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

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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 $\mu M\ L^{-1}$ 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 $\mu M\ P\ L^{-1}$ 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 × 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):

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

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 ± 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 ± 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 ± 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.

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 μM 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 ± 0.01 μM L^{-1}, and TP values were 0.22 ± 0.01 and 0.21 ± 0.01 μM 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 μM L^{-1}; silicate >30 μM L^{-1}).

Seston carbon content

Seston carbon concentrations were lower in the *Daphnia* "migration" treatment group (513 ± 16 μg POC L^{-1}) than in the *Daphnia* "no migration" group (790 ± 80 μg 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 ± 0.4 μg L^{-1}) than in the *Daphnia* "no migration" group (9.4 ± 1.1 μg 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 ± 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 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 μm^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 ($5.4 \times 10^8 \pm 1.0 \times 10^8$ $\mu m^3 L^{-1}$) than in the *Daphnia* "no migration" group ($2.0 \times 10^9 \pm 3.8 \times 10^8$ $\mu m^3 L^{-1}$), 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

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.

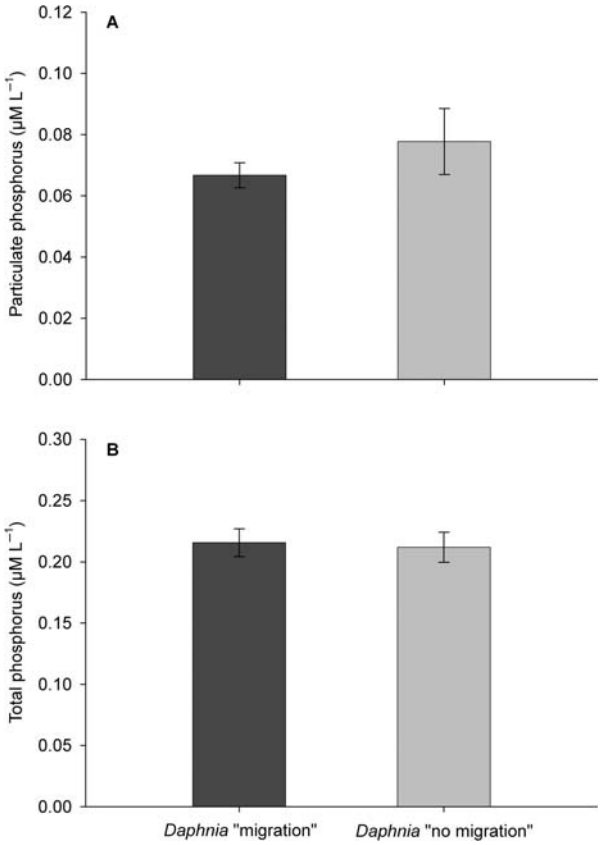

Fig. 1. Mean values of particulate phosphorus (**A**) and total phosphorus (**B**) concentrations in *Daphnia* "migration" and *Daphnia* "no migration" treatment groups in the epilimnion. Error bars represent ± 1 SE.

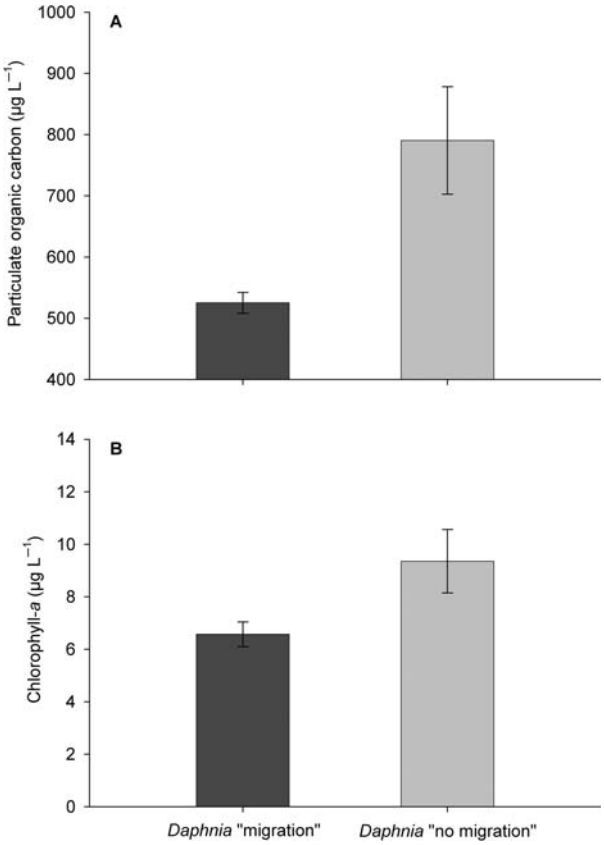

Fig. 2. Mean values of particulate organic carbon (**A**) and chlorophyll-*a* (**B**) concentrations in *Daphnia* "migration" and *Daphnia* "no migration" treatment groups in the epilimnion. Error bars represent ± 1 SE.

species, *Achnanthes microcephala* and *Cymbella helvetica*. In the *Daphnia* "migration" treatment group, the abundances of both *A. microcephala* ($7.0 \times 10^6 \pm 4.6 \times 10^5$ μm^3 L^{-1}) and *C. helvetica* ($8.0 \times 10^7 \pm 6.4 \times 10^6$ μm^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 $5.0 \times 10^7 \pm 7.0 \times 10^6$ μm^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).

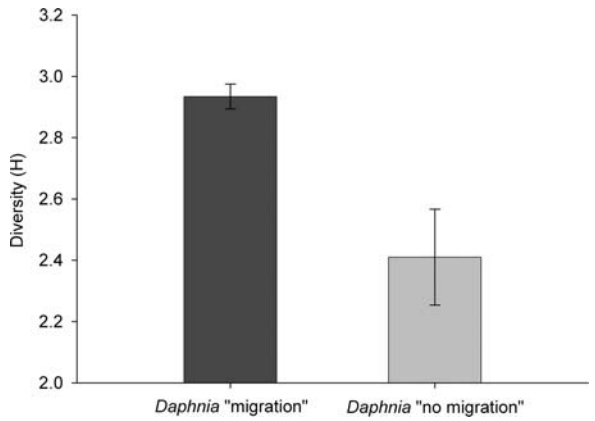

Fig. 3. Mean values of phytoplankton community diversity (H) in *Daphnia* "migration" and *Daphnia* "no migration" treatment groups in the epilimnion. Error bars represent ± 1 SE.

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. 4B). The abundance of all 11 phytoplankton species differed significantly between hypolimnion and epilimnion, indicating the presence of two different water layers (Table I).

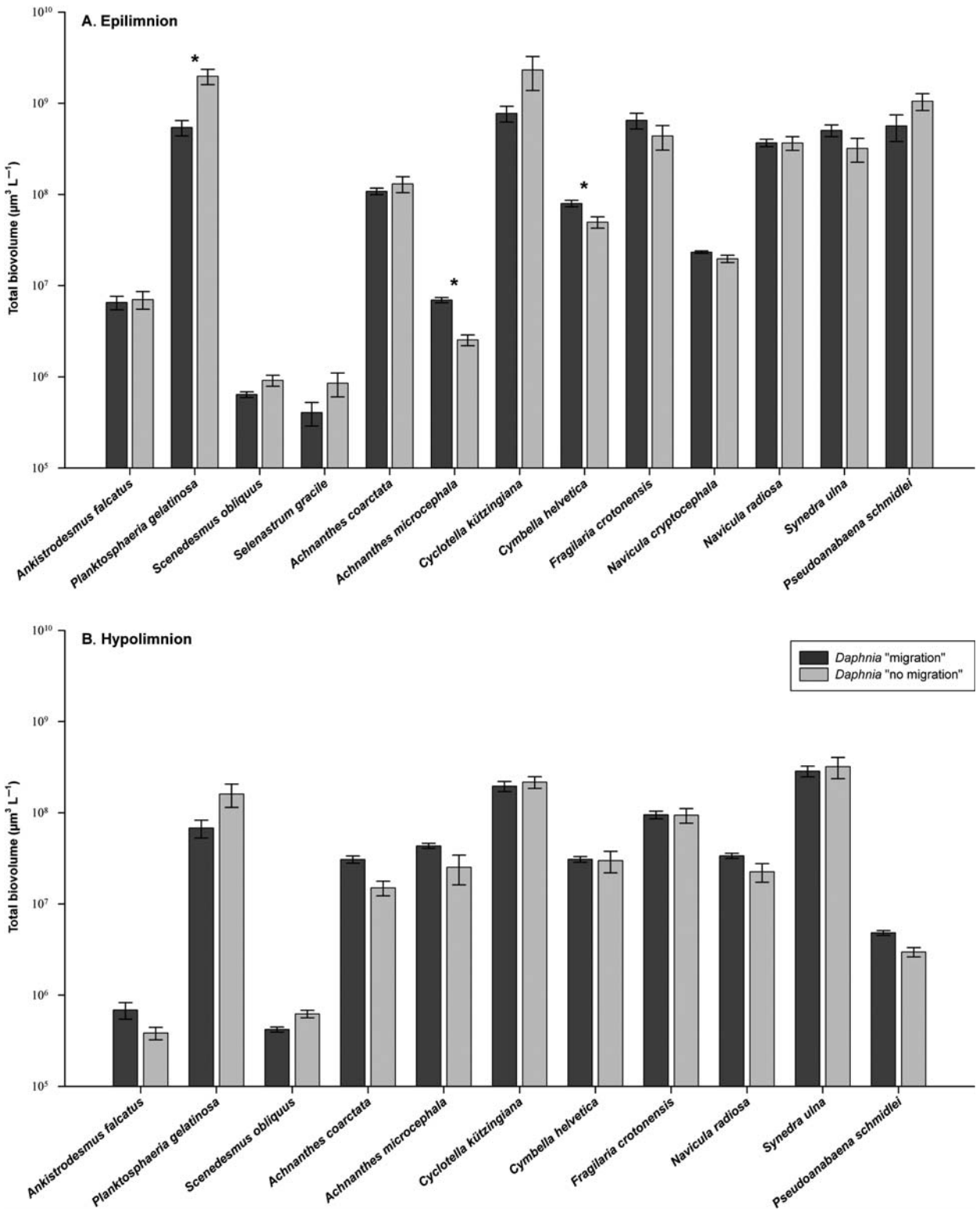

Fig. 4. Mean values of algal species abundance in *Daphnia* "migration" and *Daphnia* "no migration" treatment groups in the epilimnion (**A**) and hypolimnion (**B**). Error bars represent ± 1 SE. Significant differences between *Daphnia* "migration" and *Daphnia* "no migration" treatment groups in 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 interactions. 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 abundances 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 mesocosms. 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 concentration 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 phytoplankton communities under *Daphnia* "migration" and *Daphnia* "no migration" treatment conditions are unlikely to be caused by differences in nutrient availability. Therefore, the differences between phytoplankton communities 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 representing the phytoplankton trophic level. However, strong responses of individual plant species to the presence 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 phytoplankton 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 phytoplankton diversity, our results provide insights into the mechanisms 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 phytoplankton dynamics in our experiment was the gelatinous 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 observation that *P. gelatinosa* abundance was higher in the *Daphnia* "no migration" treatment group than in the *Daphnia* "migration" group.

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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