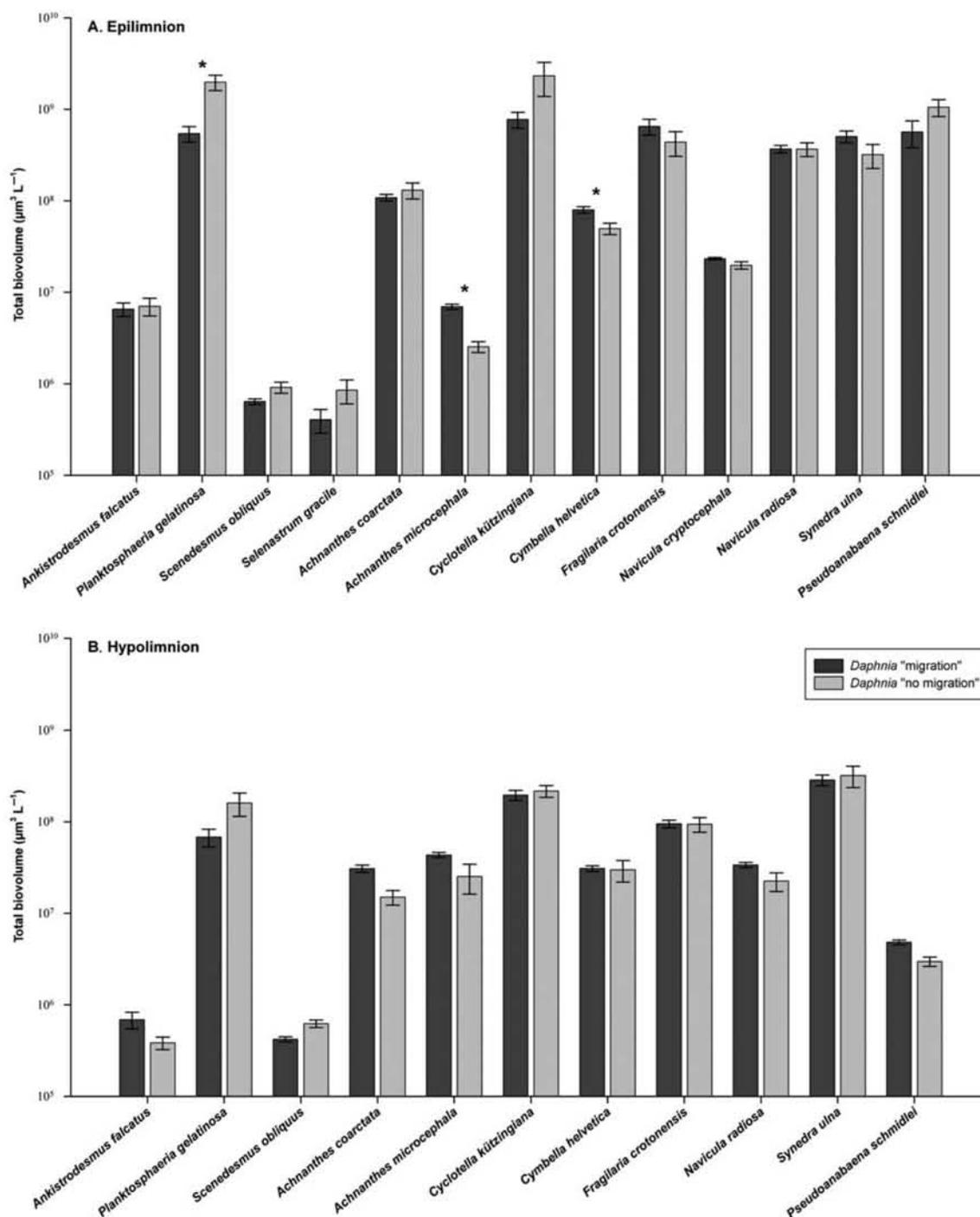
Fig. 4. Mean values of algal species abundance in *Daphnia* "migration" and *Daphnia* "no migration" treatment groups in the epilimnion (**A**) and hypolimnion (**B**). Error bars represent ± 1 SE. Significant differences between *Daphnia* "migration" and *Daphnia* "no migration" treatment groups in the epilimnion are marked by asterisks.

DISCUSSION

The aim of this study was to determine if *Daphnia* DVM can result in a refuge for phytoplankton growth and influence nutrient dynamics. We were able to maintain a near-constant temperature within our enclosures, thereby negating potential confounding influences of temperature on zooplankton–phytoplankton inter-actions. Because food conditions within the water columns were also similar, we were able to directly compare *Daphnia* populations under "migration" and "no migration" treatment conditions. The combination of forced physical mixing of the epilimnion and absence of mixing in the hypolimnion as well as the high depth-to-width ratio of our enclosures resulted in a lack of mixing between epilimnetic and hypolimnetic water layers. Additionally, the cone-shaped design of our cages diminished the effect of cage-migration treatment on total water-column mixing. The fact that the abun-dances of individual algal species were significantly different in the epilimnion and hypolimnion indeed indicated the presence of two distinct water layers.

In this study, we found no influence of *Daphnia* DVM on nutrient dynamics in the epilimnion of our meso-cosms. In particular, the availability of phosphorus was not affected by the *Daphnia* "migration" treatments. There could be two reasons for the absence of an increase or decrease in the total phosphorous concen-tration in the epilimnion. First, nutrient transport between epilimnion and hypolimnion was equal in both directions. Second, the net nutrient transport associated with migrating *Daphnia* populations was below the detection limit of common nutrient analysis methods. As a result, the differences in the development of phyto-plankton communities under *Daphnia* "migration" and *Daphnia* "no migration" treatment conditions are unli-kely to be caused by differences in nutrient availability. Therefore, the differences between phytoplankton com-munities are most likely the result of the different grazing regimes.

Trophic cascades are often difficult to detect by investigating whole trophic-level responses. In pelagic communities, total phytoplankton biomass or chlorophyll-*a* is often used as a bulk parameter repre-senting the phytoplankton trophic level. However, strong responses of individual plant species to the pre-sence of predators can be masked by opposing growth responses of other species, resulting in an undetectable response in the plant community as a whole (Sommer *et al.*, 2003). For this reason, we followed the growth responses of individual algal species to investigate the consequences of *Daphnia* DVM on phytoplankton dynamics.

In a recent study that included more than 3000 phy-toplankton samples, phytoplankton diversity was shown to be the best predictor of resource-use efficiency and stability of natural phytoplankton communities (Ptacnik *et al.*, 2008). Therefore, phytoplankton diversity and knowledge of the mechanisms that influence diversity are essential for developing a detailed understanding of pelagic food web dynamics. In this study, we observed that there was a strong influence of DVM behaviour on phytoplankton diversity, with the highest phytoplankton diversity occurring in those enclosures with migrating *Daphnia*. It is well known that nutrient availability (Interlandi and Kilham, 2001) and grazing (e.g. Leibold, 1996; Sarnelle, 2005) can influence phytoplankton diversity. Our results show for the first time that not only do the abundance and taxonomy of herbivores influence phytoplankton diversity, but herbivore behaviour also affects this important ecological parameter.

In addition to showing that DVM affects phytoplank-ton diversity, our results provide insights into the mech-anisms by which zooplankton behaviour influences phytoplankton community composition. The DVM behaviour of zooplankton resulted in a species-specific pattern of algal development. Most algae did not show a significant response to our DVM manipulations, whereas others showed either a negative or positive response to migrating *Daphnia*. The main driver of phy-toplankton dynamics in our experiment was the gelati-nous green algae, *P. gelatinosa*. Its species-specific response to DVM influenced the biomass pattern and diversity of the total phytoplankton community. The higher phytoplankton diversity in the *Daphnia* "migration" treatment group was mainly caused by the lower percentage of *P. gelatinosa* in the total phytoplankton composition.

Planktosphaeria gelatinosa, which is readily ingestable by daphnids due to its small size, was a dominant member of the phytoplankton community in Lake Brunnensee during our study. However, in addition to the positive effects of grazing attributable to nutrient recycling and release from competition, these algae might also benefit directly from grazing as a result of being eaten by *Daphnia*. Because *P. gelatinosa* has a gelatinous cover, which partially protects individual cells from being digested in the gut of the daphnids (Porter, 1976), they might be supported by gut nutrients. Thus, within the epilimnion, *P. gelatinosa* could profit from the greater possibility of being eaten by continuously grazing *Daphnia*. This positive effect of continuous grazing on the growth of this species is supported by the obser-vation that *P. gelatinosa* abundance was higher in the *Daphnia* "no migration" treatment group than in the *Daphnia* "migration" group.

F. HAUPT *ET AL.* | DAPHNIA DVM

In a study by Elser *et al.* (Elser *et al.*, 1987), the authors showed empirically that the relation between grazing intensity and algal growth could be complex, with increasing zooplankton grazing resulting in a variety of linear and non-linear positive and negative growth responses. Sommer (Sommer, 1994) summarized these positive and negative effects of zooplankton grazing on phytoplankton growth schematically. The prediction was that, under nutrient-limited conditions, phytoplankton species could even profit from low zooplankton grazing. Increasing zooplankton grazing and high nutrient availability would decrease the importance of the positive effects of zooplankton grazing on phytoplankton growth. In our case, the strong phosphorus limitation and the low *Daphnia* densities would favour a positive effect of zooplankton grazing and phytoplankton growth. Under these conditions, the positive effects of grazing can be as important for algal growth as the negative impact of grazing-dependent mortality.

The two diatoms, *A. microcephala* and *C. helvetica*, responded differently to DVM compared to *P. gelatinosa*. Their abundance was higher in the *Daphnia* "migration" treatment group than in the *Daphnia* "no migration" group. This could result from two effects. First, because both diatoms are edible for *D. hyalina* from a size standpoint (Geller and Müller, 1981), they could benefit from not being grazed during the day in the DVM treatments. Second, as both algae are semibenthic species capable of growing on the wall of the enclosures, we cannot fully exclude the possibility that the movement of cages in the migration treatments resulted in some of the wall-growing diatoms becoming re-suspended in the water column.

Our results are consistent with earlier observations from field experiments that showed large individual variations in the response of algal species to grazing (Elser *et al.*, 1987). In these previous studies, most algae showed no response to a wide range of *Daphnia* grazing-intensity manipulations. The authors argued that the type of grazer was more important for phytoplankton dynamics than changes in the intensity of the grazing pressure by a single zooplankton species. Similar observations were made in large field mesocosm experiments, where it could be shown that the grazer type affected the phytoplankton community more than variations in grazing pressure from a single zooplankton species (Sommer *et al.*, 2001, 2003). These studies also showed that grazing has a greater effect on the composition of phytoplankton communities than on total phytoplankton biomass.

The strong impact of zooplankton migration on a single algal species supports the contention that trait-mediated effects are strongly species-specific (Schmitz *et al.*, 2004). We might have expected our study to yield a different effect of DVM on the phytoplankton community if mainly highly edible and digestible algae were present. Under such conditions, it is possible for discontinuous grazing resulting from zooplankton DVM to achieve a higher phytoplankton biomass (Reichwaldt and Stibor, 2005). However, our results show that trait-mediated effects could also be observed in plankton communities. Thus, the effects of predators on phytoplankton are not only attributable to a decrease in grazer density, but also reflect escape-responses on the part of grazers. In our study, we used *Daphnia* densities typical for an oligotrophic lake to investigate trait-mediated effects instead of forcing trophic cascades using unnaturally high grazer densities. Even at these low densities, we were able to observe effects of DVM on phytoplankton. However, an increase in *Daphnia* densities (e.g. along a gradient of trophic conditions) would be predicted to result in stronger effects of DVM than those observed in our study.

The response of the phytoplankton community in terms of bulk parameters was contrary to the predictions presented in the introduction. We expected a higher total biomass of algae within the *Daphnia* "migration" treatment groups; instead, we found that this treatment resulted in lower algal biomasses. Reichwaldt and Stibor (Reichwaldt and Stibor, 2005) showed that higher phytoplankton biomass can develop under DVM conditions. However, it is obvious that the complex effects of zooplankton on phytoplankton development are highly species-specific. Our results show that general predictions about how DVM influences phytoplankton dynamics are not possible using bulk parameters to characterize phytoplankton abundance. Species-specific responses of individual algal species to zooplankton DVM determine total phytoplankton patterns. Different phytoplankton species compositions, different degrees of nutrient limitation and differences in zooplankton grazing influence how DVM affects phytoplankton dynamics. Therefore, we do not expect to find a clear relationship between zooplankton DVM and phytoplankton dynamics across lakes.

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5.3   Initial size structure of natural phytoplankton communities determines the response to *Daphnia* diel vertical migration

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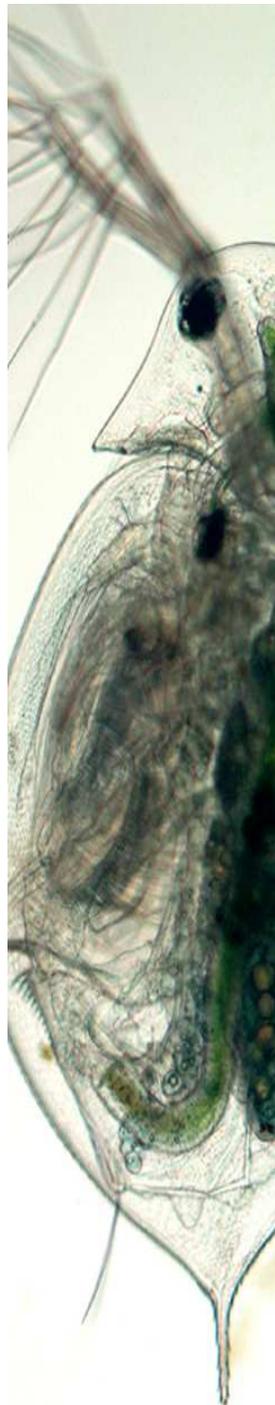
Abstract

Body size plays a central role in a number of ecological interactions, such as competition between species or prey selection by predators. Previous studies have shown that the direction and strength of phytoplankton responses to zooplankton diel vertical migration (DVM) most probably depends on the size of phytoplankton species. To examine the influence of zooplankton DVM on different sized phytoplankton communities, we designed an experiment where we manipulated the size distribution of a natural phytoplankton community *a priori* in field mesocosms. The results indicated that DVM had contrasting effects on the two evaluated phytoplankton communities. Comparison of "migration" and "no migration" zooplankton treatments showed that nutrient availability and total phytoplankton biovolume was higher in (1) "no migration" treatments with phytoplankton communities comprising mainly small algae and (2) "migration" treatments with phytoplankton communities of a broader size spectrum of algae. Hence our study showed two different mechanisms of how zooplankton DVM may influence the phytoplankton community dynamics. Nutrient cycling was an important factor in phytoplankton communities of mainly small algae, whereas the refuge effect was the main driver of phytoplankton dynamics in phytoplankton communities of a large size spectrum of algae.

Keywords: phytoplankton community, size structure, *Daphnia*, diel vertical migration, global change

Introduction

Natural primary producer communities typically comprise many species of various taxonomic levels with vastly different body sizes (Gaston and Lawton 1988). Body size is an important feature in many food web models because of its importance in numerous ecological interactions, including inter-species competition and prey selection by predators (Cohen et al. 1993; Williams and Martinez 2000). The impact of size structure on ecological interactions in pelagic ecosystems may be substantial. For example, predator-prey relationships are almost exclusively based on larger organism eating smaller organisms, with non-lethal herbivory being practically absent in these systems.

The PEG model (Sommer et al. 1986) of annual plankton succession in lakes demonstrates the significant relationship between size structure and pelagic ecosystem dynamics. Interactions are linked to the size structure and composition of the plankton community, which are subject to substantial seasonal fluctuations. Seasonal changes are further influenced by, and linked to, other abiotic and biotic factors, such as temperature gradients, nutrient availability, intra- and interspecific competition, and predation. The greatest annual community shift in temperate pelagic freshwater ecosystems, described by the PEG model, generally occurs at the end of the "clear water" phase in late spring/early summer. At this time, the community of small algae transforms into the summer community of large, grazing-resistant algae, thus influencing food availability for zooplankton.

There is growing evidence indicating that global warming may alter these processes of succession in temperate lakes, with spring algae blooms occurring earlier (Straile 2002; Winder and Schindler 2004; Berger et al. 2010). This shift is mainly caused by the earlier stratification of lakes, due to increased spring air temperatures, which accelerate the thermal stratification process of the water column. The depth of the stratified layer influences the underwater light regime of the surface layer substantially, and hence the onset of the phytoplankton growing season (Diehl et al. 2002). Peeters et al. (2007) proposed that the direct effects of temperature on phytoplankton production are weak under light limited conditions in unstratified lakes, with stratification being required to trigger the onset of phytoplankton spring blooms (Behrenfeld 2010).

In contrast, zooplankton dynamics are primarily governed by water temperature (Bottrell 1975; Reichwaldt et al. 2004) and, to a lesser extent, by earlier stratification and concurrently higher light depending algal food production (Schalau et al. 2008; Berger et al. 2010). Therefore, bloom forming algae could be subject to significant grazing after a lag phase in zooplankton population development. This time lag may result in a mismatch between phytoplankton and zooplankton cycles. If zooplankton communities fail to capitalize on the opportunity of highly edible spring blooms of small algal species, they may be forced to graze on less edible post-bloom phytoplankton communities.

The transition between spring and summer phytoplankton communities (late spring to early summer) is often characterized by a high abundance of juvenile fish, which start to populate the pelagic zone and prey on the zooplankton. Strong predation pressure triggers avoidance behavior in many zooplankton species (such as *Daphnia*), resulting in "diel vertical migration" (DVM) behavior. DVM is one of the most important escape responses exhibited by aquatic herbivores (Hays 2003). Zooplankton spend the night primarily in upper water layers, migrating down the water column at dawn to spend the day in deeper, darker and, colder waters (Lampert 1989). Because fish feed visually, behavioral studies have clearly established predator avoidance as the primary ultimate driver for DVM (Zaret and Suffern 1976). The immediate triggers initiating vertical migration behavior by zooplankton are the changes in light intensity around dawn and dusk (Ringelberg 1991), while the presence of a chemical substance (kairomone) that is released by predatory fish affects the motivation of zooplankton to respond to these triggers (Loose et al. 1993).

There are multiple routes through which DVM may influence epilimnetic phytoplankton communities. Perhaps the most substantial impact is reduced grazing pressure, due to lower zooplankton densities. For example, migrating zooplankton populations encounter lower temperatures in the hypolimnion than in the epilimnion. These lower temperatures lead to slower somatic growth, which may ultimately lead to lower population growth (Loose and Dawidowicz 1994). The lower density of migrating compared to non-migrating zooplankton populations is certainly only the case if one leaves out predation as a potential mortality factor, because if predation was present, a non-migrating population would also have lower growth due to this predation (Stich and Lampert 1984). The second possible mechanism affecting epilimnetic phytoplankton communities is that zooplankton migration leads to the daytime period being generally

free of grazing, which results in intermittent grazing pressure on the phytoplankton community in the epilimnion. Because both of these mechanisms may lead to reduced grazing pressure on phytoplankton, it is assumed that both may significantly enhance phytoplankton biomass (Lampert et al. 1986; Reichwaldt et al. 2004). Results of recent studies also suggest a third mechanism, whereby the migration of zooplankton may have a significant effect on epilimnetic nutrient supplies due to a change in nutrient recycling. Specifically, Lampert and Grey (2003) showed that DVM by *Daphnia* may result in the upward transport of nitrogen, while Haupt et al. (2010) showed an enrichment of upper water layers with phosphorus by *Daphnia* DVM.

Theoretical models have been developed using available data to estimate the impacts of zooplankton DVM on phytoplankton communities, in which discontinuous zooplankton grazing under DVM is indicated to enhance phytoplankton biomass by fostering small and fast growing algal species (Lampert 1987). A model developed by Petzoldt et al. (2009) showed that reduced zooplankton grazing and changed nutrient recycling under DVM are important mechanisms influencing plankton dynamics. The few experiments that have been conducted to investigate the effects of zooplankton DVM on pelagic ecosystems show that it may affect phytoplankton abundance, species composition, and diversity (Reichwaldt and Stibor 2005; Haupt et al. 2009).

One emerging hypothesis from limited experimental studies on changes in phytoplankton community structure as a result of zooplankton DVM, is that the effects are species-specific, probably depending on the size of particular phytoplankton taxon and the acceptable food-size range of zooplankton. Accelerated stratification processes caused by global warming may lead to an earlier "clear water" phase, which would lead to earlier phytoplankton community succession (Berger et al. 2010) from smaller fast growing spring species to larger and slower growing summer phytoplankton species. Phytoplankton succession generally arises from seasonally influenced changes in zooplankton grazing intensity (Sommer et al. 1986). However, Tirok and Gaedke (2006) showed that a "clear water" phase may occur even if *Daphnia* biomass is very low, and grazing is mainly performed by ciliates and rotifers. Basically, less mixing (by early stratification) may facilitate the early growth of phytoplankton, ciliates, and rotifers despite cold spring water temperatures, which prevent *Daphnia* development. The resulting enhanced grazing by ciliates and rotifers may shift the phytoplankton community composition from smaller to larger algae, which are consequently less edible for *Daphnia*.

Irrespective of the status of phytoplankton succession, the presence of fish stimulates mesozooplankton to perform DVM behavior. Because small phytoplankton species normally have higher growth rates than larger species (Reynolds and Irish 1997), they may use spatial and temporal refuges created by zooplankton DVM more efficiently. Hence, small fast growing edible algae may benefit more from DVM than larger slow-growing taxa. Conversely, communities that consist mainly of large inedible algae may benefit from relatively constant uninterrupted grazing by non-migrating zooplankton on the few edible taxa. Additionally, zooplankton release nutrients through sloppy feeding and excretion, which further increases the abundance of inedible algae.

Hence, we hypothesize that differences in the size structure of phytoplankton communities (such as between spring and summer phytoplankton communities) will affect the response of phytoplankton communities to zooplankton DVM. If correct, experimental manipulation of the size distribution of a phytoplankton community should alter its response to zooplankton DVM. To investigate this hypothesis, we manipulated the size distribution of a natural phytoplankton community in large (7000 L) field mesocosms, representing two different phytoplankton communities. Size control was achieved through the selective filtration of a summer phytoplankton community with two different mesh sizes (11 and 64 µm), each representing spring and summer phytoplankton communities. The resulting communities were exposed to migrating and non-migrating populations of *Daphnia*. We consider our results against theories of phytoplankton community responses to *Daphnia* DVM, and potential trophic web impacts.

Methods

The study was conducted in an experimental enclosure system deployed in oligotrophic Lake Brunnensee, southern Germany (47°59´N, 12°26´E), in the summer (June-July) of 2007. This small (5.8 ha), deep (18.6 m), hardwater lake is strongly phosphorus-limited (total P: 0.4 µM L^{-1}), with a high nitrate concentration (NO$_3^-$: 80 µM L^{-1}) during the summer. To investigate the effects of vertically migrating zooplankton on two different phytoplankton communities, we moved *Daphnia* populations up and down the water column using cages. To create the two different phytoplankton communities, epilimnetic lake water containing a summer phytoplankton community was filtered using meshes (Sefar Petex, Sefar AG, Switzerland) with either an 11 µm ("spring" phytoplankton community) or 64 µm ("summer" phytoplankton community) mesh size.

The submersible cages used in this study had already been successfully applied in earlier experiments (Reichwaldt and Stibor 2005; Haupt et al. 2009). Although predation is considered to be one of the most important causes of zooplankton DVM (Zaret and Suffern 1976), attempts to establish a predatory dynamic by fish stocking have proven very difficult, primarily due to potential indirect effects on phytoplankton caused by nutrients excreted by enclosed fish (Schindler 1992; Vanni and Layne 1997; Attayde and Hansson 1999). In practice, it is also not possible to induce zooplankton DVM behavior using kairomones because too little is known about the structure and dose-effect relationship of these chemical signals.

Experimental design

Twenty four cylindrical enclosures (transparent Trikoron bags, Rheinische Kunststoffwerke Worms, Germany) were suspended vertically from a raft to a depth of 10 m. Each 0.9 m diameter enclosure was heat-sealed at the bottom and open to the atmosphere. In the enclosures, we mimicked an unmixed, 6 m deep hypolimnion and a well-mixed, 4 m deep epilimnion. The latter was produced by intermittently bubbling compressed air (3 min on, 40 min off) through PVC-tubes at a depth of 4 m. To prevent a vertical temperature gradient in the enclosures, all were surrounded by a 15-m deep, transparent silage film (0.2 mm), which acted as a homogenous, tempered water bath. Uniform mixing in the water bath was achieved by the intermittent injection of compressed air (5 min on, 20 min off) at a depth of 12 m.

Homogenous temperature along the vertical gradient was necessary to achieve similar growth in migrating and non-migrating *Daphnia* zooplankton populations. Reichwaldt

and Stibor (2005) showed a fluctuating temperature regime had a significantly negative impact on the population growth and hence abundance of migrating *Daphnia*. In this study, we aimed to investigate the refuge effect of *Daphnia* DVM on phytoplankton communities of different size structures, and the consequences of DVM on nutrient dynamics in such communities. Therefore, we used a modification of the experimental setup of Reichwaldt and Stibor (2005) to separate refuge effects from temperature effects. This method, constructs a well-mixed water bath around all enclosures, allowing the refuge effect of zooplankton DVM to be examined under field conditions without significant temperature differences between upper and deeper water layers.

Twelve enclosures were filled with 64 µm-filtered epilimnetic water, and another 12 were filled with 11 µm filtered epilimnetic water. From this point onwards, we refer to the 11µm filtered communities as "spring" communities and the 64 µm-filtered communities as "summer" communities. Filtration and the filling of the enclosures began on 19 June 2007, which took approximately 48 h. The enclosures were filled at random with either "spring" or "summer" phytoplankton. After filling the enclosures, the "spring" community enclosures were enriched with 10 µg P L^{-1} to attain similar particulate phosphorus concentrations in all treatments, due to the particulate material having been removed from these enclosures.

Daphnia were placed in a cylindrical mesh cage (224 µm mesh aperture, diameter 0.7 m, length 3.5 m; Sefar Petex, Sefar AG, Switzerland) inside each enclosure. This mesh aperture ensured that all *Daphnia* were retained within the cages, while allowing the free exchange of algal cells. Each cage had a mesh cap that could be resealed to allow sampling. The volume of the cages was approximately 50% of the epilimnion. To simulate DVM, cages were moved up and down the water column within the enclosures in a diurnal rhythm. For the "migration" treatment group, cages containing *Daphnia* were kept in the epilimnion (top of cage: 0.25 m depth) at night (20:00–08:00 h), and then lowered into the hypolimnion (top of cage: 5.5 m depth) during the day (08:00 h to 20:00 h). Cages were manually moved as slowly as possible (maximum speed: 0.05 m s^{-1}). For the "no migration" treatment group, the cages containing *Daphnia* were kept permanently in the epilimnion. Although previous studies detected no plankton or nutrient dynamic effects from the movement of the cages (Reichwaldt and Stibor 2005; Haupt et al. 2009), we again evaluated this possibility by installing enclosures with migrating empty (no *Daphnia*) cages, and enclosures with non-migrating empty cages. Therefore, the twelve "spring" enclosures and the twelve "summer" enclosures included three *Daphnia* "migration" treatments, three *Daphnia* "no

migration" treatments, three migrating empty cages, and three non-migrating empty cages.

We used a clone of *Daphnia hyalina* originating from Lake Brunnensee, which is known to perform DVM in this lake (H. Stibor, unpublished data), to stock the cages. Prior to the experiment, *Daphnia* were reared in 30 L buckets, with an artificial culture medium in an environmental chamber at a constant temperature of 20℃. They were fed *Scenedesmus obliquus* (>1 mg C L^{-1}) every other day, and 50% of their medium was renewed every 5 d. Two days before the beginning of the experiment, all *Daphnia* were transferred to 30 µm filtered, epilimnetic lake water. At the beginning of the experiment, *Daphnia* were released into the *Daphnia* "migration" and *Daphnia* "no migration" treatment cages at a starting density of five individuals L^{-1} within the epilimnion, which is a density that is typical for this species in Lake Brunnensee (H. Stibor, unpublished data). The experiment began with the stocking of *Daphnia* on 25 June 2007, 5 d after filling the enclosures, to compensate phytoplankton growth from the losses caused by the 11µm filtration in the "spring" community treatments. The experiment lasted for four weeks until 24 July 2007. This has proven to be an ecologically rational time span for enclosure experiments, because it is long enough to show strong effects on the monitored parameters, but short enough to prevent the occurrence of artificial effects in the enclosures, such as extensive wall growth (Reichwaldt and Stibor 2005; Haupt et al. 2009).

Sampling program

Water temperature was measured weekly at 1 m vertical intervals using a WTW model Lf 191 meter with LT1/T probe (Wissenschaftlich-Technische Werkstätten, Germany). Vertical profiles of photosynthetically-active radiation (PAR) were measured in all enclosures on day 14, using a LI-139SA spherical quantum sensor (Licor, USA). In the "no migration" treatment groups, where cages remained in the epilimnion throughout the day, light intensity was measured with the cages in place, to account for possible shading effects. In both "migration" and "no migration" conditions, PAR was measured stepwise at 1 m intervals from the surface to a depth of 7 m, and was used to calculate the depth-averaged light attenuation coefficient (Diehl *et al.* 2002) for each enclosure. A *t*-test revealed no significant differences in PAR between the "migration" and "no migration" treatments in the "spring" and "summer" enclosures ("spring" community: $t_{(10)} = 0.02$; $P = 0.98$; "summer" community: $t_{(10)} = 0.39$; $P = 0.70$). This data validated

that "migration" and "no migration" treatments were not impacted by different shading regimes in either phytoplankton community.

Once a week, water samples were collected from outside the cages in each enclosure at a depth of 0.5 m (epilimnion) and 7 m (hypolimnion) using a hand pump. All samples were collected before the "migration" treatment cages were lowered to the hypolimnion. The samples were filtered through a 250 µm mesh screen, and immediately analyzed for biological and chemical parameters. Water from each sample was filtered over precombusted and acid-washed glass-fiber filters (Whatman GF/F) to determine seston carbon concentration as particular organic carbon (POC) (Elemental Analyzer, CE Instruments, UK). Concentrations of total phosphorus (TP), soluble reactive phosphorus (SRP), particulate phosphorus (PP), and silicate (SiO_2) were measured following standard methods (Wetzel and Likens 1991). Nitrate concentration was measured by ion chromatography (Model 300, Dionex Corporation, USA). Chlorophyll-*a* concentrations were determined fluorometrically (TD 700, Turner Design, USA).

To analyze the total biovolume and size spectrum of the two phytoplankton communities, we immediately preserved subsamples of the collected water samples with acid Lugol´s iodine. These samples were measured with a particle counter (Casy 1, Schärfe Systems, Germany). Plankton particles were sorted according to equivalent spherical diameter (ESD). The ESD was then used to determine 22 size classes. For each size class, we pooled the biovolume of all particles around ±0.5 µm of each respective ESD size class. Hence the smallest size class was 4 µm ESD, including the biovolume of all particles between 3.5 µm and 4.5 µm ESD, while the largest size class was 25 µm ESD, including the biovolume of all particles between 24.5 µm and 25.5 µm ESD.

At the end of the experiment zooplankton samples from all cages were collected to test the potential effects of the migrating cage on *Daphnia* growth. To accomplish this, in the morning before the migrating cages were lowered, all cages were opened at the top and mixed with a Secchi disc (the Secchi disc was lowered and brought up two times in each cage) to uniformly distribute the zooplankton. A vertical net haul from the bottom to the top inside the cage (net diameter: 0.25 m; mesh size: 150 µm) was then taken. This sampling method allowed direct comparisons between enclosures, although it probably under-sampled actual *Daphnia* densities inside the cages, because *Daphnia* that remain near to the cage bottom are not effectively caught (Haupt et al. 2009). The

samples were preserved in 4% sucrose-formaldehyde solution (Haney and Hall 1973), and all zooplankton individuals were counted under a dissecting microscope.

Data processing

In this study we were interested mainly in the mechanisms of how zooplankton DVM may influence phytoplankton communities. Therefore, we used the last sampling date, in which we expected to observe the largest effects on the monitored parameters, for the analysis of the algal communities. Because all available theoretical models investigating DVM are focused on the effects of DVM on epilimnetic algal communities, we primarily report data from this layer.

The total biovolume and biovolume of each size class of the phytoplankton communities were used to calculate the percentage biovolume of each size class at the start (day 0) and the end of the experiment (day 29). We used this data to predict the development of phytoplankton biomass $r_{(i)}$ of each size class during the experiment from the logarithms of the biovolume percentage:

$$r_{(i)} = (\ln BVP(i)_{end} - \ln BVP(i)_{start}) \tag{1}$$

where $BVP(i)_{end}$ is the biovolume percentage in size class i at the end of the experiment, and $BVP(i)_{start}$ is the biovolume percentage in size class i at the start of the experiment. We analyzed the biomass development r of the phytoplankton size classes in "migration" and "no migration" treatments by using standard regression models. Lack of fit tests were used to determine the validity of linear models, and ANCOVA methods were used to compare the slope and intercepts of linear regressions.

Cage effects were analyzed using *t*-tests to compare migrating and non-migrating empty cage data. Two-way analysis of variance (ANOVA) (with phytoplankton community type and *Daphnia* migration treatment as fixed factors) was used to compare soluble reactive phosphorus concentrations, chlorophyll-*a* and total phytoplankton biovolume between *Daphnia* "migration" and *Daphnia* "no migration" treatments. If a significant interaction between fixed factors was indicated, we performed *post hoc* tests using all pair wise multiple comparison procedures (Holm-Sidak method). Data are mainly presented as mean ± one standard error of the mean. Where appropriate to meet statistical assumptions (Sokal and Rohlf 1981), data were ln-transformed.

Results

Success of the experimental design

Filtration and initial conditions

Total phytoplankton biovolume at the start of the experiment (five days after filling the mesocosm) was $2.8 \times 10^9 \pm 1.6 \times 10^8 \, \mu m^3 \, L^{-1}$ in the "spring" and $2.7 \times 10^9 \pm 3.8 \times 10^8 \, \mu m^3 \, L^{-1}$ in the "summer" communities. *T*-tests revealed no significant differences in biovolume between both phytoplankton communities: $t_{(22)} = 0.67$, $P = 0.51$.

Linear regressions were calculated to test for significant differences between size class biovolume percentages in the "spring" and "summer" phytoplankton communities at the start of the experiment. Biovolume percentages after filtration may be described as a linear function of size classes, with the linear regressions being significant for both communities: "spring" community: y = - 0.57 x + 12.08, $R^2 = 0.56$, $F_{1,87} = 111.91$, $P < 0.001$; "summer" community: y = - 0.29 x + 7.85, $R^2 = 0.31$, $F_{1,85} = 37.61$, $P < 0.001$ (Fig. 1). The analysis of covariance revealed statistical differences in the biovolume percentage of size classes in both communities: slopes: $F_{1,172} = 14.88$, $P < 0.001$; intercepts: $F_{1,173} = 15.02$, $P < 0.001$. Therefore, filtration was successful, with the "spring" phytoplankton community containing more small algae size classes (size < 15 µm ESD), while the summer community contained larger algae size classes (individual size > 15 µm ESD).

Initial particulate phosphorus (PP) concentrations showed no significant differences between "spring" ($4.5 \pm 0.5 \, \mu g \, P \, L^{-1}$) and "summer" ($5.2 \pm 0.3 \, \mu g \, P \, L^{-1}$) phytoplankton communities: $t_{(10)} = 1.43$, $P = 0.18$.

General conditions during the experiment

Water temperature was constant in all enclosures, averaging 17.4 ℃ ± 0.03 at all depths. There was virtually no vertical temperature gradient, with the difference between temperature at the surface and maximum depth (10 m) being just 1.5 ℃ ± 0.07.

Dissolved nitrate ($>50 \, \mu M \, L^{-1}$) and silicate ($>30 \, \mu M \, L^{-1}$) were measurable in high concentrations, and obviously were not limiting during the experimental duration. There were no significant differences ($P \geq 0.11$ in all treatments) in seston carbon

concentrations between the epilimnion and the hypolimnion in all enclosures. Differences in seston carbon concentrations between water depths of 0.5 m and 7 m never exceeded 0.05 mg C L^{-1}.

Daphnia densities inside the cages averaged 4.7 ± 0.6 individuals L^{-1} based on total epilimnion volume. We found no significant differences in *Daphnia* densities between *Daphnia* "migration" and *Daphnia* "no migration" treatment groups for both phytoplankton communities: "spring": $t_{(4)} = 0.65$, $P = 0.55$; "summer": $t_{(4)} = 1.07$, $P = 0.35$. Although control treatments were not initially stocked with *Daphnia*, some animals were present in the water, and a *Daphnia* population did develop. However, *Daphnia* densities in the control treatments were always less than 0.1 individuals L^{-1}. Additional mesozooplanktonic organisms were, for the most part, excluded by the initial filtration, although some animals, mainly copepods, were found at densities of less than 0.1 individuals L^{-1}.

Control treatments (empty cages)

Analysis using *t*-tests revealed no significant differences between migrating and non-migrating control treatments for any of the measured parameters: "spring" communities: soluble reactive phosphorus (SRP) concentration: $t_{(4)} = 0.85$, $P = 0.44$; chlorophyll-*a* concentration: $t_{(4)} = 0.02$, $P = 0.98$; total phytoplankton biovolume: $t_{(4)} = 0.07$, $P = 0.95$. "Summer" communities: soluble reactive phosphorus (SRP) concentration: $t_{(4)} = 1.84$, $P = 0.14$; chlorophyll-*a* concentration: $t_{(4)} = 1.81$, $P = 0.14$; total phytoplankton biovolume: $t_{(4)} = 1.65$, $P = 0.17$. To evaluate the possible effects of the cages on large diatoms we compared the silicate (SiO_2) concentrations between migrating and non-migrating empty cages, with no significant differences being found: "spring" communities: $t_{(4)} = 1.74$, $P = 0.16$; "summer" communities: $t_{(4)} = 0.91$, $P = 0.41$.

Experimental results

Nutrients

In the "spring" communities, SRP concentrations were lower in the "migration" treatments (2.0 ± 0.03 µg P L^{-1}) than in the "no migration" treatments (2.3 ± 0.3 µg P L^{-1}) (Fig. 2). The pattern was reversed in the "summer" communities, with SRP concentrations being higher in the "migration" treatments (2.1 ± 0.01 µg P L^{-1}) than in the "no migration" treatments (1.5 ± 0.02 µg P L^{-1}). Two-way ANOVA indicated a significant interaction effect of phytoplankton community type and migration behavior on SRP ($F_{(1,8)} = 5.67$, $P = 0.044$). *Post hoc* analyses showed that the SRP concentrations in "summer" communities were significantly higher in the "migration" treatments ($P = 0.036$). When considering only the "no migration" treatments, SRP concentrations in the "spring" communities were significantly higher than in the "summer" communities ($P = 0.018$).

Phytoplankton abundance

In the "spring" communities, measured mean chlorophyll-*a* concentrations were lower (3.5 ± 0.4 µg chl-*a* L^{-1}) in the "migration" treatments than in the "no migration" treatments (6.0 ± 2.0 µg chl-*a* L^{-1}) (Fig. 3). The order was reversed in the "summer" communities, where the mean chlorophyll-*a* concentrations were higher in the "migration" treatments (5.5 ± 0.7 µg chl-*a* L^{-1}) than in the "no migration" treatments (2.2 ± 0.1 µg chl-*a* L^{-1}). There was a significant interaction effect of phytoplankton community type and migration behavior on chlorophyll-*a* concentrations ($F_{(1,8)} = 7.01$, $P = 0.029$). *Post hoc* analyses indicated that there was not a significant difference between "migration" and "no migration" treatments in the "spring" communities. However, in the "summer" communities, chlorophyll-*a* of the "migration" treatments was significantly higher ($P = 0.029$) than in the "no migration" treatments. Also, when considering only the "migration" treatments, chlorophyll-*a* of the "summer" communities were significantly higher ($P = 0.038$) than in the "spring" communities.

As with chlorophyll-*a*, mean total phytoplankton biovolume in the "migration" treatments of the "spring" communities was lower (4.6 x 10^8 ± 5.4 x 10^7 µm³ L^{-1}) than in the "no migration" treatments (8.4 x 10^8 ± 2.7 x 10^8 µm³ L^{-1}; Fig. 4). Similar to chlorophyll-*a* measurements, "migration" treatments in the "summer" communities had higher biovolume (1.0 x 10^9 ± 4.6 x 10^7 µm³ L^{-1}) than in the "no migration" treatments (5.1 x 10^8 ± 4.8 x 10^7 µm³ L^{-1}). There was a significant interaction effect of phytoplankton community type and migration behavior on total phytoplankton biovolume ($F_{(1,8)} = 7.55$, $P = 0.025$). *Post hoc* analyses indicated that in the "summer"

communities, "migration" biovolume was significantly higher ($P = 0.044$) than in the "no migration" treatments. Also, considering only the "migration" treatments, phytoplankton biovolume of the "summer" communities was significantly higher ($P = 0.030$) than the "spring" communities.

Phytoplankton community size dependent growth rates

To identify size dependent responses of both phytoplankton communities to the "migration" and "no migration" treatments, we analyzed the biomass development of phytoplankton (*r*) as a function of size.

Linear regressions of phytoplankton biomass development as a function of size in the "spring" communities were significant for both migration treatments: "migration": y = 0.09 x − 1.24, $R^2 = 0.41$, $F_{1,43} = 29.63$, $P < 0.001$; "no migration": y = 0.08 x - 0.87, $R^2 = 0.22$, $F_{1,42} = 12.16$, $P = 0.001$. Analysis of covariance revealed no statistical differences between slopes ($F_{1,85} = 0.12$, $P = 0.73$), but there were statistical differences between the intercepts of the regression ($F_{1,85} = 15.02$, $P < 0.001$). These results allow a new calculation of linear regressions with a combined mean slope: "migration" treatments, y = 0.09 x − 1.19, $R^2 = 0.41$, $P < 0.001$; "no migration" treatments, y = 0.09 x − 0.92, $R^2 = 0.22$, $P = 0.001$ (Fig. 5). The results indicate higher biomass development in the "no migration" treatments compared to "migration" treatments of the "spring" communities. Additionally, growth rates were positive for phytoplankton species larger than 11 µm ESD for "no migration" treatments, whereas this was only the case for size classes larger than 14 µm ESD in the migration treatments.

Linear regressions of phytoplankton biomass development as a function of size in the "summer" communities were only significant for "migration" treatments: y = 0.07 x − 0.33, $R^2 = 0.27$, $F_{1,46} = 17.32$, $P < 0.001$. "No migration" treatments showed no significant relationship between biomass development and size: y = 0.03 x - 0.23, $R^2 = 0.07$, $F_{1,36} = 2.53$, $P = 0.12$. Analysis of covariance revealed no statistical differences in the slopes ($F_{1,82} = 2.70$, $P = 0.10$), but there were statistical differences in the intercepts ($F_{1,83} = 9.88$, $P = 0.002$) between regressions. These results allow a new calculation of linear regressions with a combined mean slope: "migration" treatments, y = 0.05 x − 0.18, $R^2 = 0.27$; "no migration" treatments, y = 0.05 x − 0.50, $R^2 = 0.07$ (Fig. 6). The results indicate higher biomass development in the "migration" treatments compared to "no migration" treatments of the "summer" communities. Additionally, biomass development was positive for all phytoplankton size classes in "migration" treatments.

"No migration" treatments had no clear effect on size dependent biomass development in the "summer" communities.

Discussion

We experimentally manipulated the size distribution of a natural summer phytoplankton community in a small oligotrophic lake. We exposed the resulting communities to migrating and non-migrating zooplankton populations. In general, both phytoplankton communities responded with the higher growth of larger algae when exposed to grazing by *Daphnia*, which was indicated by the positive relationship between biomass development and algal size. This general response was similar between "migration" and "no migration" treatments, as shown by the similar slopes of the size-biomass development relationships.

Nevertheless, zooplankton DVM had a different effect on phytoplankton growth, which was dependent on phytoplankton size structure. Our hypothesis that different phytoplankton size distributions could affect the direction and strength of the community response to zooplankton DVM is therefore supported by the results. We were able to show experimentally, that the effects of zooplankton DVM on phytoplankton may be modified by phytoplankton size structure manipulations. However, our general expectations were mainly met by the results from treatments with the "summer" communities.

The "summer" communities, which represented early summer algal populations in small oligotrophic temperate lakes, followed the general predictions (stated in the introduction) that zooplankton DVM would cause higher phytoplankton abundance by promoting algae that are able to use the temporal refuge from grazing for growth. However, it seems that a full phytoplankton community size spectrum was necessary for zooplankton DVM to induce a refuge effect for algae. Phytoplankton only profited from zooplankton DVM in treatments containing large algae. However, contrary to the expectations stated in the introduction that mainly small algae should profit, larger algae also profited from "migration" treatments in the "summer" communities. The results obtained from the "spring" communities, which were mainly absent of large algae, suggest impacts to the contrary. For example, continuous grazing instead of discontinuous grazing resulted in higher phytoplankton biomass.

However, permanent grazing may result in higher phytoplankton abundance (Haupt et al. 2009) by fostering small phytoplankton species with gelatinous sheaths (Porter 1973). Therefore, the results of the spring treatments fit well to an earlier mesocosm

study in the same lake, in which a non-manipulated phytoplankton community was exposed to zooplankton DVM (Haupt et al. 2009). Additionally, theoretical concepts and empirical studies suggest that under oligotrophic conditions, the benefits of grazing mediated by nutrient recycling may balance or even over-yield mortality related grazing losses (Sterner 1990; Elser and Urabe 1999; Nugraha et al. 2010). Other possible explanations could be based on the interactions between microzooplankton, such as ciliates, and *Daphnia* (Juergens 1994). "Spring" phytoplankton communities suffering from serious predation by ciliates could benefit from the continuous presence of *Daphnia*, which are known to be able to drastically reduce microzooplankton biomass (Zoellner et al. 2003). Hence, more detailed studies are necessary to disentangle the different possibilities of how small, ingestible algae in natural lake communities are able to still profit from permanent grazing.

Since all other variables were controlled in the experiment, the observed differences in phytoplankton response to zooplankton DVM were directly associated with the manipulation of phytoplankton size structure. The phosphorus data also suggest that nutrient recycling by *Daphnia* appeared to be crucial for phytoplankton development in "spring" treatments containing high proportions of small, algae. In the "spring" treatments with continuous grazing, sustained removal of edible algae resulted in noticeably more dynamic nutrient recycling with higher phosphorus availability. Boersma and Wiltshire (2006) showed that *Daphnia* excrete up to about 80% phosphorus as soluble reactive phosphorus (SRP), which means that higher nutrient recycling by grazing should be coupled with a higher release of SRP. This hypothesis is supported in our study, whereby significantly higher SRP concentrations in the "spring" community "no migration" treatments compared to the "migration" treatments with discontinuous zooplankton grazing.

Obviously, the response of phytoplankton communities to zooplankton DVM was dependent on the presence or absence of large algae. The phytoplankton data, together with the nutrient measurements, indicate that the refuge effects of zooplankton DVM were larger in communities with a higher proportion of large algae ("summer" communities) compared to the effects of nutrient recycling. Large algae have the potential to store nutrients more effectively, and remove larger parts of the dissolved phosphorus pool (Wen et al. 1997). Furthermore, their lower edibility would also lead to lower recycling of phosphorus in communities with a higher proportion of large algae. In direct contrast, small algae with lower storage abilities for phosphorus and higher

edibility would foster higher nutrient turnover and recycling. Therefore, in communities mainly consisting of small algae ("spring" communities) the effect of nutrient recycling (which would be even higher in "no migration" treatments with constant grazing) may be more important than the refuge effects of zooplankton DVM. The observed size dependent interactions of zooplankton DVM with phytoplankton community structure support that both the refuge effects and size structure depend on nutrient recycling as the main drivers of how zooplankton DVM affects phytoplankton abundance.

Diel vertical migration is a classic example of a so called trait mediated effect. Trait mediated effects describe trophic cascades that are not mediated by direct mortality but by the behavioral responses of herbivores through predators (Schmitz et al. 2004). Our experimental results suggest that the direction and strength of trait mediated effects may depend on the distribution of functional traits within a community. If functional traits, such as body size, determine the flow of energy and matter within trophic cascades, the distribution of these functional traits should also influence the strength and the direction of cascade flows. In our experimental system, algal cell size not only influenced direct mortality by grazers, but also the supply of dissolved nutrients available for total phytoplankton growth. Substantial dominance by small algae resulted in trait mediated trophic cascades that were different in strength and direction from that observed for the community in which size classes were more evenly distributed, and where large species were more common. Whether the indirect trophic cascade mediated by zooplankton DVM resulted in a positive or negative effect on the trophic level of primary producers, it was clearly a function of the size distribution of the phytoplankton.

Since trait mediated trophic cascades appear to depend on functional trait distributions within primary producer communities, significant alterations in environmental factors could severely affect conditions within lake ecosystems. Global warming may be one such factor. For example, increasing temperatures could result in earlier stratification and spring algae blooms (Winder et al. 2004; Berger et al. 2010). Zooplankton communities are more restricted by cold water temperature (Bottrell 1975), and may therefore miss the opportunity to graze on a spring phytoplankton communities in which small edible algal species are present. Hence, zooplankton species may be forced to rely on nutrient poor post-bloom summer phytoplankton communities with a broader size class distribution. This negative impact on zooplankton growth could cascade to young fish, which consume zooplankton (including *Daphnia*) as a significant part of

their diet. Therefore, the complex interaction between phytoplankton size structure, fish predation, and zooplankton DVM may adjust in response to increasing warming.

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Figure legends

Fig.1: Biovolume percentages of size classes in "spring" (filled circles) and "summer" (open circles) phytoplankton communities at the beginning of the experiment. Lines represent linear regressions: "spring" community, $y = -0.29\ x + 7.85$, $R^2 = 12.08$, $R^2 = 0.56$, $P < 0.001$ (solid line); "summer" community, $y = -0.29\ x + 7.85$, $R^2 = 0.31$, $P < 0.001$ (dotted line).

Fig. 2: Mean ($\pm\ 1$ SE) SRP (soluble reactive phosphorus) concentrations in *Daphnia* "migration" (light grey) and *Daphnia* "no migration" (dark grey) treatments of the "spring" and "summer" communities.

Fig. 3: Mean ($\pm\ 1$ SE) chlorophyll-*a* concentrations in *Daphnia* "migration" (light grey) and *Daphnia* "no migration" (dark grey) treatments of the "spring" and "summer" communities.

Fig. 4: Mean ($\pm\ 1$ SE) total phytoplankton biovolume in *Daphnia* "migration" (light grey) and *Daphnia* "no migration" (dark grey) treatments of the "spring" and "summer" communities.

Fig. 5: Phytoplankton biomass size class development in "migration" (gray circles) and "no migration" (black circles) treatments of "spring" communities. Lines represent combined linear regressions: "migration" treatments, $y = 0.09\ x - 1.19$; $R^2 = 0.41$ (gray line); "no migration" treatments, $y = 0.09\ x - 0.92$; $R^2 = 0.22$ (dotted line).

Fig.6: Phytoplankton biomass size class development in "migration" (gray circles) and "no migration" (black circles) treatments of "summer" communities. Lines represent combined linear regressions: "migration" treatments, $y = 0.05\ x - 0.18$; $R^2 = 0.27$ (gray line); "no migration" treatments, $y = 0.05\ x - 0.50$; $R^2 = 0.07$ (dotted line).
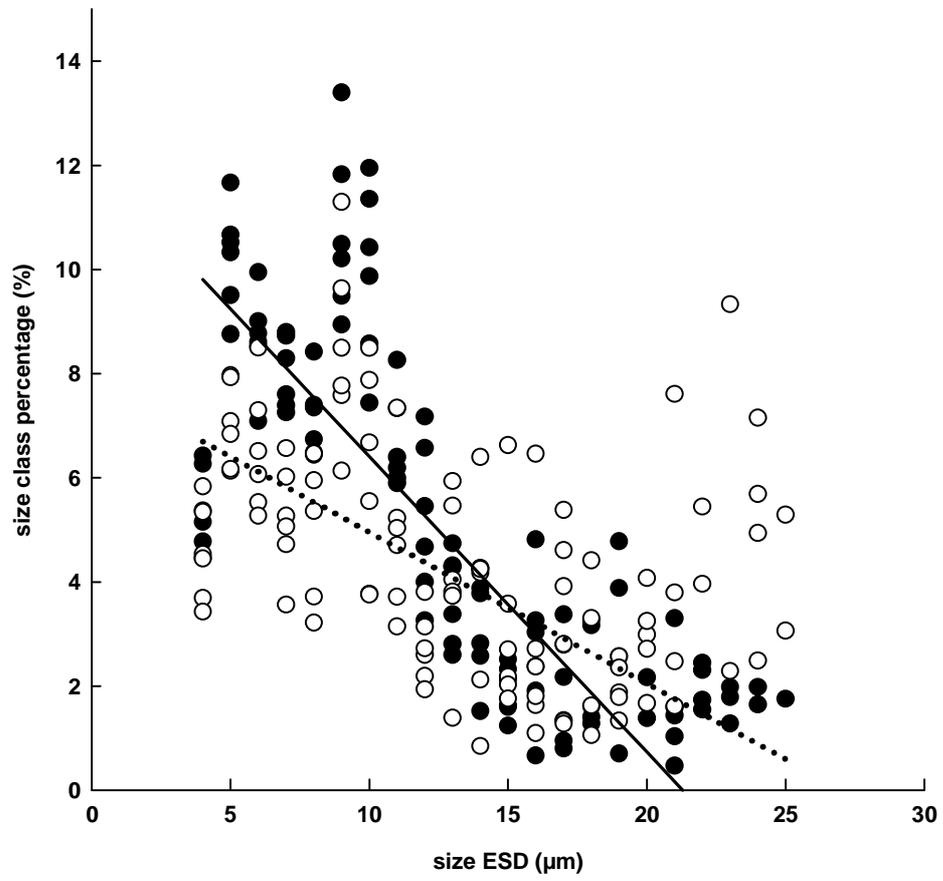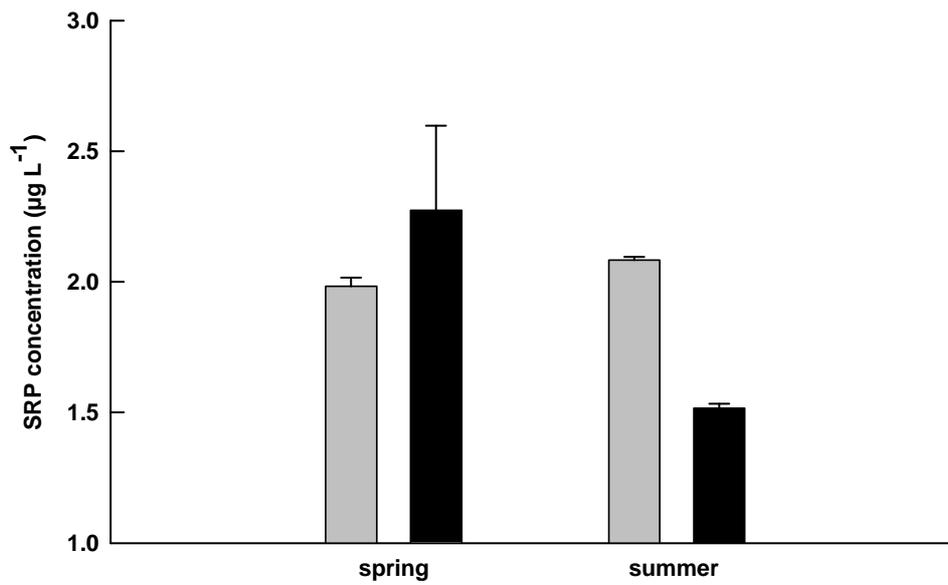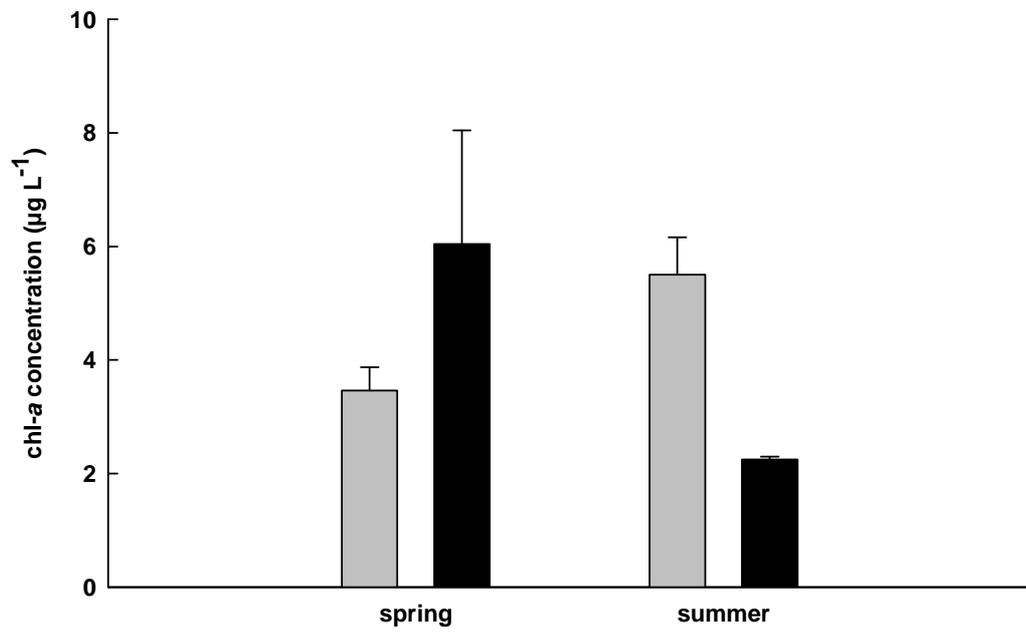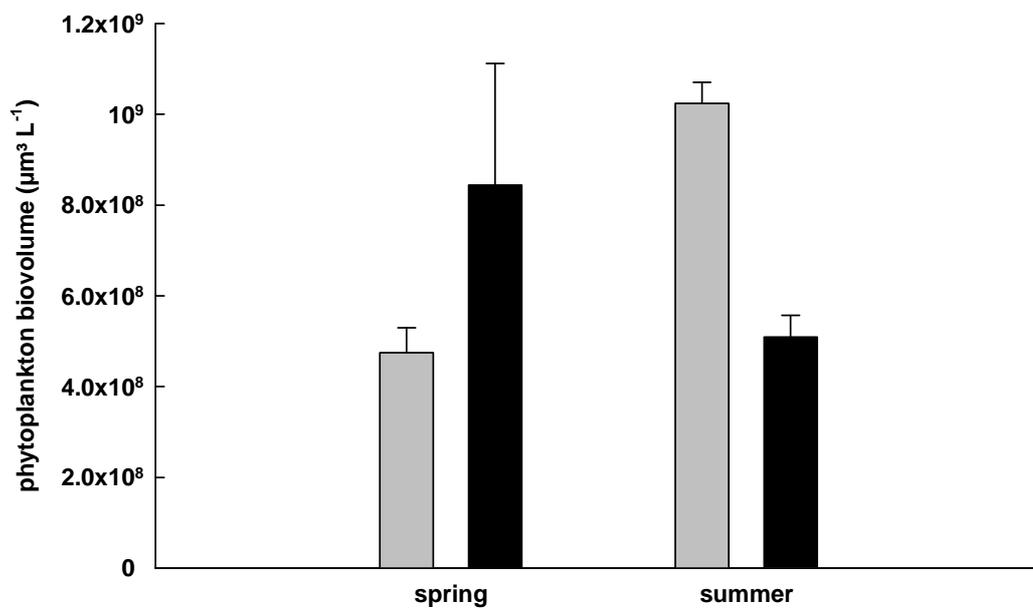
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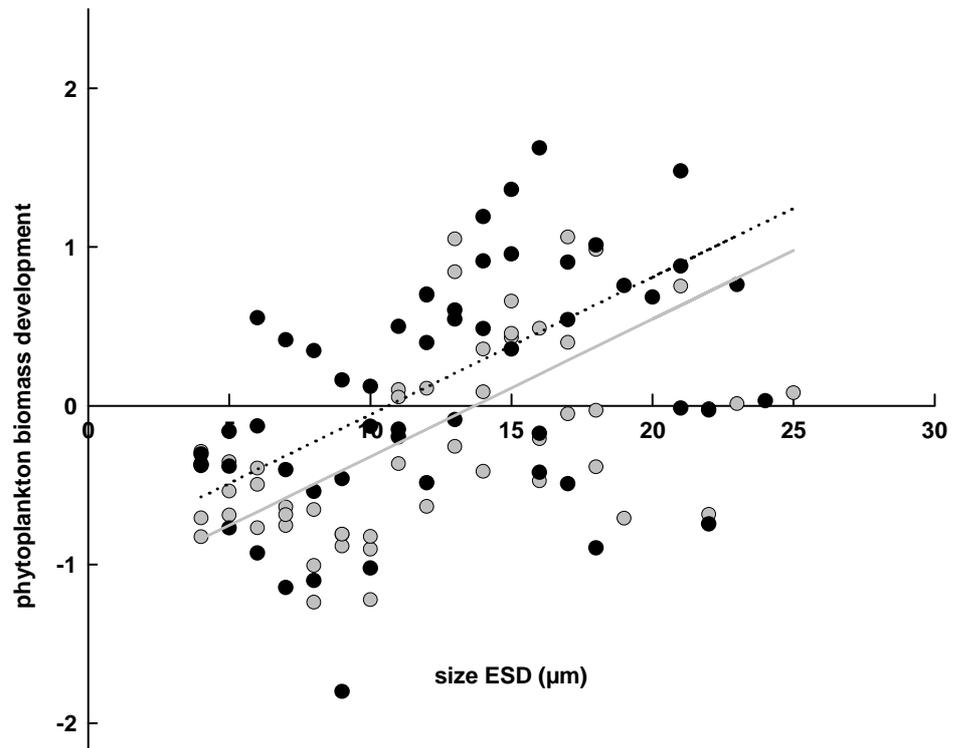
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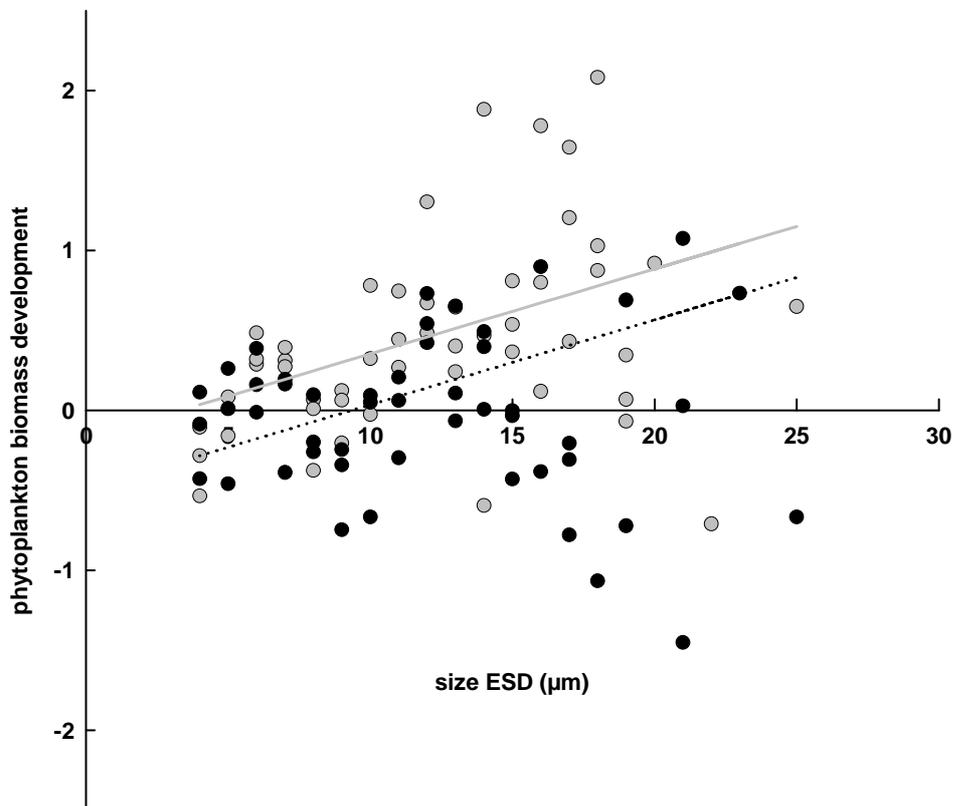
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5.4 Carbon sequestration and stoichiometry of motile and nonmotile green algae
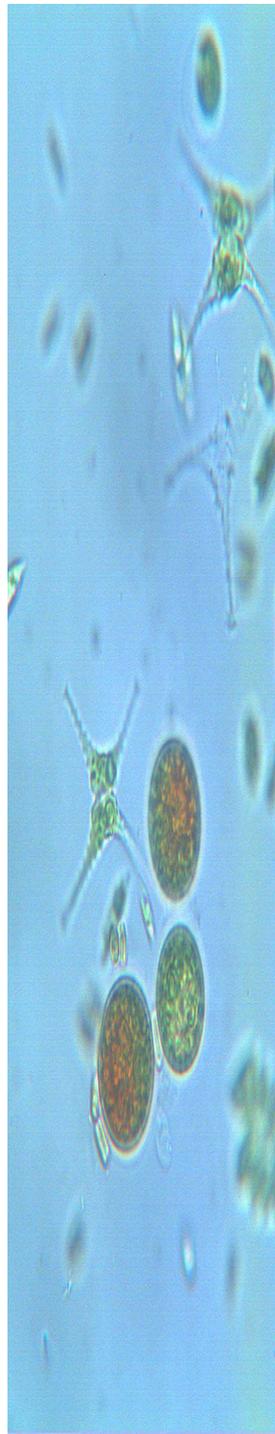
Carbon sequestration and stoichiometry of motile and nonmotile green algae

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Abstract

Actively motile, flagellated phytoplankton taxa often exploit vertical gradients in the availability of light and nutrients. The ability to move actively usually goes along with low investment in structural carbon components and should entail costs in terms of increased energy expenditure. This should be reflected in higher metabolic rates and higher light requirements for growth and, consequently, in lower light-dependent specific production rates, carrying capacities, and carbon-to-phosphorus (C:P) ratios (during phosphorus limitation) of flagellated compared to nonmotile taxa. Laboratory experiments with four flagellated and five nonmotile species of green algae, performed over a light gradient, corroborated these expectations. Parameter fits to short-term production-irradiance measurements suggest that flagellated taxa had higher respiration rates and higher light requirements for growth than nonmotile taxa. Accordingly, both short-term photosynthetic rates and longer-term (14 d) biomass accrual were lower for flagellated than for nonmotile taxa. While most of the variance in algal C:P ratios was explained by species-specific effects, there was also a tendency for algal C:P ratios to be lower in flagellated that in nonmotile taxa. Collectively, these results point at significant costs of motility, which may explain why flagellated taxa are often outcompeted by nonmotile taxa in turbulently mixed environments, where active motility is of little use.

Primary production in aquatic systems is strongly dependent on the supply with two fundamentally different kinds of essential resources: light and nutrients. While light is always supplied externally from above and decreases exponentially with depth, mineral nutrients are often scarce in the upper, illuminated water layers but abundant in deeper zones, where mineralization rates exceed uptake rates. The availabilities of light and mineral nutrients therefore frequently exhibit opposing vertical gradients (Klausmeier and Litchman 2001; Huisman et al. 2006; Jäger et al. 2008). Many phytoplankton taxa cope with this environmental challenge by influencing their vertical position in the water column through active movement or buoyancy regulation. For example, periodic vertical migrations allow motile algae to access deeper, nutrient-rich water and to adjust for optimal irradiance (Olli 1999). Hence, motile species seem to have a considerable advantage over nonmotile species, especially at low turbidity and in stratified water columns (Jones 1993; Ralston et al. 2007; Jäger et al. 2008).

The capacity to perform vertical movements is, however, of little use in deeply and well-mixed water columns, where both motile and nonmotile phytoplankton taxa are displaced passively by turbulent forces. Correspondingly, shifts from stratified to well-mixed conditions are commonly associated with shifts from flagellated or buoyant to nonmotile phytoplankton taxa (Reynolds et al. 1983; Jones and Gowen 1990; Huisman et al. 2004). The latter suggests

that there are costs to motility that play out when its potential benefits cannot be exploited. Motility should indeed involve costs in terms of energy expenditure and the provision of specific cell structures that are required for movement. While costs of motility are thus very plausible, surprisingly little is known about the nature of these costs. Theoretical considerations suggest that most planktonic protists should expend only a low ($<1\%$) to moderate (1–10%) proportion of their total metabolic rate on motility (Raven and Richardson 1984; Crawford 1992). Intriguingly, however, empirical estimates of the cost of locomotion in rotifers amounted to 38% of total metabolism, which greatly exceeded a theoretical expectation of 1% (Epp and Lewis 1984). We are unaware of any actual measurements of the costs of motility in unicellular algae. Clearly, there is a need for more research in this area.

The uptake of dissolved inorganic nutrients and the rate of light-dependent carbon fixation by phytoplankton are not tightly coupled. For nutrients that can be effectively stored (such as phosphorus), the carbon-to-nutrient ratio in phytoplankton biomass is therefore often highly flexible and varies with environmental conditions (Sterner et al. 1997; Berger et al. 2006). For example, the carbon-to-phosphorus (C:P) ratio of phytoplankton can vary more than 20-fold as a function of the supply ratio with light and phosphorus (Urabe and Sterner 1996; Sterner et al. 1997; Striebel et al. 2008). For a number of reasons, it seems plausible to expect that actively motile and nonmotile phytoplankton taxa should, on average, differ in the flexibility and range of their biomass C:P ratios. First, in nonturbulent water columns, motile taxa can perform periodical vertical migrations between upper, illuminated, and deeper, nutrient-rich layers and should thus achieve more balanced ratios of carbon fixation to phosphorus uptake compared to nonmotile taxa. Second, everything

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else being equal, motile taxa, in particular flagellated ones, should respire more carbon and need more phosphorus than nonmotile taxa because active movement requires increased metabolism and a sufficient turnover of the phosphorus-rich molecule adenosine triphosphate (ATP). Higher metabolic rates and higher phosphorus requirements should, in turn, be reflected in lower C:P ratios of flagellated taxa. Finally, many nonmotile species of (green) algae possess cell walls containing large amounts of phosphorus-free, structural carbon compounds, such as cellulose, which shifts their C:P ratio toward higher values, especially compared to flagellated taxa.

On the basis of the previously mentioned considerations, we hypothesized that flagellated and nonmotile phytoplankton taxa should, on average, differ with respect to their energetic costs, their phosphorus requirements, and their structural carbon requirements. These differences should be reflected in on average higher metabolic rates and higher light requirements for growth and, consequently, in lower light-dependent specific production rates, carrying capacities, and C:P ratios of flagellated compared to nonmotile taxa. To test these expectations, we performed short-term production and longer-term biomass accrual experiments with four flagellated and five nonmotile phytoplankton species over a gradient of light supplies.

Methods

To minimize trait variability among the study species stemming from other sources than motility (e.g., major differences in pigment constitution), all taxa were chosen from a single taxonomic group, that is, green algae. All in all, we used nine different species: four motile, flagellated ones (*Chlamydomonas* sp., *Haematococcus pluvialis*, *Phacotus lenticularis*, and *Carteria* sp., all Chlorophyceae, order Volvocales) and five nonmotile ones (*Scenedesmus* sp. and *Golenkinia brevispicula*, both Chlorophyceae, order Chlorococcales; *Tetraedron minimum* and *Monoraphidium* sp., both Chlorophyceae, order Sphaeropleales; and *Staurastrum tetracerum*, Zygnematophyceae, order Zygnematales). We tested for group-specific differences concerning biovolume per cell ($t = 1.747$; df = 7; $p = 0.12$), carbon content per cell ($t = -1.25$; df = 7; $p = 0.25$) and per biovolume ($t = 0.597$; df = 7; $p = 0.57$). The t-tests revealed no significant differences between groups.

The algae were precultured 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 levels of continuous incident photosynthetically active radiation: 3, 10, 20, 110, and 290 μmol quanta m^{-2} s^{-1}. Each algal treatment was established with the same initial biovolume (2×10^6 μm^3 mL^{-1}) and replicated three times. The treatments were arranged as semibatch cultures (10% exchange d^{-1}) in 250-mL translucent cell culture bottles in a climate chamber at 20°C. To maintain a homogeneous distribution of both algae and nutrients and

to counteract sedimentation of nonmotile species, bottles were shaken two times per day.

The growth experiments lasted for 14 d, at which point phytoplankton populations had reached the stationary phase; that is, no increase in algal biomass (as measured with an automatic particle counter) had been observed for three or more consecutive days. Particulate organic carbon and particulate phosphorus were analyzed at the start of the experiment and after 14 d 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), and particulate phosphorus was measured photometrically after sulfuric acid digestion followed by molybdate reaction.

We measured specific net primary production of all nine species over the same gradient of light intensities as used in the growth experiments. We quantified primary production with the oxygen method after 4 h of incubation (Wetzel and Likens 2003). The algae were precultured, and the measurements were conducted in the same phosphorus-reduced growth medium as was used in the growth experiments. Again, each algal treatment (taxon $\times$ light intensity combination) was started at the same initial algal biovolume and replicated three times.

The data on biomass accrual (particulate organic carbon), phytoplankton C:P ratios, and specific net primary production were all analyzed with a nested 2-way analysis of covariance with incident radiation as a continuous treatment factor, algal motility as a categorical treatment factor, and species as a random factor nested within motility. All response variables and incident radiation were log transformed prior to statistical analyses.

To quantitatively describe the dependence of specific net primary production of each species on light intensity, we used nonlinear least square regressions to fit a modified Michaelis–Menten–type model to the data from the primary production assays:

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

This allowed us to estimate light saturated specific photosynthetic rates (P_{max}), half-saturation constants (k_s), and basal respiration rates (y_0) for each species. In addition, the intercept of the fitted net photosynthesis curves with the x-axis was used to estimate the light intensity (light$_{comp}$) at which gross photosynthesis is sufficient to compensate for respiratory losses. As an additional measure of the costs of motility, we determined the ratio of specific respiration to maximal specific production (Harris 1978).

Results

Specific net primary production—Specific net primary production in the short-term incubations increased with light availability (Fig. 1; Table 1). Equation 1 gave excellent fits to the relationships between light intensity

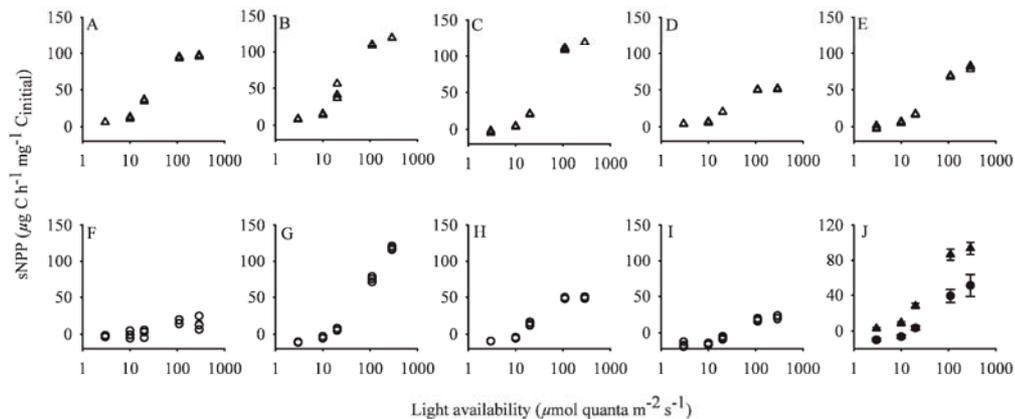1748   *Striebel et al.*

Fig. 1.   Specific net primary production (µg carbon assimilation per mg initial biomass [POC] per hour) of nonmotile species—triangles; (A) *Golenkinia brevispicula*; (B) *Monoraphidium* sp.; (C) *Scenedesmus* sp.; (D) *Staurastrum tetracerum*; (E) *Tetraedron minimum*—and motile species—circles; (F) *Carteria* sp.; (G) *Chlamydomonas* sp.; (H) *Haematococcus pluvialis*; (I) *Phacotus lenticularis*—as a function of incident radiation. (J) Mean values of motile (circles) and nonmotile (triangles) species with standard errors; note different y-axis scaling.

and specific net primary production of all nine species (R^2 ≥ 0.97 and $p < 0.0001$ for all species except *Carteria*, where $R^2 = 0.71$ and $p = 0.0006$). There was no clear difference in the estimated half-saturation constants (k_s) between motility categories (flagellated vs. nonmotile species; Table 2). In contrast, there was a trend for lower maximum photosynthetic rates (P_{max}) and higher specific respiration rates in flagellated compared to nonmotile species (Table 2). While none of these trends was statistically significant, their combination yielded significant differences in primary production between flagellated and nonmotile taxa. In particular, flagellated species needed higher light intensities to balance respiratory losses ($light_{comp}$; Table 2) and had a higher ratio of respiration to P_{max} (Table 2), and their specific net primary production was on average lower over the entire range of light intensities compared to nonmotile species (Fig. 1J; Table 1).

Phytoplankton biomass and C:P ratios—In agreement with the short-term primary production essays, the biomasses of all flagellated and nonmotile taxa in the stationary phase increased with increasing light availability in the longer-term experiments, reaching maximum values close to 5 mg C L^{-1} and about 3 mg C L^{-1} for the most

productive nonmotile and flagellated species, respectively (Fig. 2; Table 1). The light levels at which different species reached their maximum biomasses ranged from 20 to 290 µmol quanta m^{-2} s^{-1}. On average, flagellated species attained lower biomasses than nonmotile species, the differences being small at light intensities ≤10 µmol quanta m^{-2} s^{-1} but rather large at light intensities of 20 µmol quanta m^{-2} s^{-1} and above (Fig. 2J; motility × light interaction, Table 1).

Phytoplankton C:P ratios also increased with increasing light availability, with several species reaching molar ratios of up to 1200 at light intensities of 110 µmol quanta m^{-2} s^{-1} and above (Fig. 3; Table 1). On average, nonmotile species tended to have higher C:P ratios than flagellated ones at all but the lowest light intensity (Fig. 3J; motility × light interaction, Table 1). Most of the variance in algal C:P ratios was, however, explained by species-specific differences within rather than between motility categories, suggesting that some species within each motility category have inherently low C:P ratios (e.g., *Tetraedron* sp., *Phacotus* sp., and *Carteria* sp.), while others attain inherently high C:P ratios (e.g., *Scenedesmus* sp., *Staurastrum* sp., and *Haematococcus pluvialis*) in high-light environments.

Table 1.   Summary of nested analyses of covariance of the contributions of light availability, motility, and species (nested within motility) to the total variances in specific net primary production (sNPP), carbon biomass accrual (POC), and molar carbon-to-phosphorus ratios (C:P).

	sNPP			POC			C:P		
	SS	df	p	SS	df	p	SS	df	p
Light	12.86	1, 124	0.0001	3.63	1, 122	0.0001	3.58	1, 122	0.0001
Motility	3.82	1, 7	0.011	1.29	1, 7	0.175	0.33	1, 7	0.588
Motility × light	0.15	1, 124	0.0001	0.39	1, 122	0.0001	0.13	1, 122	0.097
Species	2.26	7, 124	0.011	3.97	7, 122	0.0001	7.16	7, 122	0.0001

Table 2. Photosynthetic parameters estimated from photosynthesis–light intensity curves fitted according to Eq. 1. Shown are separate parameter estimates (with standard error in parentheses) for each species with values of the regression fits (all $p \leq 0.0006$) as well as the average parameter values (with standard errors) for motile and nonmotile species. Also shown are the p-values of t-tests comparing average parameter values of motile and nonmotile species.

	Golenkinia sp.	Monoraphidium sp.	Scenedesmus sp.	Staurastrum sp.	Tetraedron sp.	Mean nonmotile species	Carteria sp.	Chlamydomonas sp.	Haematococcus sp.	Phacotus sp.	Mean motile species	Comparison motile vs. nonmotile (p)
$Light_{comp}$ (μmol photons $m^{-2}\,s^{-1}$)	2.5	2.03	6.6	2.16	5.11	3.98 (1.01)	10.78	12.95	9.67	34.03	16.86 (5.77)	0.005
Respiration (μg C mg $C_{init}^{-1}\,h^{-1}$)	8.06 (5.39)	7.48 (5.8)	19.55 (6.34)	3.94 (3.08)	9.40 (2.59)	9.69 (2.63)	5.29 (4.21)	18.05 (1.47)	19.9 (3.9)	21.43 (2.17)	16.17 (3.69)	0.25
P_{max} (μg C mg $C_{init}^{-1}\,h^{-1}$)	123.99 (5.59)	149.02 (6.3)	171.64 (8.43)	64.79 (3.11)	110.25 (3.74)	123.94 (18.15)	24.29 (4.47)	197.98 (6.36)	80.24 (3.81)	52.43 (2.82)	88.74 (38.16)	0.23
k_s (μmol photons $m^{-2}\,s^{-1}$)	35.93 (8.58)	38.38 (8.78)	51.32 (13.03)	33.26 (8.3)	54.86 (9.48)	42.75 (4.33)	38.64 (37.10)	129.04 (12.48)	29.32 (6.98)	49.23 (14.04)	61.56 (22.86)	0.51
Respiration × P_{max}^{-1}	0.07	0.05	0.11	0.06	0.09	0.08 (0.01)	0.22	0.09	0.25	0.41	0.24 (0.07)	0.031
r^2	0.98	0.98	0.98	0.97	0.99	—	0.71	0.99	0.97	0.97	—	—

Discussion

We had hypothesized that, compared to nonmotile algae, flagellated algal taxa should have higher metabolic rates and higher light requirements for growth, and, as a result, lower light-dependent specific production rates, carrying capacities, and C:P ratios. These expectations were largely fulfilled in our comparison of nonmotile and motile, flagellated green algae. While the trend for higher respiration rates of flagellated compared to nonmotile species was not statistically significant, flagellated taxa required a higher light intensity for positive net photosynthesis and attained lower photosynthetic rates at all levels of incident radiation than did nonmotile taxa. Indeed, our photosynthetic measurements corroborate earlier measurements (Harris 1978; Cushing 1989) showing that the ratio of specific respiration to maximal specific production is higher for motile than for nonmotile species.

Lower photosynthetic rates of flagellated taxa in short-term incubations were subsequently reflected in lower steady-state biomasses and C:P ratios, the differences between flagellated and nonmotile taxa being most pronounced at the highest levels of incident radiation. Specific net primary production, for example, was negative for all flagellated taxa at the lowest incident radiation but positive for nonmotile taxa. Nevertheless, longer-term biomass accrual did not differ between flagellated and nonmotile taxa at the lowest incident radiation. This suggests that compensatory physiological and/or ecological adaptations (e.g., increased mixotrophic nutrition at low light levels) may have taken place over the course of the biomass accrual experiments. It also suggests that the most important costs of motility may be in terms of a reduced capacity to effectively use a higher light supply rather than in terms of a higher basic metabolism.

The primary production measurements suggest that, at least in the short term, flagellated species needed higher minimal light intensities to have a positive carbon balance (Table 2), indicating that actively motile species needed more energy to compensate for respiration losses. Our laboratory experiment was conducted in well-mixed vessels with limited volume. Thus, significant spatial heterogeneity in resource availability was absent from our experiment, and flagellated species could not take advantage of their ability to move. The lower biomasses attained by flagellated species compared to nonmotile species therefore point at the costs and disadvantages of this strategy in a spatially homogeneous environment. In turbulent pelagic environments, where directional movement is impossible, flagellated species would thus be expected to be inferior competitors. Conversely, flagellated species would be expected to be superior competitors in stably stratified environments, where the spatial separation of high-light and high-nutrient environments is often pronounced. A recent mesocosm experiment investigating the effects of turbulence and water column depth on phytoplankton community composition is in agreement with this hypothesis. Turbulent water columns were dominated by nonmotile species such as diatoms, whereas stratified water

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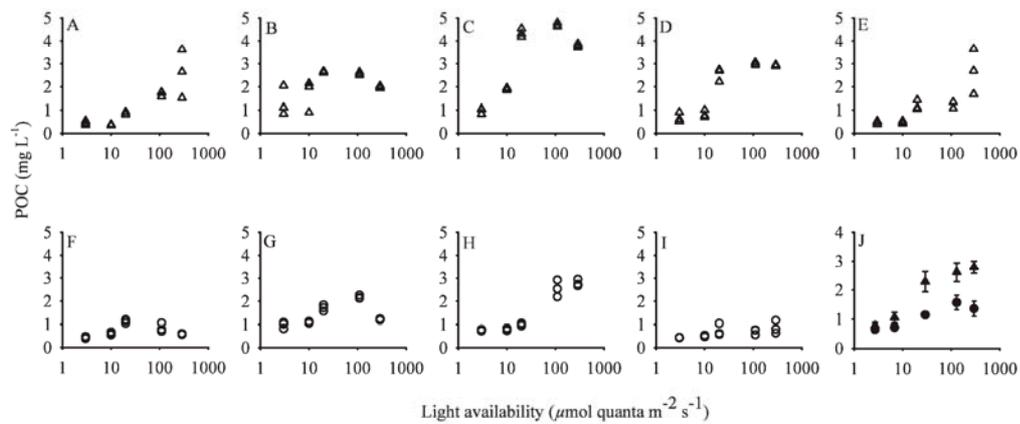

Fig. 2.   Biomass, determined as particulate organic carbon at the end of the experiment (day 14), of (A–E) nonmotile (triangles) and (F–I) motile (circles) species (labeling as in Fig. 1) as a function of incident radiation. (J) Mean values of motile (circles) and nonmotile (triangles) species with standard errors; note different y-axis scaling.

columns were dominated by flagellated taxa (Jäger et al. 2008).

Nearly all flagellates use a combination of phototrophic and phagotrophic production (Raven 1997), suggesting that they might use mixotrophic nutrition as a means of gaining sufficient phosphorus for motility and growth 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). Mixotrophic algae that feed on bacteria could access this additional phosphorus source. In a study of the effect of mixotrophy on phytoplankton carbon to phosphorus stoichiometry, mixotrophic species showed indeed considerably lower C : P ratios than purely autotrophic algae (Katechakis et al. 2005). The mixotrophic species

studied by Katechakis et al. (2005) were all flagellated, while the autotrophs were all nonmotile. Thus, the question remains whether lower C : P ratios of flagellates are a consequence of motility, mixotrophic nutrition, or both. Our data suggest that motility per se may not be sufficient to explain the generally lower C : P ratios of flagellates since we found substantial variation among species within motility categories, indicating that other traits in addition to motility contribute to species-specific responses. Therefore, future research should examine the amount of mixotrophic nutrition within motile phytoplankton species and the contribution of mixotrophy and motility to phytoplankton biomass stoichiometry.

While experimental manipulations of light and phosphorus availability have frequently resulted in strong
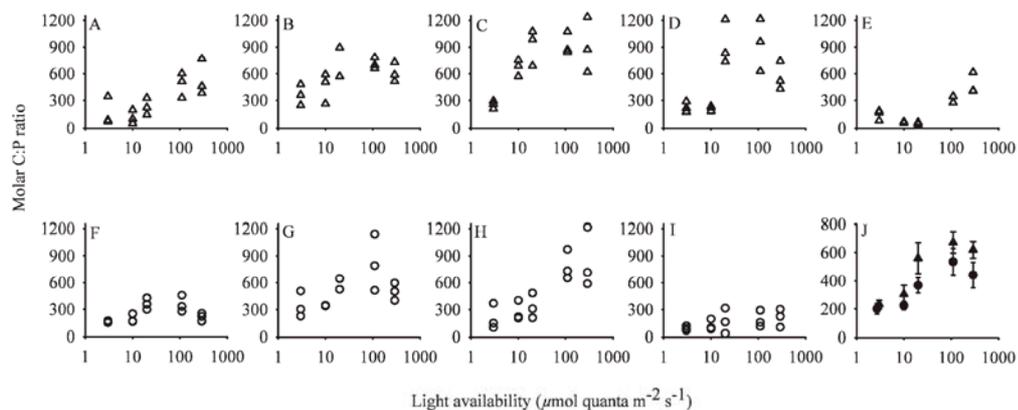

Fig. 3.   Molar carbon-to-phosphorus ratios at the end of the experiment (day 14) of (A–E) nonmotile (triangles) and (F–I) motile (circles) species (labeling as in Fig. 1) as a function of incident radiation. (J) Mean values of motile (circles) and nonmotile (triangles) species with standard errors; note different y-axis scaling.

effects on the carbon-to-phosphorus stoichiometry of phytoplankton biomass (Urabe et al. 2002; Diehl et al. 2005; Striebel et al. 2008), sometimes such manipulations may induce only weak stoichiometric responses. On the basis of our data, we suggest that the relative contribution to total phytoplankton biomass by taxa with highly flexible C:P ratios (such as nonmotile green algae) vs. taxa with limited stoichiometric flexibility (such as flagellates) can explain some of the variation in phytoplankton stoichiometric responses to variations in light and nutrient supply. In line with this, Jäger et al. (2008) observed that variation in algal C:P ratios among mesocosms differing in water column depth (and thus in ratios of light to phosphorus supply) was considerably lower in stratified water columns with a high proportion of motile algae than in turbulently mixed water columns dominated by nonmotile taxa.

Shifts in biomass composition of phytoplankton communities may have consequences beyond phytoplankton ecophysiology. Phytoplankton–zooplankton interactions can be strongly influenced by phytoplankton stoichiometry (Sterner and Hessen 1994; Andersen et al. 2004; Diehl 2007). Fast-growing herbivorous zooplankton species, such as *Daphnia*, have high demands for phosphorus and therefore exhibit relatively low biomass C:P ratios. Consequently, low biomass C:P ratios and the absence of distinct cell walls can result in high assimilation efficiencies of actively motile phytoplankton taxa when grazed by *Daphnia* (Katechakis et al. 2005). The proportion of motile species within phytoplankton communities may therefore influence the transfer efficiency between phytoplankton and fast-growing zooplankton and thus potentially strongly affect pelagic ecosystem dynamics. Studies manipulating the proportion of motile species in phytoplankton communities grazed by *Daphnia* are needed to investigate this possible link between the functional composition of phytoplankton communities and herbivorous zooplankton dynamics.

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6   Discussion of methods

The studies presented in the papers I – IV have shown that strong effects on the pelagic ecosystem can arise from zooplankton DVM and that effects of migrating phytoplankton species on individual stoichiometry can be examined. Preceding the general discussion of the results, I will discuss why, despite the indisputable effects of plankton vertical migrations, there is only a small number of studies dealing with those migration effects and how I dealt with the problems that arise from studying effects of plankton vertical migrations.

6.1   Studying zooplankton DVM – problems and consequences

It is very surprising that the consequences of large plankton mass movements on pelagic ecosystem dynamics have not been well studied, even though reasons of zooplankton migrations and related costs for zooplankton species are well understood (Zaret and Suffern 1976, Stich and Lampert 1981, Ringelberg1991, Loose 1993, Lass et al. 2000, Reichwaldt et al. 2004). Although phytoplankton are very important to global carbon dynamics (Geider et al. 2001) and are the basis of freshwater and marine food webs, the consequences of zooplankton DVM for phytoplankton dynamics have received astonishingly little detailed study. A reason may be the difficulties in inducing, regulating and controlling migration behavior in experiments.

Predation is considered one of the most important causes of DVM (Zaret and Suffern 1976) and has also proven to induce DVM under a changing light regime in mesocosm studies (Loose et al. 1993, own study on the upward phosphorus transport by zooplankton DVM paper I). However, stocking experimental setups with predatory fish is associated with uncertainties because excreted nutrients by fish can also affect phytoplankton dynamics (Schindler 1992, Vanni and Layne 1997, Attayde and Hansson 1999). Using only the chemical signals released by fish, the kairomones would be an elegant solution to overcome those hindrances. However, it is not practicable to induce DVM behavior by using kairomones alone, because the chemical structure of the kairomones is not well known.

6.2   Experimental setup – field mesocosm studies

In my studies on the effects of zooplankton DVM on natural lake phytoplankton communities, I used an innovative new technique of using mesh cages in enclosure systems, that was developed in the group I was working in, to artificially induce zooplankton DVM. The experimental setup was first described by Reichwaldt and Stibor (2005) and gave me full control of *Daphnia* DVM with no significant artificial impact of "migration" and "non-migration" treatments on any of the reported parameters in my studies. Therefore, I was able to investigate the consequences of DVM for phytoplankton growth, composition and dynamics in natural lake communities while avoiding experimental artefacts arising from using fish.

Another important aspect of zooplankton DVM in temperate regions is the decreasing water temperature with depth. The main aim of my studies with natural lake phytoplankton communities was to determine if *Daphnia* DVM can create a grazer-free refuge for phytoplankton species. However, it is difficult to assess the consequences of such a refuge effect of zooplankton DVM on phytoplankton because depth and temperature can normally not be separated in temperate pelagic environments. Migrating zooplankton populations experience lower temperatures in the hypolimnion, leading to slower individual growth, which would result in decreased zooplankton densities compared to non-migrating populations (assuming that mortality by predators is absent, as it was the case in my enclosure systems). Thus, the refuge effect of DVM on phytoplankton cannot be examined separately from the temperature effect, which also causes a decrease in zooplankton populations and diminished grazing. Reichwaldt and Stibor (2005) showed the drastic impact of a fluctuating temperature regime experienced by migrating *Daphnia* on their population growth, which resulted in a significant lower *Daphnia* abundance in migrating populations. However, such temperature related costs and effects of DVM were not part of my study.

The strong temperature effects in the study by Reichwaldt and Stibor (2005) impressively showed that for an understanding of zooplankton DVM effects on phytoplankton, it is important to separate refuge effects from temperature effects. Therefore, I excluded the temperature factor by surrounding the mesocosms with a fully mixed water bath to prevent vertical temperature gradients. This method allowed me to investigate the refuge effect of DVM on natural phytoplankton communities without significant differences in the temperatures experienced by zooplankton

migrating between the hypolimnion and epilimnion. As a result, *Daphnia* densities did not differ between "migration" and "non-migration" treatments.

6.3   Experimental setup – laboratory mesocosm studies

As mentioned above, it is also possible to work with predatory fish in enclosures to induce zooplankton DVM. However, this approach has several restrictions. These restrictions are mainly related to the nutrient excretions of enclosed fish that can additionally affect phytoplankton growth and mask or promote the effects of migrating zooplankton. It is difficult to distinguish the effects of DVM on phytoplankton from the effects of fish nutrient excretions on phytoplankton. The use of fish to induce zooplankton DVM was therefore restricted to experiments where nutrients are strictly controlled, as was the case in my study in the well controlled laboratory "plankton towers" at the former Max-Planck-Institute for Limnology in Plön, Germany, now Max-Planck-Institute for Evolutionary Biology (paper I).

Nutrient measurements, especially analyses of phosphorus dynamics, are difficult to perform in DVM studies, because it is impossible to distinguish phosphorus originating from the hypolimnion and epilimnion. Additionally, the concentrations of phosphorus during migration may be too small to be analyzed by classical photometric techniques. The detection limit for dissolved phosphorus using photometric methods is far above levels that are relevant for biological dynamics such as primary production or bacterial growth (Vadstein 2000). The use of radioactive tracers provides an elegant solution to overcome both hindrances. My setup with stocking ^{33}P labeled algae in the hypolimnion enabled a differentiation between phosphorus from the epi- and hypolimnion, combined with a high resolution of the transported phosphorus.

6.4   Experimental setup – laboratory microcosm studies

Phytoplankton can also perform vertical migrations, and species showing such behavior have to be motile. In my study regarding phytoplankton migrations I investigated the energetic costs, phosphorus requirements, and structural carbon requirements of motile and non-motile phytoplankton taxa. To investigate these parameters, I performed microcosm laboratory experiments with four flagellated and five non-motile phytoplankton species. These laboratory experiments were conducted in vessels with limited volume, where mobile species had no possibility to profit from their ability to move and all samples received the same amount of the limiting nutrient,

phosphorus. For this reason, motile and non-motile species had the same general conditions, which was important to point out advantages, disadvantages and costs of both strategies.

7   General discussion of results

According to a recent review by Vellend (2010), community ecology is often perceived as a confusion. The seemingly endless number of processes that can underlie the investigated patterns and the apparent uniqueness of each study system accounts for that. For example, Palmer (1994) already identified 120 different hypotheses to explain the maintenance of species diversity. Adding real organisms to simple Lotka – Volterra formulations of interactions between two species can result easily in more than 2000 different model solutions (Vellend 2010). Therefore, Vellend (2010) recommended using only four classes of processes: selection, drift, speciation and dispersal to understand community dynamics.

Ecology studies mainly assume ecological interactions in fixed habitat space but seldom consider interactions that regularly vary between different habitats. The movement of organisms across space, or dispersal, is usually considered from a biogeography point of view. The influence of habitat selection on community dynamics is hardly addressed in experimental studies investigating ecosystem processes. However, habitat selection behavior is an important part of predator avoidance behavior of many organisms. The actual expression of this behavior is thereby often explained as the result of a trade off between avoiding predators and gaining resources. Vertical migration of organisms in the pelagic zone is a characteristic example of this trade off.

Decaestecker et al. (2002) stated: "In the face of antagonistic interactions, habitat selection strategies in time and space are essential for the survival of many organisms." Many studies on predation have documented the ecological costs of anti-predatory habitat selection behavior, such as reduced food intake, reduced competitive strength, and increased susceptibility to predation by a different kind of predator (Orcutt and Porter 1983, Loose and Dawidowicz 1994, Tollrian and Harvel 1999, Lass et al. 2000).

In pelagic ecosystems no clear habitat structure exists, with the exception of vertical light attenuation. Hiding is only possible in deeper and therefore darker water. Additionally, light clearly separates the water column in two habitats: one with enough

light for photosynthesis and one without. Inorganic nutrients, such as nitrogen or phosphorus, are the second factor that changes with depth. In aquatic environments light and nutrients are not distributed as in terrestrial ecosystems, but clearly show opposite trends that light decreases with depth, whereas nutrients increase with depth (Diehl 2002), which separates the water column in different habitats. Migrations of plankton in pelagic water columns must therefore be viewed from this perspective considering the clear separation of light and nutrients in space.

7.1   Predator avoidance migrations

Migrations can occur regularly between habitats of different environmental conditions. This is the case with large herbivorous zooplankton species, which make a habitat shift twice daily between the epi- and hypolimnion. In this context, I studied the effects of zooplankton DVM on ecosystem dynamics.

DVM of zooplankton can act as a nutrient vector in the water column; nutrient availability for the phytoplankton community in the epilimnion can be influenced by DVM. I was able to show that *Daphnia* DVM can cause a measurable upward phosphorus transport (paper I). Furthermore, the amount of phosphorus transported and released by *Daphnia* was within a biologically meaningful range. Therefore, nutrient transport by *Daphnia* DVM could be a significant mechanism in fuelling primary production in the nutrient limited epilimnion.

Although the upward flux of phosphorus by zooplankton DVM may be low compared to other processes influencing phosphorus dynamics in the epilimnion, it is a regular and most probably the only internal nutrient supply during periods of stratification. The phosphorus transported by DVM may comprise a substantial phosphorus source for primary production. Phosphorus concentration is often extremely low within the epilimnion of stratified lakes due to a constant gravity-driven flux of phosphorus bound in particles from the epilimnion to the benthos. Therefore, any new input of phosphorus will be immediately translated into an increase in primary production (Schindler 1987).

DVM of zooplankton can also be a significant vector in the mortality and growth for the phytoplankton community. My studies suggest that DVM of zooplankton had significant effects on quantitative and qualitative characteristics of the phytoplankton community. DVM also influenced the diversity of the phytoplankton community, which was higher in

those treatments with migrating *Daphnia*. Nutrient availability (Interlandi and Kilham 2001) and grazing (e.g. Leibold 1996, Sarnelle 2005) can influence phytoplankton diversity. My results show for the first time that phytoplankton diversity is not only influenced by the abundance and grazing characteristics of herbivores but also by predator induced herbivorous behavior.

On the other hand, my results show that under nutrient-limited conditions, phytoplankton species could even profit from permanent grazing by zooplankton (Sommer 1994). Under certain conditions the positive effects of grazing, mediated by nutrient recycling, can be just as influential on algal growth as the negative impact of grazing-dependent mortality. Therefore, trophic cascades are often difficult to detect by investigating whole trophic-level responses, represented by bulk parameters, such as chlorophyll-*a* or particulate organic carbon.

The strong impact of *Daphnia* DVM on a single algal species (*Planktosphaeria gelatinosa*) in my study (paper II) shows that the effects of DVM are highly species-specific. This means that general predictions about how DVM influences phytoplankton dynamics are not possible using only bulk parameters to characterize phytoplankton abundance. Instead, species-specific responses of individual algal species to zooplankton DVM may determine total phytoplankton community patterns. Different phytoplankton species compositions, different degrees of nutrient limitation strength, and differences in zooplankton grazing all influence how DVM affects phytoplankton dynamics. It was therefore necessary to study the effects of DVM on differently size structured communities.

The results in the study with size manipulated phytoplankton communities (paper III) showed that zooplankton DVM affected phytoplankton growth and nutrient recycling differently, depending on phytoplankton size structure. A comparison of *Daphnia* "migration" and "no migration" treatments showed that total phytoplankton abundance and nutrient availability was higher in "no migration" treatments of communities with mainly small algae, whereas it was higher in "migration" treatments of communities with a wider size range of algae. Also the size structure of the two communities was influenced differently by DVM.

The results provide evidence that the direction and strength of trait mediated effects depend on the dominance or evenness of functional traits within a community. If functional traits such as body size determine the flow of energy and matter within trophic cascades, the distribution of these functional traits will also influence the strength and the direction of cascade flows. My results also show that a regular migration of grazers between habitats with high and low or with no primary production has consequences for the structure and the dynamics of the primary producer community in the epilimnion. Additionally, the results indicate that zooplankton migration can change the phytoplankton community composition and its growth through several mechanisms. In summary, zooplankton migration affects pelagic ecosystems not only by a single trait mediated effect but also by a combination of mechanisms related to both the biomass composition and the activity of migrating animals.

7.2   Migrations to optimize resource uptake

As it is the case in many motile phytoplankton species, migrations can also be motivated by optimization of resource uptake within the water column. These migrations are characterized by a steady habitat shift along light and nutrient gradients within the water column.

Most of the phytoflagellates use a combination of phototrophy and phagotrophy (Raven 1997), which suggests that this mixotrophy is an important additional phosphorus source for motility and growth, especially in environments where phosphorus is scarce and nutrient transport and/or recycling by *Daphnia* is low or absent. Phosphorus is often several orders of magnitude more concentrated in the biomass of bacteria than in the water (Vadstein 2000). Mixotrophic algae that feed on bacteria could access this additional phosphorus source. Therefore, mixotrophic algae are known to show lower C: P ratios than autotrophic ones.

My results in the study about the carbon sequestration and stoichiometry of motile and non-motile algae (paper IV) point at significant costs of motility, which may explain why flagellated taxa are often outcompeted by non-motile taxa in turbulently mixed environments where active motility is of little use. But there was also a tendency for algal C: P ratios to be lower in flagellated than in non-motile taxa. The shifts in biomass composition of phytoplankton could have consequences beyond phytoplankton ecophysiology. Fast-growing herbivorous zooplankton species, like *Daphnia* sp., have a high demand for phosphorus and therefore low inherent C: P ratios. Therefore, the

low biomass C: P ratios and the easily digestible cell walls of active motile taxa can result in high assimilation efficiency when grazed upon by zooplankton (Katechakis et al. 2005). Thus, the proportion of motile, migrating species within phytoplankton communities can strongly influence the transfer efficiency between phytoplankton and zooplankton, which could have consequences for ecosystem dynamics.

7.3   Resume

In summary, my results indicate that migration behavior must not only be viewed from the perspective that organisms move across space and therefore change the community composition of different habitats. The migrating organisms also act as a vector transporting energy, organic matter and "ecological interactions" determined by the organisms' activity and characterized by their life history. Aquatic pelagic ecosystems, including phytoplankton, zooplankton and predators, have a long evolutionary history and are most likely among the longest existing communities consisting of multicellular organisms on earth. Migration between habitats must therefore have had a clear evolutionary imprint on pelagic ecosystem dynamics. Still, the question remains how strongly existing pelagic ecological dynamics have been shaped by migrations of organisms within water columns.

8   Outlook

8.1   Zooplankton DVM effects on a global scale

Zooplankton DVM behavior has been described in all marine environments, from polar to tropical regions (Hays 2003). While cladocerans are the most important migrating zooplankton species in freshwater ecosystems, copepods are the dominate zooplankton species performing DVM in marine ecosystems and copepods are possibly the most common animal species in the world (Humes 1994). Marine phytoplankton are responsible for 50 % of global primary production, and indeed marine primary production strongly influences the composition of the atmosphere and major global nutrient and element fluxes (Falkowski and Raven 2007). In addition to being the basis of marine food webs, phytoplankton are also the basis of global fish production, which is the main food source for a large part of human population.

Due to the importance of marine zoo- and phytoplankton, the potential effects of DVM on marine pelagic ecosystems can have global impacts. There is no reason to doubt that the basic mechanisms observed in my studies on how DVM influences freshwater ecosystems effects are also valid for marine ecosystems. Although mesocosm experiments in marine environments are often difficult and expensive to maintain, it is important to study DVM effects on marine phytoplankton and marine nutrient fluxes under natural conditions. The next step in DVM research should be the study DVM effects on marine pelagic ecosystems.

Additionally, DVM is extremely important for distributing particulate organic matter (POM) in the ocean. Small, slow sinking particles are concentrated by detrivores, such as copepods, into larger fecal pellets (Smetacek 1980). These fecal pellets sink very rapidly into the ocean and represent the main faction of sinking POM. This organic matter transfer is fostered by vertical migrating organisms, which feed at night in the epilimnion and migrate down at dawn and release their fecal pellets in the hypolimnion. The POM in these pellets act as a basal resource in the benthic zone of the ocean. DVM can therefore be seen as one of the most important biomass vectors in the ocean, with significant consequences for deep sea ecosystems.

8.2   Research with other zooplankton groups

Zooplankton DVM research in marine ecosystems also raises the questions whether DVM effects on phytoplankton are related to zooplankton taxonomy. As mentioned above, cladocerans represent the most important zooplankton species in freshwater ecosystems, and copepods are the dominating zooplankton in marine ecosystems (Humes 1994). Although both groups perform DVM, their impacts on the phytoplankton community differ. This is mainly due to differing feeding strategies and differing elemental growth demands of both mesozooplankton groups.

Herbivorous cladocerans feed as unselective grazers with a food spectrum only limited by the mesh size of the filtration apparatus (Geller and Müller 1981), whereas copepods are active predators (Fryer 1957), selecting their prey, which mainly consists of algae. Therefore, only little overlap in the food spectrum of both groups exists: cladocerans mainly feed on small algal species and copepods, in most cases, on larger algae. This different size selectivity can have diverse effects on phytoplankton community composition (Sommer et al. 2001). Sommer et al. (2001) tested these theories and could demonstrate the consequences of the different feeding behavior of both zooplankton groups in experiments. Additionally, the food spectrum of cladocerans and copepods in their study showed no overlap, and the effects of both zooplankton groups on the phytoplankton were completely contrary.

My results showed that the altered grazing pattern of migrating zooplankton species is one of the most important mechanisms of how zooplankton DVM influences phytoplankton dynamics. Therefore, the feeding strategy (grazer vs. predator) of the migrating species could have additional, yet completely unknown consequences.

Besides the altered feeding pattern, nutrient recycling and transportation was an important mechanism of how zooplankton DVM can influence the pelagic ecosystem. Hence, copepods and cladocerans differ not only in their feeding behavior, but also in their nutrient recycling. Cladocerans excrete diffuse fecal material (Boersma and Wiltshire 2006), which is quickly dissolved in the water so that the nutrients are available in the epilimnion. Copepods produce compact and solid "fecal pellets" (Smetacek 1980) that tend to quickly sink. Therefore, the nutrients bound in fecal pellets are unavailable to the epilimnion. A DVM based upward nutrient transport caused by migrating copepods, as demonstrated in my studies with the cladoceran

Daphnia, seems therefore unlikely. Experimental studies have yet to study these theories in detail.

Another important difference in the comparison of copepod and cladoceran DVM consequences for pelagic ecosystems may arise from the different biomass stoichiometry of copepods and cladocerans. Both groups differ in their biomass carbon to phosphorus (C: P) and carbon to nitrogen (C: N) ratios. These differences also influence the elemental composition of the feces and subsequently the nutrient recycling of both groups. In general, fast growing cladocerans have a high P demand resulting in low biomass C: P ratios, whereas copepods exhibit low biomass C: N ratios, therefore, in relative terms, cladocerans recycle more N, whereas copepods recycle more P (Sommer and Stibor 2002). The biomass C: P and C: N ratios of phytoplankton in the epi- and hypolimnion of a stratified water column could additionally influence the different nutrient recycling of cladocerans and copepods during DVM.

In summary, different feeding behaviors, nutrient recycling and stoichiometric needs of copepods and cladocerans could affect phytoplankton dynamics in different ways. This has already been shown in experiments; however, these experiments did not involve zooplankton DVM. Future experimental studies investigating these contrary effects of copepods and cladocerans under DVM conditions are needed to unravel the conspicuous effects of both zooplankton groups in natural pelagic ecosystems were DVM is nearly always present.

8.3   Research with migrating phytoplankton

Studies manipulating the proportion of motile species in phytoplankton communities grazed upon by *Daphnia* are needed to investigate this possible link between the functional composition of phytoplankton communities and herbivorous zooplankton dynamics. The differing biomass stoichiometry of mobile and non-motile species may influence the transfer efficiency between phytoplankton and zooplankton. Low phytoplankton C: P ratios are seen as a high food quality for cladocerans and herbivorous zooplankton species may therefore follow migrating phytoplankton species because of their lower C: P ratios.

The question remains whether the observed lower biomass C: P ratios of flagellates are a consequence of motility, mixotrophic nutrition, or both (Katechakis et al. 2005).

My data suggest that motility per se may not be sufficient to explain the lower C: P ratios of flagellates, since we found substantial variation among species within motility categories, indicating that other traits in addition to motility contribute to species-specific responses. Additionally, estimates of phytoplankton DVM across different lakes are completely missing. Future research should bridge the gap between phytoplankton mobility and phytoplankton migrations to optimize resource uptake of light and nutrients and zooplankton performance.

8.4 Methodological improvements in studying zooplankton DVM effects

My experimental studies made clear that the effects of zooplankton DVM on phytoplankton (caused by nutrient transport, refuge effects or induced phytoplankton migration behavior) are in a range that is detectable and measurable. However, the experimental study and analysis of the effects of zooplankton DVM, by inducing DVM without the kairomones, is very laborious due to the necessity for large and sophisticated experimental setups with a high number of controls to test for experimental cage effects. Therefore, only strong effects of DVM are likely to be found by such experiments, even if a high number of replicates are included into the experimental design. With these experimental restrictions minor effects of zooplankton DVM are unlikely to be found.

The use of fish kairomones for inducing zooplankton DVM would allow easier and more detailed experiments. Unfortunately, the chemical structure of the kairomones is not yet known in detail, although there are some first results suggesting some molecules (Akkas et al. 2010, Bentkowski et al. 2010). The large mesh cages I used in my field experiments ensured a full control over zooplankton DVM but are accompanied by the risk of artefacts. Inducing DVM by fish kairomones would allow to ability to carry out more precise experiments under more natural conditions without the nutrient input by fish. Even whole lake experiments based on complete new research questions regarding effects of DVM would be possible, e.g. inducing DVM in naturally fish-free lakes or the comparison of DVM effects between fish-free lakes and lakes with fish. Additionally, it would be possible to induce DVM in seasons when normally no DVM takes place. In short, the use of kairomones would allow easier and more precise DVM studies and give new insights into mechanistic aspects of pelagic ecosystem functioning.

8.5   Modelling the effects of migrations on pelagic ecosystems

The results of my studies can be used to improve existing models regarding effects of migrating zoo- and phytoplankton on pelagic ecosystem functioning. One advantage of modeling plankton migrations is the possibility of manipulating time. Experimental studies (such as the mesocosm experiments in my studies) are often limited to a time span of a couple of weeks, due to an increase of experimental structure effects , e.g. extensive growth of algae at the enclosure walls, whereas theoretical models can easily cover longer time spans, e.g. a complete succession period between spring and fall circulations. Additionally, models can include more environmental variation and conditions than experiments can. Therefore, models can help construct a framework of possible theoretical effects of migrating plankton on pelagic ecosystem functioning. My experiments can provide initial data in helping to parameterize such models.

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Publications

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Presentations

<u>Oral presentations</u>

Haupt, F., Striebel, M. and Diehl, S. The influence of mixing depth on phytoplankton-*Daphnia*-interactions: A field experiment. ASLO international meeting, June 2005, Santiago de Compostella, Spain.

Haupt, F., Stockenreiter, M., Baumgartner, M., Boersma, M. and Stibor, H. Does diel vertical migration of *Daphnia* influence the phytoplankton community structure? SIL international meeting, August 2007, Montreal, Canada.

Haupt, F., Stockenreiter, M., Baumgartner, M., Reichwaldt, E. S., Boersma, M. und Stibor, H. Aufwärtsgerichteter Nährstofftransport durch vertikalwandernde Daphnien. DGL Jahrestagung, September 2008, Konstanz, Germany.

Haupt, F., Stockenreiter, M., Baumgartner, M., Reichwaldt, E. S., Boersma, M. and Stibor, H. Upward nutrient transport by *Daphnia* diel vertical migration. ASLO aquatic science meeting, January 2009, Nice, France.

Haupt, F., Stockenreiter, M., Boersma, M., and Stibor, H. Does diel vertical migration of *Daphnia* influence the phytoplankton community structure? ASLO aquatic science meeting, Feburay 2011, San Juan, Puerto Rico.

<u>Poster presentations</u>

Haupt, F., Stockenreiter, M., Baumgartner, M., Reichwaldt, E. S., Boersma, M. und Stibor, H. Aufwärtsgerichteter Nährstofftransport durch vertikalwandernde Daphnien. Munich Interact, April 2009, München, Germany.

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I want to thank:

Declaration

Diese Promotion wurde im Sinne §12 der Promotionsordnung von Prof. 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.

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Ort, Datum   Unterschrift

Beitrag der Koautoren und eigener Beitrag

<u>Paper I</u>

Maria Stockenreiter und Michaela Baumgartner waren im Rahmen ihrer Diplomarbeit an der Durchführung und Auswertung (Auszählen der Zooplanktonproben) des Experiments beteiligt. Elke Reichwaldt half bei der Planung und Durchführung des Experiments und half bei der Fertigstellung des Manuskripts. Winfried Lampert half bei der Fertigstellung des Manuskripts.

<u>Paper II</u>

Maria Stockenreiter und Michaela Baumgartner waren im Rahmen ihrer Diplomarbeit an der Durchführung und Auswertung (Auszählen der Phytoplanktonproben) des Experiments beteiligt.

<u>Paper III</u>

Maria Stockenreiter war an der Durchführung und Auswertung (Auszählen der Phytoplanktonproben) des Experiments beteiligt und half bei der Fertigstellung des Manuskripts.

<u>Paper IV</u>

Ich war an der Konzeption, Durchführung und Auswertung der Experimente beteiligt und half beim Verfassen des Manuskripts.

Maarten Boersma unterstütze mich bei der Konzipierung der Versuche und half beim Anfertigen der Manuskripte I – III. Herwig Stibor unterstütze mich bei der Konzipierung der Versuche, war an der Diskussion der Ergebnisse beteiligt und half beim Anfertigen der Manuskripte I – IV.