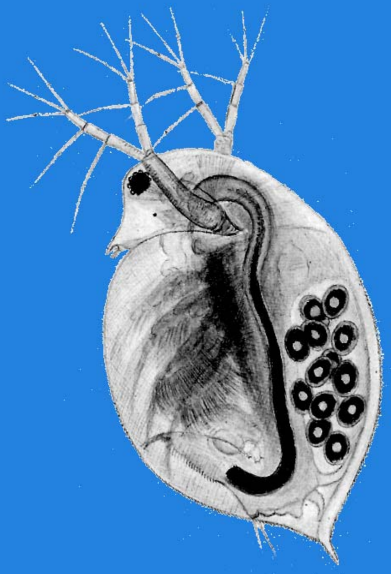
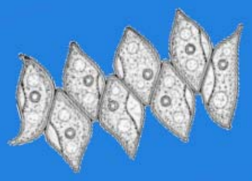
The effects of diel vertical migration of *Daphnia* on zooplankton-phytoplankton interactions: laboratory and field experiments

Elke S. Reichwaldt





Dissertation

zur Erlangung des Doktorgrades

der Fakultät für Biologie

der Ludwig-Maximilians-Universität München

März 2004

The effects of diel vertical migration of *Daphnia* on zooplankton-phytoplankton interactions: laboratory and field experiments

Elke S. Reichwaldt

Dissertation

an der Fakultät für Biologie

der Ludwig-Maximilians-Universität München

vorgelegt von Elke S. Reichwaldt

aus München

München, den 22.3.2004

Cover:

Daphnia by G.O.Sars, 1861

Scenedesmus by H.Streble and D.Krauter, 1988

Erstgutachter: PD Dr. Herwig Stibor

Zweitgutachter: PD Dr. Ralph Tollrian

Tag der mündlichen Prüfung: 25.5.2004

Summary

Zooplankton populations which perform diel vertical migration (DVM) only spend the night in surface water layers but migrate downwards into the lower water layers during the day. The intention of this study was to investigate effects of DVM of *Daphnia* on phytoplankton dynamics and *Daphnia* life history parameters in a lake. I conducted field and laboratory experiments in which I compared ‘migration’ with ‘no-migration’ situations. It is generally assumed that phytoplankton communities in the epilimnion of stratified lakes profit from the presence of DVM. This might be caused by less grazing due to intermittent grazing and/or less grazing due to lower population densities of migrating *Daphnia* populations (as they migrate into the colder, lower hypolimnion during the day which leads to a lower temperature-dependent population growth). In a first laboratory experiment I showed that an enhanced phytoplankton biomass could develop under a migration regime solely due to intermittent grazing. I further showed that edible phytoplankton species with higher intrinsic growth rates benefited more from intermittent grazing than edible species with lower intrinsic growth rates. Field experiments also indicated that phytoplankton biomass in the epilimnion was higher when subject to a migrating zooplankton population and that additionally different phytoplankton community compositions arose from different migration regimes (‘migration’/’no-migration’). For example edible algae were at an advantage when zooplankton migrated, whereas large, inedible algae species had an advantage when zooplankton populations did not migrate. In an additional laboratory experiment I also showed that these changes in phytoplankton composition had strong feedback effects on life history parameters of daphnids and that food conditions experienced by migrating daphnids were advantageous. In a further laboratory experiment I exposed two *Daphnia* species to either constant or regularly changing temperature regimes to study whether a fluctuating temperature regime – as experienced by migrating daphnids – implies costs for daphnids. Somatic growth rates of juvenile *Daphnia* in the regularly changing temperature regime were almost as low as under constant low temperature conditions indicating that a regular change in temperature involves high costs.

The results of my study indicate that DVM has a strong modulating effect on zooplankton-phytoplankton interactions in a lake.

Zusammenfassung

Zooplankter, die eine tagesperiodischen Vertikalwanderung (TPV) durchführen halten sich nur nachts in der Oberflächenschicht der Gewässer auf, den Tag dagegen verbringen sie in tieferen Schichten. Die vorliegende Arbeit beschäftigt sich mit den Auswirkungen der TPV von Daphnien auf Phytoplanktondynamiken und Populationsparameter der Daphnien. Dazu führte ich sowohl Freiland- als auch Laborversuche durch, in denen ich jeweils 'Migrations-' mit 'Nicht-Migrations-' Ansätzen verglich. Es wird generell angenommen, dass Phytoplanktongemeinschaften in den Epilimnien von geschichteten Seen der gemäßigten Zone von einer TPV profitieren können. Das kann durch zwei Mechanismen hervorgerufen werden: Erstens durch einen zeitlich gepulsten Fraßdruck (Grazing nur nachts), und zweitens durch einen geringeren Fraßdruck, hervorgerufen durch die meist geringere Dichte wandernder Zooplanktonpopulationen, da diese, bedingt durch die Wanderung tagsüber in die kalten, tiefergelegenen Wasserschichten, ein reduziertes Wachstum haben. In einem ersten Laborversuch konnte ich zeigen, dass allein durch einen zeitlich gepulsten, diskontinuierlichen Fraßdruck ein erhöhtes Algenwachstum entstehen konnte. Ich konnte weiterhin zeigen, dass fressbare Algenarten mit einer höheren intrinsischen Wachstumsrate einen größeren Vorteil von der TPV hatten als fressbare Arten mit einer niedrigeren Wachstumsrate. In Freilandexperimenten konnte ich zum einen ebenfalls zeigen, dass die Algenbiomasse bei Wanderung der Daphnien erhöht war, zum anderen konnte ich auch vom Wanderregime abhängige Veränderungen in der Phytoplanktongemeinschaft nachweisen. So hatten fressbare Phytoplanktonarten einen Vorteil von einer TPV, unfressbare Algenarten hatten dagegen einen Vorteil, wenn keine Wanderung stattfand. Ich konnte in einem weiteren Laborversuch zeigen, dass die oben genannten Veränderungen in der Phytoplanktondynamik starke rückwirkende Effekte auf Populationsparameter der Daphnien hatten. In einem weiteren Laborversuch setzte ich die Daphnien entweder konstanten, oder tageszeitenabhängig fluktuierenden Temperaturregimes aus. Dadurch untersuchte ich, ob eine sich zwei mal pro Tag ändernde Temperatur (wie Daphnien es bei einer Wanderung erleben) Kosten für Daphnien verursacht. Dabei war die somatische Wachstumsrate der juvenilen Daphnien bei einem fluktuierenden Temperaturregime fast genauso gering wie bei konstant tiefer Temperatur. Dies gibt Hinweise auf Kosten, die durch ein fluktuierendes Temperaturregime entstehen.

Die Ergebnisse zeigen, dass eine TPV von Daphnien starke, modulierende Auswirkungen auf Zooplankton-Phytoplankton Interaktionen hat.

Table of contents

Introduction

Summary of the papers

Papers

Paper 1

The effect of different zooplankton grazing patterns resulting from diel vertical migration on phytoplankton growth and composition: a laboratory experiment
(Reichwaldt, Wolf and Stibor, Oecologia in revision)

Paper 2

The impact of diel vertical migration of *Daphnia* on phytoplankton dynamics
(Reichwaldt and Stibor, submitted to Nature)

Paper 3

The Effects of *Daphnia* Diel Vertical Migration on Phytoplankton Dynamics and their implictions for *Daphnia* life history Parameters
(Reichwaldt, Wolf and Stibor, submitted to Limnology and Oceanography)

Paper 4

Effects of a fluctuating temperature regime experienced by *Daphnia* during diel vertical migration on *Daphnia* life history parameters
(Reichwaldt, Wolf and Stibor, submitted to Hydrobiologia)

Acknowledgements

Curriculum vitae

Introduction

The phenomenon of diel vertical migration

The behavioural phenomenon of diel vertical migration (DVM) of mesozooplankton in marine and freshwater ecosystems is widely known. In the presence of hazards like visual predation by planktivorous fish large zooplankton individuals or species (e.g. cladocerans, copepods) only spend the night in surface waters (epilimnion). During the day they stay in the lower and darker water layers (hypolimnion) often crossing the thermocline during their migration downwards and upwards. Thus, in stratified lakes of the temperate region zooplankton regularly experiences strong differences in temperature between day and night.

DVM is one of the world's most massive animal migrations as an enormous amount of herbivorous biomass moves daily up and down the water column. Early research on DVM was mainly interested in the investigation of ultimate and proximate causes of this behaviour. The relative change in light intensity is thought to be the proximate cause (Enright and Hamner 1967; Ringelberg 1993) and predator avoidance against optically orientated fish is considered as the ultimate cause of DVM (Zaret and Suffern 1976; Stich and Lampert 1981; Lampert 1993). Vertical migration is induced by chemical trigger substances, so called kairomones (Dodson 1988; Loose and Dawidowicz 1994).

It was recognized early that DVM represents an induced behaviour which is not fixed, and thus, the question about the trade-off for this behaviour arose. As mentioned above, the main benefit of this behaviour is reduced mortality due to predator avoidance. In contrast, main costs seem to be connected to low temperatures experienced in the hypolimnion of stratified lakes during the day (Dawidowicz and Loose 1992; Loose and Dawidowicz 1994). Biological processes are generally temperature-dependent and are slowed down by low temperatures. Therefore low temperatures have a strong direct, negative influence on zooplankton population dynamics. Experiments on the effect of temperature on population parameters indicated for example that somatic growth rate is slower (Orcutt and Porter 1984) and time until first reproduction and egg development time are longer at constant low temperatures (Bottrell 1975; Orcutt and Porter 1983; Orcutt and Porter 1984; Sakwinska 1998; Giebelhausen and Lampert 2001). All these experiments were conducted with constant temperature regimes. However, in temperate lakes migrating zooplankton population experience a regular change in temperature twice a day due to the temperature gradient in the water column. In this context, the question then arises whether migrating zooplankton populations have costs because of a regularly changing temperature regime additionally to the costs due to a lower temperature experienced during the day (e.g. costs for permanently re-adjusting metabolic rates). To my knowledge there are only two studies in which zooplankton performance has been studied under a fluctuating temperature regime (Orcutt and Porter 1983; Stich and Lampert 1984). Stich & Lampert (1984) exposed zooplankton to a fluctuating temperature regime but they simultaneously varied the food regime to simulate conditions of migrating populations as precisely as possible. Thus, from this experiment it is not possible to infer the direct influence of a fluctuating temperature regime on the zooplankton. Orcutt & Porter (1983) compared the values of life history parameters of *Daphnia* under a fluctuating temperature regime with the values at an intermediated temperature. However, this comparison is not appropriate if we want to assess the costs for a migrating population because migrating zooplankton populations do not experience an intermediated temperature. Thus, if we want to assess the costs of a fluctuating temperature regime we have to compare the values of life history parameters in the fluctuating temperature regime with the calculated mean of the values of the life history parameters in a correspondingly warm and cold temperature regime (following the principle of degree-day calculations used to control pests (Begon et al. 1990)). One part of my study was the assessment of the costs due to a fluctuating temperature regime experienced by daphnids during migration twice a day, as to my knowledge this has not been studied so far.

However, temperature is not the only important factor that affects migrating and non-migrating zooplankton populations differently. Migrating zooplankton also experience different food conditions during the day as non-migrating daphnids do. Early studies on this subject suggested that migrating zooplankton experience lower amounts of food during the day due to less food in the hypolimnion than in the epilimnion (Orcutt and Porter 1984; Lampert 1987; Duncan et al. 1993). Consequently it was assumed that not only a lower temperature but also less food had a negative impact on migrating zooplankton populations. However, recent studies showed that food conditions are not always worse in the hypolimnion due to deep-chlorophyll-maxima in some lakes (Williamson et al. 1996; Winder et al. 2003). These studies indicated that food conditions for the zooplankton were actually better in the hypolimnion than in the epilimnion. However the studies also suggest that negative temperature effects might be stronger than positive food effects in those lakes because zooplankton still migrated into the epilimnion.

Possible effects of diel vertical migration on phytoplankton

Although diel vertical migration represents a well-studied phenomenon, the experimental investigation of its influence on other components of the food web has been ignored so far. DVM leads to a daily shift of large amounts of herbivorous zooplankton biomass between the epilimnion and the hypolimnion and this should have substantial consequences for the whole pelagic food web. The assessment of the effects of DVM on phytoplankton communities was a further emphasis in my study.

In stratified lakes, alternative migration regimes of zooplankton result in different conditions for the phytoplankton in the epilimnion. In the absence of migration the phytoplankton in the epilimnion is grazed continuously. On the other hand, if the zooplankton migrates, the phytoplankton experiences less grazing caused by an intermittent grazing pressure (grazing only during the night). Additional to less grazing due to an intermittent grazing pressure, the phytoplankton in the epilimnion might also be confronted with a reduced grazing pressure during the night due to lower zooplankton densities of migrating populations (because of a lower temperature experienced by the zooplankton accompanied with low growth rates in the hypolimnion during the day). Consequently, both intermittent grazing and lower zooplankton abundance lead to a reduced grazing pressure for the phytoplankton in the epilimnion if zooplankton populations migrate. There are several theoretical models concerning migration of zooplankton and its influence on phytoplankton (McAllister 1969; Petipa and Makarova 1969; Gabriel in Lampert 1986, 1987). These models predict that the rhythm of particle elimination is – besides the grazing intensity – also important for phytoplankton production. They all stress that phytoplankton can benefit from migrating zooplankton due to an intermittent grazing pressure which leads to a more or less grazing-free period during the day in which the algae can grow undisturbed. It is obvious that phytoplankton should benefit from migration because intermittent grazing is usually equivalent with a lower grazing time. To separate the effect of a lower grazing time from the effect of a pulsed grazing pressure, Gabriel (in Lampert 1986, 1987) incorporated the following assumption into his theoretical model which makes it possible to identify the direct effects of intermittent grazing on the phytoplankton: migrating zooplankton should consume the same amount of phytoplankton biomass per day as non-migrating zooplankton (Figure 1). As with the other models, this model also predicts an enhanced population growth for phytoplankton under a migration regime, even after taking this assumption into account. Furthermore, it predicts that phytoplankton species with higher intrinsic growth rates benefit more from a nocturnal grazing regime than species with lower intrinsic growth rates. Consequently, this leads to shifts within the phytoplankton community to fast growing species under a migration regime.

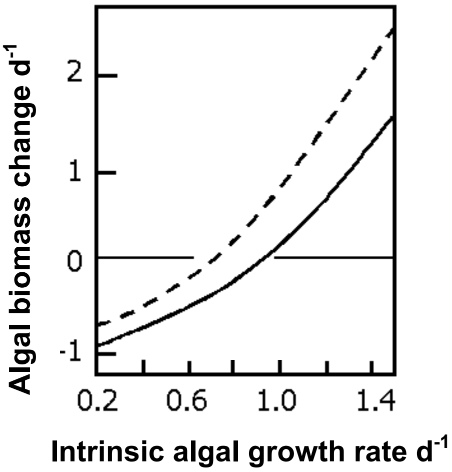


Figure 1 Model calculation of the effect of different diel grazing patterns of zooplankton on edible phytoplankton net production. In both patterns the same total algal biomass is consumed by the zooplankton per day. The lower line estimates algal biomass change if grazing is continuously (no migration), the upper broken line estimates algal biomass change if grazing takes place only during the night (with vertical migration). 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 (Gabriel in Lampert 1986, 1987) (changed by the author).

In lakes it is not easy to distinguish whether an existing effect on phytoplankton is due to less grazing caused by a lower zooplankton abundance or by intermittent grazing. In fact, both mechanisms are combined in lakes and will affect phytoplankton dynamics at the same time. However, in laboratory experiments in which the assumption of Gabriel's model can be met accurately, separation of these two mechanisms can be achieved and this is of fundamental importance for the understanding of food web dynamics in combination with DVM.

DVM and the trophic cascade concept

To emphasize the importance of the possible consequences of DVM on phytoplankton dynamics, DVM has to be considered in a broader context. The trophic cascade concept has recently drawn attention to indirect interactions in food webs. The concept emphasizes the indirect effects of predators on their prey’s resource, either by influencing the density of the prey (density-mediated indirect interactions) or by changing traits of the prey, such as behaviour, life history parameters or morphology (trait-mediated indirect interactions) (Abrams 1995; Peacor and Werner 2001) (Figure 2). This was already shown by many studies in a variety of different ecosystems (reviewed in Schmitz et al. 2000; Schmitz et al. 2004). For example Trussell et al. (2002) studied trait-mediated indirect effects in a rocky intertidal food web composed of algae, herbivorous snails, and snail-eating crabs. They found that a chemical cue released by the caged crab was sufficient to reduce the grazing impact of snails on algae by changing the growth and behaviour of the snail. The presence of crabs led to an enhanced algae biomass indicating the strong indirect effect of the predator (crab) on the snail’s resource (algae). Another example is the study by Tessier and Woodruff (2002) who investigated indirect interactions in lakes with phytoplankton, herbivorous zooplankton and zooplanktivorous fish. They showed that the indirect effect of fish on phytoplankton could affect the community composition of the phytoplankton without changing total algae biomass.

DVM is a typical example of a trait-mediated indirect interaction because kairomones released by fish change the zooplankton’s behaviour by inducing migration, and, thus, influence the phytoplankton community. However, it is surprising that research on this well-understood behaviour has not yet involved experimental studies on the effects of this kairomone-mediated trophic cascade on phytoplankton dynamics.

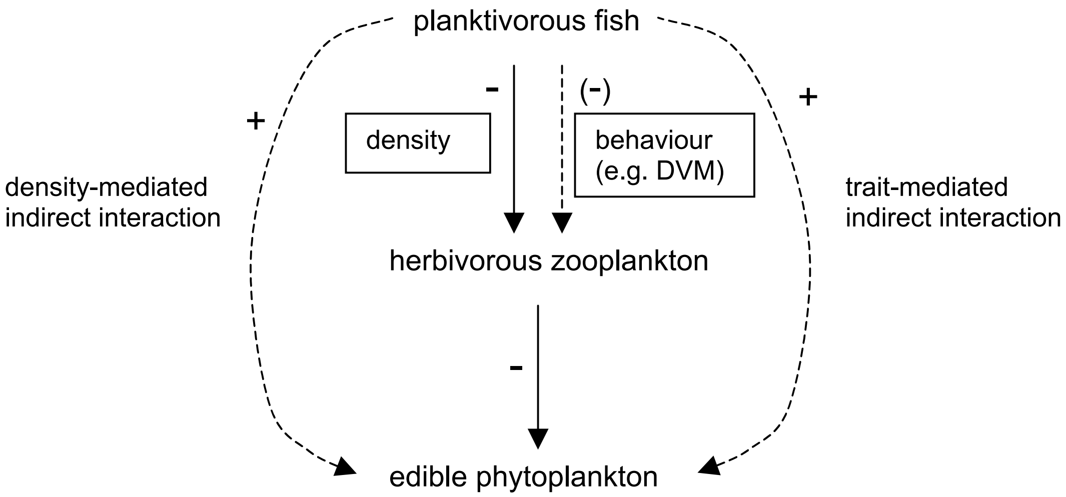


Figure 2 Simple trophic cascade in a limnetic food web. The presented organisms are those treated in the present study

The project

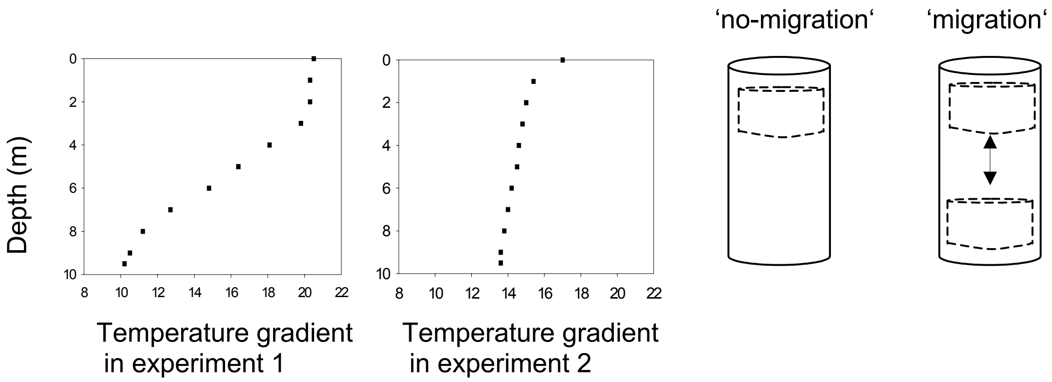
This study gives a broad insight into the effects of DVM of *Daphnia* on phytoplankton dynamics and zooplankton life history parameters.

1. The first set of five laboratory experiments was conducted to test the theoretical model of Gabriel (in Lampert 1986, 1987) working with the following hypotheses (Paper 1):
    - Phytoplankton biomass development is higher under a *Daphnia* 'migration' regime (intermittent grazing) than under a 'no-migration' regime (continuous grazing), even if the same amount of biomass is eliminated in both grazing regimes per day
    - Edible phytoplankton species with higher intrinsic growth rates benefit more from migration (intermittent grazing) than edible species with lower intrinsic growth rates
    - Relative performance of phytoplankton species (e.g. dominance of one algae species) can change with the grazing pattern (continuous / intermittent)
2. In two mesocosm experiments conducted at Lake Brunnsee (Germany), I investigated the effects of DVM of *Daphnia hyalina* on natural phytoplankton community dynamics. Consequently I compared phytoplankton dynamics in the presence of a non-migrating zooplankton population (continuous grazing) with the dynamics in the presence of a migrating population (intermittent grazing). I also measured life history parameters of the migrating and non-migrating *Daphnia* populations. Migration of daphnids was achieved by confining them into cages (made out of gauze) that were either moved to the

appropriate layers twice a day (‘migration’ treatment), or left continuously in the epilimnion (‘no-migration’ treatment) (Figure 3). Experiment 1 was conducted with a natural temperature gradient in the water columns of the mesocosms (Paper 2), whereas in experiment 2 temperature was held constant over the water columns (Paper 3). I did the latter because zooplankton growth rates are largely dependent on temperature and I wanted to achieve similar zooplankton population growth rates in both regimes in this experiment to separate the effects of intermittent grazing from the impact of lower grazing pressure due to lower densities of *Daphnia*.

In these field experiments I dealt with the following hypotheses and questions:

- Phytoplankton biomass development in the epilimnion of the ‘migration’ treatment (intermittent grazing) is enhanced compared to biomass development in the ‘no-migration’ treatment (continuous grazing)
- Phytoplankton community compositions differ depending on the grazing regime (continuous / intermittent)
- In experiment 1, *D. hyalina* has a lower population growth in the ‘migration’ treatment compared to population growth in the ‘no-migration’ treatment because of the temperature gradient
- In experiment 2, the differences in population growth of *D. hyalina* in the two treatments are only small or non-existent due to the absence of a temperature gradient



Temperature gradient in experiment 1

Temperature gradient in experiment 2

Figure 3 Experimental design of the mesocosm experiments 1 and 2.

3. The second laboratory experiment I conducted ran parallel to the second mesocosm experiment (both are described in Paper 3). As the interaction between zooplankton and phytoplankton is mutual (which means that the zooplankton influences the phytoplankton and vice versa), I was interested in the feedback effects of the DVM-related changes in seston composition on population parameters of *Daphnia hyalina*. In this bioassay, juvenile daphnids in the laboratory were fed with seston from the mesocosms in a way that mimicked food conditions experienced by their counterparts in the field (migrating / non-migrating / non-migrating in the presence of a migrating population) (Figure 4).

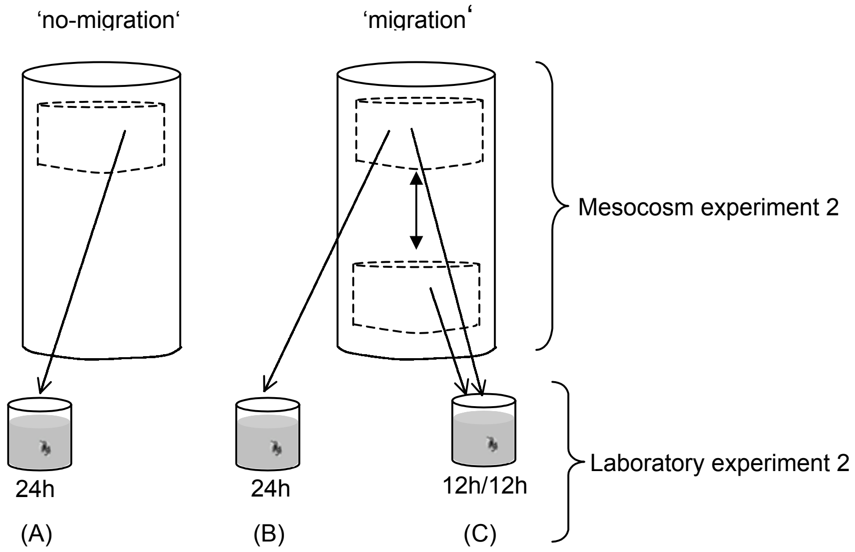


Figure 4 Experimental design of laboratory experiment 2. Juvenile daphnids were fed with food mimicking a situation (A) without migration, (B) without migration although the rest of the population migrates, and (C) with migration.

4. Migrating zooplankton populations in stratified lakes generally have costs due to a lower temperature experienced during the day in the hypolimnion. Additional to these costs, they can also have costs due to a regular change in temperature because they cross the thermocline twice a day. In a third laboratory experiment I studied the effects of a regularly changing temperature regime on life history parameters of *Daphnia hyalina* and *Daphnia magna* (Paper 4). Applied temperatures were similar to temperatures in Lake Brunnsee during the mesocosm experiments in summer.

Summary of the papers

PAPER 1

The effect of different zooplankton grazing patterns resulting from diel vertical migration on phytoplankton growth and composition: a laboratory experiment

(Reichwaldt, Wolf and Stibor, Oecologia in revision)

Herbivorous zooplankton has a strong influence on phytoplankton dynamics, as algae are one of their main food sources. If the zooplankton performs a diel vertical migration (DVM), the phytoplankton in the epilimnion experiences an intermittent, nocturnal grazing pressure, whereas the phytoplankton is grazed continuously if no-migration is present. To my knowledge no experimental studies on the effects of intermittent grazing of zooplankton on phytoplankton have been performed so far. A theoretical model by Gabriel (in Lampert 1986, 1987; see also Figure 1 on page 11) predicts that DVM enhances phytoplankton biomass and changes phytoplankton community composition in the epilimnion of lakes. An important assumption of this model is that the same amount of carbon (as an equivalent for phytoplankton biomass) is eliminated by the grazers per day regardless of the grazing regime (continuous grazing or intermittent grazing due to migration). This assumption then allows us to identify the direct effects of intermittent grazing on the phytoplankton.

To test the predictions of the model, I conducted five laboratory experiments using both *Daphnia hyalina* and *Daphnia magna* in which I compared the effects of a ‘migration’ regime with the effects of a ‘no-migration’ regime. In each treatment the daphnids were kept inside cages so that they could easily be taken out of the experimental ‘migration’ vessels during the day. This was a simple method to mimic DVM in the laboratory. The cages from the ‘migration’ treatments that were taken out of the experimental vessel during the day were stored in intermediate vessels during that time. These intermediate vessels were in any way identical to the experimental vessels. Each experiment consisted of three different *Daphnia* treatments: (1) 13 daphnids/l grazing for 24 hours (’continuous’ = ’no-migration’), (2) 13 daphnids/l grazing for 12 hours at night (’nocturnal’ = ‘migration’) and (3) 26 daphnids/l grazing for 12 hours at night (’enhanced nocturnal’ = ‘migration’). The phytoplankton thus experienced either a continuous grazing pattern or a discontinuous grazing pattern only during the night (mimicking grazing by migrating daphnids). By taking the double amount of daphnids in the ‘enhanced nocturnal migration’ treatment the same amount of phytoplankton biomass was eliminated in this ‘migration’ treatment as in the ‘continuous no-migration’ treatment over time. This was confirmed in additional experiments where the community-grazing rate of both populations was determined. I additionally conducted two control treatments without daphnids: (1) the cages were left in the experimental vessel for 24 hours or (2) the cages were taken out of the experimental vessel for 12 hours during the day. The two control treatments should discover any differences that arose from cage handling. I had 6 different phytoplankton species and each of the five experiments was stocked with two algal species assigned randomly. I tried to cover a broad spectrum of edible phytoplankton species with different intrinsic growth rates. The species used were *Monoraphidium minutum* (Chlorophyceae), *Scenedesmus acuminatus* (Chlorophyceae), *Scenedesmus obliquus* (Chlorophyceae), *Chlamydomonas sphaeroides* (Chlorophyceae), *Cyclotella pseudostelligaria* (Bacillariophyceae) and *Rhodomonas minuta* (Cryptophyceae).

The results of all experiments indicated that all phytoplankton species benefited from both nocturnal grazing regimes (‘nocturnal’ and ‘enhanced nocturnal’). Even if the same amount of biomass was eliminated in ‘migration’ (‘enhanced nocturnal’) and ‘no-migration’ regimes, phytoplankton species had an enhanced biomass development in the ‘migration’ treatment. This might have been due to the fact that algae can grow undisturbed during the day and thus are able to produce a higher biomass. The results of the experiments with *D. magna* additionally showed a significant correlation between the intrinsic growth rate of an algae and the magnitude of the advantage it had from nocturnal grazing: the higher the intrinsic growth rate the more the advantage. This advantage might arise from the faster growth of these algae species during the time when no grazing occurs. This leads to the idea that the grazing regime itself (continuous / intermittent) can be responsible for the dominance of an alga species depending on its growth rate. Therefore I compared the ratios of the two algae species (that are present in each experiment) between the treatments. Results indicated, that the ratios differed, depending not only on the presence or absence of grazers (comparison of *Daphnia* treatments with control treatments) but also on the existing grazing pattern (comparison within the *Daphnia* treatments). For example in the experiment with *C. sphaeroides* and *S. obliquus*, *C. sphaeroides* was dominant under a ‘continuous’ grazing pattern but *S. obliquus* was dominant under an ‘enhanced nocturnal’ discontinuous grazing pattern. This emphasizes that the grazing pattern itself can lead to a shift in dominance.

The results of these experiments indicate that the grazing regime itself (continuous / intermittent) has a strong influence on phytoplankton dynamics, as predicted by theoretical models. The effects seen here were solely due to intermittent grazing. We can therefore imply that the effect of DVM on phytoplankton in lakes is not only due to less grazing of migrating daphnids (due to a lower zooplankton density) but also due to intermittent grazing.

PAPER 2

The impact of diel vertical migration of *Daphnia* on phytoplankton dynamics

(Reichwaldt and Stibor, submitted to Nature)

Diel vertical migration (DVM) of large zooplankton is a wide-spread behaviour in freshwater and marine pelagic ecosystems. The underlying mechanisms (Zaret and Suffern 1976; Stich and Lampert 1981; Gliwicz 1986; Neill 1990) and the consequences for the zooplankton (Dawidowicz and Loose 1992; Loose and Dawidowicz 1994) are well-known. As the zooplankton migrates downwards into the hypolimnion of a lake during the day and upwards into the epilimnion during the night, a huge amount of herbivorous biomass moves through the water column twice a day. This must have profound consequences for the phytoplankton in a lake, however, these consequences have never been investigated experimentally.

The phytoplankton in the epilimnion experiences different grazing pressures, depending on whether zooplankton migrates or not. In the absence of migration, the phytoplankton is grazed continuously in the epilimnion. In the presence of migration, the phytoplankton is only grazed during the night (intermittent grazing regime). Additional to a lower grazing pressure due to intermittent grazing, the phytoplankton also experiences less grazing due to usually lower densities of migrating zooplankton populations in stratified lakes (due to costs caused by the lower temperature in the hypolimnion). It is generally assumed that phytoplankton can benefit from DVM due to these two mechanisms in a way that phytoplankton biomass and the proportion of edible algae is enhanced if DVM is present (McAllister 1969; Petipa and Makarova 1969; Lampert 1986, 1987).

I conducted a mesocosm experiment in Lake Brunnsee (Germany) to compare the effects of a migrating *Daphnia hyalina* populations on phytoplankton dynamics with the effects of a non-migrating *Daphnia hyalina* population. Additionally, zooplankton life history parameters of migrating and non-migrating zooplankton were examined. Difficulties in testing the effects of DVM on food webs arise experimentally as it is difficult to induce DVM. The chemical composition of the fish kairomone is not exactly known and the described chemical that can induce DVM would have to be added in an amount that the carbon fixed in the kairomone would be higher than the carbon fixed in food (Boriss et al. 1999). On the other hand, DVM could be induced by fish swimming in a cage or in a separate tank to avoid predation on zooplankton (Loose 1993). However, the release of nitrogen and phosphorus of fish is high and will have direct effects on phytoplankton, making it difficult to separate the effects of fish-induced DVM and fish-released nutrients on phytoplankton. Therefore I developed a technique in which I mimicked DVM without addition of chemical cues or the presence of predators. I forced daphnids to migrate by confining them into cages (made out of gauze) that were either moved to the appropriate layers two times a day (‘migration’ treatment), or stayed continuously in the epilimnion (‘no-migration’ treatment) (Figure 3). Proper control treatments were conducted and showed that there were no differences in phytoplankton dynamics due to cage handling.

Population growth of *Daphnia hyalina* was higher in the ‘no-migration’ treatment than in the ’migration’ treatment. This can easily be explained by the lower temperature in the hypolimnion experienced only by migrating daphnids and which ultimately leads to their lower population growth.

Phytoplankton biomass (measured as chlorophyll-*a*) in the epilimnion was enhanced under a ‘migration’ regime of *Daphnia hyalina*. Additionally, edible algae benefited from a migrating zooplankton population. For example, biomass developments of *Cyclotella meneghiniana* (Bacillariophyceae) and *Monoraphidium minutum* (Chlorophyceae), the two most common edible algae species in this experiment were higher in the ’migration’ treatment than in the ’no-migration’ treatment. These results are also supported by the fact that the proportion of all edible seston (defined as all particles with a biovolume of <1000 µm^3 (Sommer et al. 2001)) was higher under a migration regime. Biomass developments of inedible pennate diatoms, such as *Asterionella formosa*, *Fragillaria crotonensis* and *Synedra* spec. (all Baccillariophyceae >60 µm) were enhanced in the ’no-migration’ treatment. This might have been caused by lower algal competition and consequently better growth conditions for those algae species in the ‘no-migration’ treatment because less edible algae were present. The results indicate that the phytoplankton community composition was different in the two treatments (‘migration’/’no-migration’) which could have been either the effect of a lower grazing pressure caused by intermittent grazing or the effect of less grazing caused by a lower zooplankton density. As I have already shown that intermittent grazing alone can have a strong effect on phytoplankton dynamics (Paper 1), I can assume that the effects seen here are at least to some part also caused by the discontinuous grazing pressure of the migrating zooplankton population.

These results give first experimental evidences that DVM of zooplankton can have strong effects on food web dynamics in a lake. Changes in phytoplankton dynamics not only involved total biomass of phytoplankton but also the structure of the phytoplankton community. As the largest part of the earth's primary production is bound to pelagic ecosystems (marine and freshwater) and DVM is also present in the marine ecosystem the effects seen here might influence the main part of the earth's plant biomass.

PAPER 3

The Effects of *Daphnia* Diel Vertical Migration on Phytoplankton Dynamics and their implications on *Daphnia* life history Parameters

(Reichwaldt, Wolf and Stibor, submitted to Limnology and Oceanography)

In the presence of hazards like planktivorous fish large zooplankton species or individuals perform a diel vertical migration (DVM). The zooplankton then only spends the night in the epilimnion of a lake but migrates downwards into the lower hypolimnion at the beginning of the day. In stratified lakes with migrating zooplankton populations phytoplankton communities in the epilimnion can benefit from DVM because of two mechanisms. Firstly, zooplankton abundance is usually lower in migrating than in non-migrating populations. This is due to the fact that zooplankton growth is largely temperature-dependent, and migrating zooplankton populations experience a lower temperature during the day in the hypolimnion than non-migrating populations. Secondly, migrating zooplankton populations only feed in the epilimnion during the night, causing an almost grazing-free period for the phytoplankton in the epilimnion during the day. Consequently grazing pressure is only intermittent for the phytoplankton if DVM is present and algae experience better growth conditions due to a period of more or less undisturbed growth in the absence of large grazers during the day.

In Paper 2 I already investigated the consequences of DVM of *Daphnia hyalina* on phytoplankton dynamics in mesocosm experiments with a natural temperature gradient, consequently assessing the combined impact of both mechanisms described above. In contrast to this, I here conducted a mesocosm experiment with a constant temperature over the water column in order to separate the effects of intermittent grazing from the impact of lower grazing pressure due to lower densities of migrating *Daphnia*. Similar to the mesocosm experiment described in Paper 2 I here compared effects of migrating and non-migrating *Daphnia hyalina* populations on phytoplankton dynamics and zooplankton life history parameters (using again cages to mimic DVM).

As I have already shown in Paper 1 that intermittent grazing alone could be responsible for changes in phytoplankton dynamics, I expected an enhanced phytoplankton biomass and a higher proportion of edible algae in the ‘migration’ treatments in the epilimnion. Additionally, as the interaction between *Daphnia* and phytoplankton is mutual, I also expected these

DVM-related changes in seston composition to have feedback effects on life history parameters of the daphnids. For this reason I conducted a laboratory experiment which ran parallel to the mesocosm experiment. In this laboratory experiment *Daphnia hyalina* was fed with seston from the mesocosms in a pattern that simulated the food conditions experienced by their counterparts in the field. Temperature and light conditions were constant in this experiment. I had three treatments: (1) daphnids were fed with food from the epilimnion of the 'no-migration' mesocosm treatment continuously, (2) daphnids were fed with food from the epilimnion of the 'migration' mesocosm treatment continuously, and (3) daphnids were fed with food from the epilimnion and hypolimnion of the 'migration' mesocosm treatment in an alternating way (Figure 4). By comparison of (1) and (2) I could directly assess the effects of a migrating zooplankton population in the mesocosm on food conditions and could decide whether these food conditions were advantageous or disadvantageous for daphnids. By comparison of (1) and (3) I could assess whether food conditions experienced by migrating daphnids were advantageous or disadvantageous compared to food conditions experienced by non-migrating daphnids.

Results of the mesocosm experiment showed that although temperature was almost constant over the whole water column, zooplankton population growth rate per day was higher in the 'no-migration' treatment than in the 'migration' treatment. I found that this could be attributed to a high mortality of juvenile daphnids in the 'migration' treatment. As a consequence I could not separate the effects of lower grazing due to intermittent grazing from the effects due to a lower density of daphnids in the migration treatment. However, this does not lessen the validity of the results concerning phytoplankton dynamics because both mechanisms also influence phytoplankton dynamics in lakes. For the same reason the requirements for the laboratory experiment (comparison of effects of 'migration' versus 'no-migration') were not violated.

In the mesocosm experiment total phytoplankton biomass (measured as chlorophyll-*a*) was enhanced under a 'migration' regime. Additionally, results indicated that edible algae in the epilimnion benefited from a nocturnal grazing regime. This was due to the fact that the proportion of edible seston (all particles <1000µm³ (Sommer et al. 2001)) and the biomass development of the most common edible algae (*Cyclotella meneghiniana*) were both higher in the 'migration' treatment than in the 'no-migration' treatment. These results are similar to those achieved in the mesocosm experiment described in Paper 2 and again support the idea that the two different migration regimes (‘migration’ / ‘no-migration’) have strong, different effects on phytoplankton dynamics.

The results of the laboratory experiment showed that the differences in phytoplankton composition caused by the different grazing regimes in the mesocosms (higher phytoplankton biomass / more edible algae) had strong effects on life history parameters of zooplankton. Firstly, I showed that migration in the mesocosms led to food conditions that were advantageous for daphnids (comparison of laboratory treatments 1 and 2). This was due to the fact that AFR was lower, and somatic growth rate and number of offspring were higher when daphnids were fed with food from the epilimnion of the ‘migration’ treatment instead of with food from the epilimnion of the ‘no-migration’ treatment. This could be connected to more and/or better food in the epilimnia of these mesocosms which was the result of migration. This emphasizes that migration of large *Daphnia* can not only lead to favourable food conditions for juvenile daphnids which usually stay I the epilimnion continuously as long as they are small (Lampert 1992), but also for other zooplankton species that do not perform DVM (e.g. ciliates, rotifer). Secondly, I showed that daphnids fed with food mimicking migration (laboratory treatment 3) also had a lower AFR, and higher somatic growth rates and numbers of offspring than daphnids fed with food mimicking no-migration (laboratory treatment 1). On the one hand, this could again be connected to more and/or better food in the epilimnion (caused by migration in the mesocosms). On the other hand this could also be connected to more food in the hypolimnia as I observed deep-chlorophyll maxima (DCM) in the mesocosms. I could therefore show that food conditions are not always worse for migrating zooplankton which is consistent with studies from Winder et al. (2003) and Williamson et al. (1996).

The results of the two experiments emphasize the strong consequences of DVM on phytoplankton dynamics because migration of *Daphnia* enhanced phytoplankton biomass and favoured edible algal species in the epilimnion. The different phytoplankton community compositions which were the result of the different grazing regimes had also substantially different effects on several life history parameters of *Daphnia*. These results emphasize the mutual character of zooplankton and phytoplankton interactions.

PAPER 4

Effects of a fluctuating temperature regime experienced by *Daphnia* during diel vertical migration on *Daphnia* life history parameters

(Reichwaldt, Wolf and Stibor, submitted to Hydrobiologia)

In stratified lakes daphnids usually cross the thermocline twice a day if performing diel vertical migration (DVM). Therefore they regularly experience strong differences in temperature between day and night. As most biological processes are temperature-dependent, temperature is one of the most important factors influencing zooplankton dynamics. There are many studies which examined the influence of constant different temperatures on life history parameters of *Daphnia* and results were unanimously similar: growth rate increased, whereas age at first reproduction and egg development time decreased with increasing temperature (at least within a reasonable temperature range) (Orcutt and Porter 1983; Orcutt and Porter 1984; Sakwinska 1998; Giebelhausen and Lampert 2001). Thus, temperature is only sub-optimal for migrating daphnids in the hypolimnion during the day. However, migrating zooplankton populations not only experience a low, but also a regular change in temperature twice a day due to the temperature gradient in stratified lakes. In this context, the question then arises whether migrating *Daphnia* populations have costs because of this diurnal regular change in temperature (e.g. costs for regularly re-adjusting metabolic rates) in addition to the costs they have due to a lower temperature in the hypolimnion.

I conducted a laboratory experiment to assess the effects of a regularly changing temperature regime on life history parameters of *Daphnia hyalina* and *Daphnia magna* under non-limiting food conditions. In this experiment the daphnids were exposed either to a permanently warm (19°C), a permanently cold (12°C), or a regularly changing temperature regime (19°C/12°C for 12h/12h). This experiment ran parallel to the extensive mesocosm study described in Paper 2. The temperatures applied in this laboratory experiment were therefore similar to those in Lake Brunnsee. The life history parameters measured were somatic growth rate, age at first reproduction (AFR), egg development time and number of offspring. To assess whether migrating daphnids have costs due to a fluctuating temperature regime, I compared the calculated mean of the values of a life history parameter in the warm and cold temperature regimes with the value of this life history parameter in the fluctuating temperature regime. In this experiment I expected the life history parameters in the fluctuating temperature regime to be exactly halfway between the values at warm and cold temperature because *Daphnia* spent half of the time in warm, and the other half of the time in cold water. However, if the values of the parameters were lower in the fluctuating temperature regime than expected from the calculated mean this would suggest costs of a fluctuating temperature regime.

The results of my experiments showed that AFR and egg development time decreased with increasing temperature, and somatic growth rate increased with increasing temperature in both *Daphnia* species. Number of offspring did not depend on temperature and was similar in all treatments which is in accordance with the study of Giebelhausen and Lampert (2001) which showed that the number of offspring depends on food conditions mainly. In a regularly changing temperature regime AFR and egg development time were exactly intermediate to high and low temperature. Therefore, fluctuations in temperature did not imply any additional costs for these parameters. However, somatic growth rate of both species was significantly lower in the regularly changing temperature regime than expected from the calculated mean. In *D. hyalina* somatic growth rate in the fluctuating temperature was even as low as at constant cold temperature, which points to high costs of fluctuating temperatures experienced by migrating daphnids. This might be due to the fact that physiological adaptations of internal processes to prevailing temperatures are slow so that the daphnids experience an only sub-optimal metabolism during the time until processes are adjusted properly.

The results indicate that *Daphnia* not only have costs due to a lower temperature in the hypolimnion during the day but also have additional costs due to a regular change in temperature twice a day. Therefore daphnids should minimize both the time they spend in the hypolimnion and the amplitude of migration in stratified lakes to avoid costs which would reduce population growth.

Synopsis

Diel vertical migration represents a trait-mediated indirect interaction between fish, zooplankton and phytoplankton in the trophic cascade of pelagic zones of waters. The experiments described here give a first impression of the importance of this behavioural change in the context of food web ecology.

I first want to briefly address the experimental design of my mesocosm experiments. I used cages to force *Daphnia* to migrate in the mesocosms. Although the relatively high mortality rate of juvenile *Daphnia* in the 'migration' treatment is somewhat problematic, I do believe that these cages provide a good method for mimicking DVM without the use of kairomones or fish, because chemical and biological parameters (e.g. total biovolume, chlorophyll-*a*, proportion of edible algae) never differed between inside and outside the cages. However, I think this method is not suitable for extraordinarily long experiments or experiments in eutrophic lakes as mesh openings become overgrown with benthic algae after some time.

The results of my experiments produced a consistent picture of the effects of DVM of *Daphnia* on phytoplankton dynamics: compared to situations with non-migrating *Daphnia* algae generally had an advantage from DVM and could achieve an enhanced biomass development under a 'migration' regime. Edible species had by far the highest advantage from migration and thus were responsible for the main increase in total biomass. Among the edible algal species those species with the highest intrinsic growth rates had the highest advantage from migration, resulting in different phytoplankton community compositions under different migration regimes. These results are consistent with theoretical predictions (Lampert 1986; 1987) and might have been due to the fact that the phytoplankton species can grow undisturbed during daylight in the presence of a discontinuous grazing pattern. They can thus increase their standing stock without mortality due to grazing during the day. In the laboratory experiment these results were solely an effect of intermittent grazing and not an effect of a lower grazing pressure due to a lower *Daphnia* density in the 'migration' treatment. However, in the field experiments there was no possibility to separate these two effects. I can therefore only rely on the results of the laboratory experiment to assume that the effects in the mesocosms were, at least partly, also an effect of intermittent grazing. Still, I think that in my field experiments a large part of the effects was probably due to a lower *Daphnia* density in the migration treatment. A lower *Daphnia* density led to a lower overall grazing pressure and to higher phytoplankton biomass growth due to the strong association between *Daphnia* density and algal community dynamics (Svensson and Stenson 1991).

In all experiments different seston compositions arose from the different grazing pressure regimes that were generated by migrating and non-migrating daphnids. Such differences in seston composition should feed back on the zooplankton population itself due to the mutual character of the interaction between *Daphnia* and its food. I therefore also assessed the feedback effects of the different seston compositions (caused by the two migration regimes) on zooplankton life history parameters in the laboratory (Paper 3). In my experiments migrating daphnids experienced a higher mean amount of food, and at the same time food of a better quality due to a higher proportion of edible algae, which was both an effect of DVM in the mesocosms. However, data suggested that the higher mean amount of food experienced by the migrating daphnids was also due to a deep-chlorophyll maximum in the hypolimnia of the mesocosms. In any case, the food conditions experienced by migrating daphnids were advantageous in that they increased somatic growth rate and number of offspring, and decreased age at first reproduction. All these parameters generally influence fitness and population growth rates of *Daphnia*, so that food conditions under a ‘migration’ regime should have a positive effect on *Daphnia* population growth in lakes. These results are consistent with findings of Williamson et al. (1996) and Winder et al. (2003) who found that food is not always worse for migrating zooplankton due to deep-chlorophyll-maxima in lakes. However, negative effects of low temperature in the hypolimnion of stratified lakes (Orcutt and Porter 1984; Dawidowicz and Loose 1992; Loose and Dawidowicz 1994) and the movement of migrating daphnids through a range of temperatures during a 24 hour period might overrule these positive effects of food in lakes. I addressed the temperature aspect in a laboratory experiment in which I assessed the costs of daphnids being exposed to a regularly changing temperature regime that mimicked conditions experienced by migrating daphnids in a stratified lake. My results indicate that daphnids might have a larger disadvantage from migration than previously thought because somatic growth rate under a regularly changing temperature regime was as low as at a constant low temperature. Possibly the process of re-adjusting metabolic rates to a prevailing temperature twice a day is slow and metabolism might therefore be sub-optimal for the daphnids for some time. This might then imply costs for migrating daphnids, which are subject to a regular change in temperature twice a day.

Application of continuous and nocturnal grazing as alternatives (as was done in my experiments) to study the effects of different grazing regimes on phytoplankton, is artificial as in lakes both types of grazers might be present simultaneously. The possibly most prominent example for this is Lake Constance in which non-migrating *Daphnia galeata* populations coexist with migrating *Daphnia hyalina* populations (Stich and Lampert 1981). But even in lakes with only migrating *Daphnia* species there are still many other grazers that usually do not migrate (e.g. ciliates, rotifer, nauplia, juvenile *Daphnia*). It seems likely that the strength of the effects of DVM on phytoplankton might depend on the diversity of the zooplankton fauna in a lake because the species and their developmental stages that constitute the zooplankton community may use different size ranges of food particles and may exhibit different migration patterns. Consequently, the effects of DVM might be less pronounced in lakes with a highly diverse zooplankton fauna and might also vary seasonally.

Peacor and Werner (2001) suggested that the effects of trait-mediated indirect interactions might be greater than one expects because density-mediated and trait-mediated indirect interactions can have effects of the same size. This might especially be true for induced behavioural changes as these changes are often immediate and affect the whole population. For example Schmitz et al. (1997) evaluated the relative strength of density-mediated and trait-mediated interactions in a field experiment composed of plants, herbivorous grasshoppers, and predacious spiders. They used two types of spiders: spiders which could actively decrease the density of grasshoppers by predation, and spiders that had their chelicerae glued so that they only affected the behaviour of the grasshoppers (antipredator behaviour of the grasshoppers because the grasshoppers could not distinguish between the two types of spiders). The authors showed that the increase of both types of spiders led to an increase in plant biomass and consequently showed the strong indirect effect of spiders on plants. As the effect of both types of spiders was similar in size Schmitz et al. concluded that indirect effects resulting from antipredator behaviour could produce trophic-level effects that are similar in form and strength to those generated by direct predation events.

The results of my study suggest that DVM as a trait-mediated interaction might be a very important feature in the food web of lakes as I could demonstrate strong effects of this behaviour on the zooplankton-phytoplankton interaction. I found a change in total phytoplankton biomass and in the composition of the phytoplankton community at the same time which had both strong feedback effects on the *Daphnia* itself. Effects within the trophic cascades do not always include changes in the total biomass as well as changes in the structure of a community composition. For example Tessier and Woodruff (2002) showed that changes in a fish community did not lead to a higher amount of phytoplankton biomass in their study but only to a change in the phytoplankton community composition. This was due to the fact that the changes in fish community composition altered the community structure and consequently also the size structure of the zooplankton level.

In the discussion about the effects of DVM of daphnids on phytoplankton dynamics one should not forget that each *Daphnia* population consists of many individuals and that phytoplankton dynamics in lakes are ultimately affected by processes of individual behaviour. Each animal will show an individual reaction to trigger substances and will - depending on its needs - search for its optimal environment. Studies indicated that swimming costs are almost non-existent in daphnids (Lampert et al. 1988) so that the vertical position of *Daphnia* individuals will only depend on their individual needs and on the environmental conditions.

This study indirectly emphasizes the cascading effect of carnivores (fish) on plants (phytoplankton) mediated by herbivores in the pelagic food web of a lake. It indicates that cascading effects can occur that are not mediated by mortality of daphnids by predation as the effect of DVM on phytoplankton is a behavioural one. My results suggest that in lakes the presence of kairomones might contribute to a reduction in the impact of zooplankton grazing on algal communities by changing zooplankton behaviour and, thus, also its feeding and growth rates which might lead to functional variations in grazer-resource couplings.

Future research

‘There is still much to be done, let’s get started!’

Although diel vertical migration is one of the best examined ecological phenomena in the world, this sentence is still valid for research in that field.

A process which is still little understood is the dynamics of nutrient recycling by zooplankton in combination with diel vertical migration. Nutrient recycling is important for phytoplankton especially in oligotrophic lakes because the amount of available nutrients is an important factor influencing phytoplankton population growth. It is obvious that the direction of the gross nutrient transport by migrating zooplankton depends on the nutritive value of the food in the epilimnion and hypolimnion of a lake. For example in a lake with low food densities in the hypolimnion, DVM can lead to a reduction of available nutrients for the phytoplankton in the epilimnion due to the fact that the zooplankton population transports more nutrients from the epilimnion into the hypolimnion than vice versa. On the other hand, recent research has drawn attention to deep-chlorophyll maxima which would ultimately lead to an opposite effect as described above (Williamson et al. 1996, Winder et al. 2003; the present study). The interactions between DVM, nutrient recycling, and deep-chlorophyll maxima is still a broad field to be worked on.

Besides phytoplankton, bacteria are a further food source used by daphnids. *Daphnia*-mediated predatory interactions are known to cascade down to the bacterial level (Vaque and Pace 1992; Degans et al. 2002; Zöllner et al. 2003). However, nothing is known about the impact of DVM on bacterioplankton. This would represent a demanding subject to study because bacteria play an important role in freshwater ecosystems. For example it is known that the largest part of inorganic phosphorus (P) is bound in bacterial biomass and that the turnover rate of P therefore strongly depends on the dynamics of bacteria (Vadstein 2000).

We additionally need more quantitative information about the effects of changes in phytoplankton communities on zooplankton production. For example changes in total biomass as well as shifts in the proportion of edible algae would affect fish production, and consequently also humans. Furthermore, DVM could possibly lead to better food conditions in the epilimnion for those organisms that do not migrate (e.g. other *Daphnia* species, ciliates) and it would be therefore interesting to study the indirect effects of DVM on other zooplankton species mediated by the phytoplankton.

Future work must determine whether the short-time effects of this trait-mediated interaction observed in my study influence long-term community dynamics such as seasonal successions of phytoplankton species in lakes as well.

Finally, daphnids are not the only zooplankton species performing DVM. Experiments were conducted with daphnids here because they are easy to handle, and they are the most common zooplankton species in lakes. But thinking of the large marine ecosystem one should not forget that copepods, which are the predominant zooplankton species in oceans, do also perform DVM. As they are not filter feeders like daphnids but feed selectively on larger phytoplankton species, the effect of DVM of copepods on phytoplankton might be different from the effect of migrating daphnids. With the growing use of mesocosm experiments in the marine system this should also be a fruitful research area in marine zooplankton ecology.

References

Abrams, P. A. 1995. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. Am. Nat. **146:** 112-134.

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

Boriss, H., M. Boersma, and K. H. Wiltshire. 1999. Timehylamine induces migration of waterfleas. Nature **398:** 382-382.

Bottrell, H. H. 1975. The relationship between temperature and duration of egg development in some epiphytic Cladocera and Copepoda from the river Thames, Reading, with a discussion of temperature functions. Oecologia **18:** 63-84.

Dawidowicz, P. and C. J. Loose. 1992. Metabolic costs during predator-induced diel vertical migration of *Daphnia.* Limnol. Oceanogr. **37:** 1589-1595.

Degans, H., E. Zöllner, K. Van der Gucht, L. De Meester, and K. Jürgens. 2002. Rapid Daphnia-mediated changes in microbial community structure: an experimental study. FEMS Microbiology Ecology **42:** 137-149.

Dodson, S. 1988. The ecological role of chemical stimuli for the zooplankton: Predator-avoidance behavior in *Daphnia*. Limnol. Oceanogr. **33:** 1431-1439.

Duncan, A., C. Guisande, and W. Lampert. 1993. Further trade-offs in *Daphnia* vertical migration strategies. Arch. Hydrobiol. Beih. Ergebn. Limnol. **39:** 99-108.

Enright, J. T. and W. M. Hamner. 1967. Vertical diurnal migration and endogenous rhythmicity. Science **157:** 937-941.

Giebelhausen, B. and W. Lampert. 2001. Temperature reaction norms of Daphnia magna: the effect of food concentration. Freshwater Biology **46:** 281-289.

Gliwicz, Z. M. 1986. Predation and the evolution of vertical migration in zooplankton. Nature **320:** 746-748.

Lampert, W. 1986. Limnologie: funktionelle Beziehungen in Ökosystemen; Kaskadeneffekte in See-Ökosystemen: die Bedeutung der tagesperiodischen Vertikalwanderung des Zooplankton. Verh. Dtsch. Zool. Gesell. **79:** 105-116.

Lampert, W. 1987. Vertical migration of freshwater zooplankton: Indirect effects of vertebrate predators on algal communities., p. 291-298. *In* [eds.] Kerfoot, W. C. and A. Sih, Predation: direct and indirect impacts on aquatic communities. University press of England.

Lampert, W. 1992. Zooplankton vertical migrations: Implications for phytoplankton-zooplankton interactions. Arch. Hydrobiol. Beih. Ergebn. Limnol. **35:** 69-78.

Lampert, W. 1993. Ultimate causes of diel vertical migration of zooplankton: New evidence for the predator-avoidance hypothesis. Arch. Hydrobiol. **39:** 79-88.

Lampert, W., R.-D. Schmitt, and P. Muck. 1988. Vertical migration of freshwater zooplankton: test of some hypotheses predicting a metabolic advantage. B. Mar. Sci. **43:** 620-640.

Loose, C. 1993. *Daphnia* diel vertical migration behaviour: Response to vertebrate predator abundance. Arch. Hydrobiol. Beih. Ergebn. Limnol. **39:** 29-36.

Loose, C. and P. Dawidowicz. 1994. Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. Ecology **75:** 2255-2263.

McAllister, C. D. 1969. Aspects of estimating zooplankton production from phytoplankton production. J. Fish. Res. Bd. Can. **26:** 199-220.

Neill, W. E. 1990. Induced vertical migration in copepods as a defence against invertebrate predation. Nature **345:** 524-526.

Orcutt, J. D. and K. G. Porter. 1983. Diel vertical migration by zooplankton: Constant and fluctuating temperature effects on life history parameters of *Daphnia*. Limnol. Oceanogr. **28:** 720-730.

Orcutt, J. D. and K. G. Porter. 1984. The synergistic effects of temperature and food concentration on life history parameters of *Daphnia*. Oecologia **63:** 300-306.

Peacor, S. D. and E. E. Werner. 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. Proc. Natl. Acad. Sci. USA **98:** 3904-3908.

Petipa, T. S. and N. P. Makarova. 1969. Dependence of phytoplankton production on rhythm and rate of elimination. Mar. Biol. **3:** 191-195.

Ringelberg, J. 1993. Phototaxis as a behavioural component of diel vertical migration in a pelagic *Daphnia*. Arch. Hydrobiol. Beih. Ergebn. Limnol. **39:** 45-55.

Sakwinska, O. 1998. Plasticity of *Daphnia magna* life history traits in response to temperature and information about a predator. Freshwater Biology **39:** 681-687.

Schmitz, O. J., V. Krivan, and O. Ovadia. 2004. Trophic-cascades: the primacy of trait-mediated indirect interactions. Ecol. Lett. **7:** 153-163.

Schmitz, O. J., A. P. Beckerman, and K. M. O'Brien. 1997. Behaviorally mediated trophic cascades: Effects of predation risk on food web interactions. Ecology **78:** 1388-1399.

Schmitz, O. J., P. A. Hambäck, and A. P. Beckerman. 2000. Trophic cascades in terrestrial systems: A review of the effects of carnivore removals on plants. Am. Nat. **155:** 141-153.

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

Stich, H.-B. and W. Lampert. 1981. Predator evasion as an explanation of diurnal vertical migration by zooplankton. Nature **293:** 396-398.

Stich, H.-B. and W. Lampert. 1984. Growth and reproduction of migrating and non-migrating *Daphnia* species under simulated food and temperature conditions of diurnal vertical migration. Oecologia **61:** 192-196.

Svensson, J.-E. and J. A. E. Stenson. 1991. Herbivoran impact on phytoplankton community structure. Hydrobiologia **226:** 71-80.

Tessier, A. J. and P. Woodruff. 2002. Cryptic trophic cascade along a gradient of lake size. Ecology **83:** 1263-1270.

Trussell, G. C., P. J. Ewanchuk, and M. D. Bertness. 2002. Field evidence of trait-mediated indirect interactions in a rocky intertidal food web. Ecol. Lett. **5:** 241-245.

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

Vaque, D. and M. L. Pace. 1992. Grazing on bacteria by flagellates and cladocerans in lakes of contrasting food-web structure. J. Plankton Res. **14:** 307-321.

Williamson, C. E., R. W. Sanders, R. E. Moeller, and P. L. Stutzman. 1996. Utilization of subsurface food resources for zooplankton reproduction: Implications for diel vertical migration theory. Limnol. Oceanogr. **41:** 224-233.

Winder, M., M. Boersma, and P. Spaak. 2003. On the cost of vertical migration: are feeding conditions really worse at greater depths? Freshwater Biology **48:** 383-393.

Zaret, T. M. and J. S. Suffern. 1976. Vertical migration in zooplankton as a predator avoidance mechanism. Limnol. Oceanogr. **21:** 804-813.

Zöllner, E., B. Santer, M. Boersma, H. G. Hoppe, and K. Jürgens. 2003. Cascading predation effects of Daphnia and copepods on microbial food web components. Freshwater Biology **48:** 2174-2193

Paper 1

The effect of different zooplankton grazing patterns resulting from diel vertical migration on phytoplankton growth and composition: a laboratory experiment

(Reichwaldt, Wolf and Stibor, Oecologia in revision)

The effect of different zooplankton grazing patterns resulting from diel vertical migration on phytoplankton growth and composition: a laboratory experiment

Elke S. Reichwaldt *, Isabelle D. Wolf, Herwig Stibor

Ludwig-Maximilians Universität, Department Biologie II, Aquatische Ökologie,
Karlstr. 25, D-80333 München, Germany

* Correspondence author
address: Ludwig-Maximilians Universität, Department Biologie II, Abteilung
Aquatische Ökologie, Karlstr. 25, D-80333 München, Germany
e-mail: ereichwaldt@web.de
fax number: +49-89-5902-461

Abstract

Diel vertical migration (DVM) of herbivorous zooplankton is a widespread behavioral phenomenon in freshwater ecosystems. So far only little attention has been paid to the migration behavior’s impact on the phytoplankton community in the epilimnion. Some theoretical models predict that algal population growth in the epilimnion should depend on the herbivore’s migration and grazing patterns: Even if migrating zooplankton consume the same total amount of algae per day in the epilimnion as non-migrating zooplankton, nocturnal grazing should result in enhanced algal growth and favor algal species with high intrinsic growth rates over species with lower intrinsic growth rates. To test these hypotheses we performed experiments in which several algal species were confronted with different feeding regimes of *Daphnia*. In the experiments algal growth did not only depend on the absolute time of grazing but was comparatively higher when grazing took place only during the night, even when the grazing pressure was the same. Furthermore, algae species with higher intrinsic growth rates had higher advantages when being grazed upon only in a discontinuous way during the night than algae species with a smaller intrinsic growth rate. The grazing pattern itself was an important factor for relative algal performance.

Key words: *Daphnia*, DVM, grazing rate, direct effects, algal performance

Introduction

Herbivore-plant interactions are one of the most important factors influencing the mutual coexistence of different species in an ecosystem. Certainly this interaction has strong effects on the lower trophic levels of the trophic cascade. The habitat choice of the herbivore, which is often mediated by an antipredator behavior against its own predator, can be substantial for the plants‘ productivity (Turner and Mittelbach 1990; McIntosh and Townsend 1996; Schmitz et al. 1997; Diehl et al. 2000). But besides this spatial scale, the temporal scale also plays an important role considering the effects of herbivores on their prey. It can generally be seen, that herbivores only forage during part of the day either to avoid their own predators or to avoid such external factors as e.g. unfavorable temperatures. In such a case, if the generation time of the prey (= plant) species is sufficiently short, such a diel habitat choice of the herbivore has substantial advantages for the prey species. Prey species with shorter generation times might then be competitively favored by a diel habitat choice because they can multiply faster during the time when no predation occurs, as species with a slower generation time. One system in which an antipredator mediated behavioral diel habitat choice takes frequently place is the generally well-studied system of diel vertical migration of zooplankton in lakes. Here the generation times of the prey (= phytoplankton) is not only short but also very variable. Thus, the effect of different diel habitat choice on the lower trophic level can be investigated.

Diel vertical migration of herbivorous zooplankton is a widespread phenomenon in freshwater ecosystems. The proximate and ultimate causes are thought to be known very well (Zaret and Suffern 1976; Stich and Lampert 1981; Gliwicz 1986; Neill 1990; Ringelberg 1991; Lampert 1993; Ringelberg 1993). Thus, change in light intensity is considered as the proximate cause and predator avoidance is considered as the ultimate cause of this behavior. According to the predator avoidance theory, large zooplankton individuals migrate downwards into the darker hypolimnion during the day to reduce the probability of an attack by their visual-orientated predators. However during the night when it cannot be seen by its predators, the zooplankton feeds within the epilimnion where temperature is higher and food is considered to be more abundant (Lampert 1986; 1987).

A lot of research has already been done on the effect of DVM on the zooplankton itself (Orcutt and Porter 1984; Stich and Lampert 1984; Dawidowicz and Loose 1992; Loose and Dawidowicz 1994; McKee and Ebert 1996; Giebelhausen and Lampert 2001; Hülsmann 2001). However, much less attention has been paid to the migration's impact on other components of the planktonic food web, e.g., the phytoplankton (but see Lampert 1992). Vertical migration leads to changes in the actual biomass of the zooplankton in the epilimnetic layer with a regular period (Geller et al. 1992). Resulting from this, the lower components of the food web in the epilimnion experience different zooplankton grazing patterns. If no migration takes place, the phytoplankton in the epilimnetic layer is confronted with a continuous grazing pressure. However, if zooplankton migrates, phytoplankton can grow uninfluenced during the day and is only faced with a nocturnal (discontinuous) grazing pressure. Because zooplankton, in particular daphnids, can have a strong effect on phytoplankton biomass by its community grazing pressure (Sarnelle 1992), it is important to get a deeper insight into the effects different grazing regimes can have.

It is not yet clear, if zooplankton increase their per capita grazing rate while grazing in the epilimnion during the night. The results of studies performed on this subject are to some extent contradictory. Thus Duval and Geen (1976) found, that *Daphnia* showed higher filtration rates at night. Haney (1973) observed no higher community filtration rate of an entire zooplankton community on one occasion but a significant higher rate on a second date. He explained this with the different species composition of the two zooplankton communities. In later studies Haney and Hall (1975) and Haney (1985) found that the per capita filtration rate of *Daphnia* was higher during its grazing time within the epilimnion during the night and that the increase of the filtration rate was size-dependent. On the other hand Gulati et al. (1982) and Lampert and Taylor (1985) showed that higher community grazing rates could be linked with higher total biomass of daphnids in the epilimnion during the night and found that filtration rates per unit biomass were the same during day and night. The latter could also be observed by Geller et al. (1992). However, in all above described possibilities the grazing pressure experienced by the phytoplankton in the epilimnion is different between day and night.

Some early models documented that algal growth in the epilimnion depends on the herbivores' grazing pattern. Even if a migrating zooplankton community increases its feeding rate and consumes the same total amount of algae per day as a non-migrating community, nocturnal grazing will result in enhanced algal growth (McAllister 1969; Petipa and Makarova 1969; Gabriel in Lampert 1986, 1987). This might be due to the fact, that when migration takes place algae are not disturbed during their period of photosynthesis but are grazed only in a discontinuous way. The models of Gabriel (in Lampert 1986, 1987) and McAllister (1969) also predict that, depending on their growth rate, different algae species can have different advantages from a diel grazing pattern. They predict, that fast-growing algae benefit more from a (discontinuous) nocturnal grazing pattern than slow-growing algae. This might be due to the fact that fast growing algae can produce a higher biomass during the day than slow growing algae. If then the same amount of algae is grazed by the zooplankton from these two algae populations in the night, the proportion of grazed algae biomass to total biomass will be lower for the fast growing algae. Therefore the faster development of population biomass of these algae during the time when no grazing occurs is a probable explanation for this phenomenon. All these models consider a system with one algae which is confronted with grazing. A further step towards field situations originate if one imagines a system where two algae species which are not grazed selectively are exposed to grazing simultaneously. Then, different algae species with distinct growth rates potentially can have different advantages depending on the grazing pattern of the zooplankton. Thus, shifts in the relative performance of a species can be mediated through the antipredator behavior of a herbivore as already shown in other predator-prey systems (Power et al. 1989; Persson 1993; Diehl and Eklöv 1995; McIntosh and Townsend 1996; Schmitz et al. 1997; Turner et al. 1999; Trussell et al. 2003).

We performed experiments in which several algae species were confronted with different daphnid feeding regimes. The effect of diel vertical migration on algae could then be estimated by comparing algal growth for the continuous and the two possible nocturnal grazing regimes. Furthermore the hypothesis of Gabriel (in Lampert 1986, 1987) and McAllister (1969) that fast-growing algae benefit more from a nocturnal grazing pattern than slow-growing algae was tested with the data set of these experiments. Additionally it was analyzed whether the relative competitive performance of different algae species changed with the grazing patterns.

Materials and Methods

To investigate the effects of different grazing patterns of daphnids on the growth dynamic of freshwater algae we carried out laboratory experiments with 6 species of algae [*Monoraphidium minutum* (Chlorophyceae), *Scenedesmus acuminatus* (Chlorophyceae), *Scenedesmus obliquus* (Chlorophyceae), *Chlamydomonas* *sphaeroides* (Chlorophyceae), *Cyclotella pseudostelligaria* (Bacillariophyceae) and *Rhodomonas minuta* (Cryptophyceae)] and two species of daphnids (*Daphnia magna* and *Daphnia hyalina*). We received the algae from the Göttinger Algensammlung prior to the experiment and cultured them in batch cultures. All of the algal species are known to provide good food for the daphnids and are not grazed selectively. Prior to each experiment both *Daphnia* species, which had been cultured in the laboratory for more than two years, were adapted to the experimental light and temperature conditions. The daphnids used in the experiments were individuals of the second clutch of synchronized females. They were put into the experiments within one day after they had released their first brood.

The experiments were carried out in batch culture in two-liter plastic beakers in a climatic chamber at a constant temperature of 18°C and with fluorescent tubes (Osram L 36W/21-840 ‘Lumilux cool white’ and Osram L 36W/77 ‘Fluora’) as light sources (40 $\mu E\ m^{-2}\ s^{-1}$). Each beaker held a volume of one liter of medium. This medium consisted of 0.45 µm-filtered water from the mesotrophic Lake Langbürgener See (Bavaria, Germany) enriched with nitrogen (16 $\mu mol\ N\ l^{-1}$), phosphorous (1 $\mu mol\ P\ l^{-1}$), a mix of vitamins, and a mix of trace elements. In trials with *C. pseudostelligaria* we also added silica (8 $\mu mol\ Si\ l^{-1}$). In experiment 1 no vitamins and micronutrients were added.

Each beaker contained a cage made almost completely out of gauze with a mesh size of 250 µm. While algae could move freely through the cage walls, daphnids (when present) were always kept inside the cage. The cage was, however, tight-fitting in the beaker so that approximately 90% of the medium in the beaker was accessible to the daphnids.

Altogether we performed five experiments in which five different pairwise combinations of the above algal species were grown under different grazing regimes. The basic design was identical in all five experiments. It consisted of eight different treatments, each replicated four times: two different control treatments (‘continuous’ and ‘nocturnal’), two ‘continuous grazing’ treatments (13 *D. magna* or 13 *D. hyalina* grazing day and night), two ‘ordinary nocturnal grazing’ treatments (13 *D. magna* or 13 *D. hyalina* grazing only during the night) and two ‘enhanced nocturnal grazing’ treatment (26 *D. magna* or 26 *D. hyalina* grazing only during the night). The beakers were intended to mimic the epilimnion of a lake. Thus, the ‘continuous grazing’ treatments should match a system where daphnids do not migrate. The ‘ordinary nocturnal grazing’ treatments mimicked a system where daphnids migrate but do not increase their filtration rate when feeding in the epilimnion at night. The ‘enhanced nocturnal grazing’ treatments mimicked the situation, where daphnids migrate and double their filtration rate at night. To simulate the ‘ordinary nocturnal grazing’ and ‘enhanced nocturnal grazing’ regimes we removed cages with *Daphnia* from the experimental beakers at the beginning of each light cycle, replaced them with identical empty cages, and held the cages with *Daphnia* in separate, ‘intermediate’ beakers containing the same medium and algae concentration as the experimental beakers. At the beginning of each dark cycle, cages with *Daphnia* were put back into the experimental beakers and the empty cages were returned to the intermediate beakers. The same beaker exchange procedure was also applied to the ‘nocturnal control’ cages. Finally, to control for mechanical effects of cage transfer (e.g., resuspension of sedimented algae) we also removed twice a day the cages from the ‘continuous control’ and ‘continuous grazing’ treatments but returned them immediately to the experiment beakers.

The experiments lasted for 13-15 days and were run at a light : dark cycle of 12 h : 12 h except for experiment 1 where the cycle was 16 h light : 8 h dark. Thus, with the exception of exp. 1, the community filtration rate of the daphnids, averaged over 24 h, should have been roughly the same in the ‘continuous grazing’ and the ‘enhanced nocturnal grazing’ treatments (13 daphnids grazing 24 h vs. 26 daphnids grazing for 12 h). The experiment was started by stocking the experimental and intermediate beakers with two different algae, each at a density equivalent to 0.5 mg C l^{-1}. The algae were then given 12 h to grow undisturbed before the first samples were taken. Immediately after that the daphnids where put either into the experimental beakers (continuous treatments) or into the intermediate beakers (nocturnal treatments). During each experiment daphnids were checked once a day, dead animals were replaced, and juveniles were removed.

We sampled the beakers every third day on midday with a flexible tube, which allowed continuous sampling across the water column. Water samples were fixed with Lugol’s iodine (5g I_2 + 10 g KI ad 20 ml aq. dest and 50 ml 10% acetic acid). We estimated algal densities from microscopic counts using Utermöhl chambers and an inverse microscope (Leitz Labovert) (Utermöhl 1958). If present at least 400 cells of each species was counted to keep estimation error <10% (Lund et al. 1958).

We expressed the performance of each algal species as the natural logarithm of its relative population change over the experimental duration using the equation:

$$(1) \qquad \text{relative algal growth} = \ln\left(\frac{N_f}{N_0}\right)$$

where N_f and N_0 are the final and initial densities ml^{-1} of algae. We analyzed treatment effects on algal performance statistically with ANOVA. We first ran separate ANOVAs across the grazing treatments with *D. magna* and *D. hyalina*, respectively, including the two control treatments in both analyses. We then analyzed the statistical significance of differences between treatment pairs with contrasts.

We also calculated the intrinsic growth rate (μ) of each algal species from the initial, exponential growth phase in the control treatments using the equation:

$$(2) \qquad \mu = \frac{(\ln N_t - \ln N_0)}{t - t_0}$$

where N_0 and N_t are the densities ml^{-1} in the beginning (t_0 = day O) and after t = 2-6 days (depending on the length of the exponential growth phase), respectively. This value reflects the maximum possible growth rate of each algal population in the presence of a second species and under the experimental conditions.

To analyze the effects of different grazing patterns on algae with different intrinsic growth rates, we calculated the log ratio $\ln(N_{EN}/N_C)$, where N_{EN} and N_C are the final densities ml^{-1} in the 'enhanced nocturnal grazing' and the 'continuous grazing' treatments, respectively. We used this ln ratio because it has clear biological meaning and good statistical properties (e.g., an approximately normal distribution) (Hedges et al. 1999). The ln ratios of all species were then plotted against their intrinsic growth rates and a linear regression was calculated to test for the existence of a positive correlation between a species' intrinsic growth rate and the advantage it has from a discontinuous ('enhanced nocturnal grazing') vs. a 'continuous grazing' pattern.

To investigate how different grazing patterns might influence the competitive algal performance, we calculated the 'algal ratio' $\ln(N_1/N_2)$, where N_1 and N_2 represent the final densities ml^{-1} of the two algal species in each experiment. For each species pair the species with the higher final density in the control treatments was labeled N_1. To test for the competitive performance a one-way ANOVA with post hoc tests was calculated for each experiment separately. Depending on whether the homogeneity of variances was given or not a Tukey post hoc test or a Games-Howell post hoc test was used, respectively.

We performed a separate community grazing experiment with ^{14}C-labelled algae to test whether *Daphnia* density had a negative effect on per capita grazing rates. Because such a crowding effect should be larger with the bigger *D. magna*, this experiment was only performed with *D. magna*. Either 13 or 26 *D. magna* were put into two-liter plastic beakers containing one liter of medium. Each treatment was replicated 5 times. Unlabelled *Scenedesmus obliquus* in a concentration equivalent to 1 mg C l^{-1} was added as food. After 12 h of acclimatization ^{14}C-labelled *S. obliquus* was added (0.058 mCi per beaker). The daphnids were allowed to graze for 16 min at a temperature of 18°C. After that they were harvested immediately, placed in scintillation vials, dried for 48 h at a temperature of 60°C and digested in 0.5 ml of tissue solubilizer (Packard Bioscience: Solvable 0.5M) for another 24 h at a temperature of 55°C. DPMs were then counted by liquid scintillation with 10 ml of Hionic Fluor Cocktail (LSC-cocktail, Packard) in a scintillation counter (Packard 2500 TR).

Results

***Daphnia* density and per capita grazing rates**

Daphnia density did not affect per capita grazing rates in the experiment with ^{14}C-labeled food. The mean per capita grazing rate in the experiment with 13 *D. magna* was 0.92 ml h^{-1} (SE = 0.07, n = 5), the mean per capita grazing rate in the experiment with 26 daphnids was 0.98 ml h^{-1} (SE = 0.05, n = 5). These two per capita grazing rates did not differ significantly (t-test, $t_{(8)}$ = -0,67, p = 0.52). Thus, our assumption that 26 daphnids grazed twice the amount of algae per unit time as did 13 daphnids was likely met also in the other experiments.

Algal performance under different grazing regimes

There were significant treatment effects in all experiments (Table 1a).

In all experiments algal species increased over time in the control treatments (Fig. 1). The growth of all algal species was always very similar in the two control treatments (‘continuous’ and ‘nocturnal’ control) and did not differ statistically in any of the 5 experiments. This suggests that any observed differences in algal performance in treatments with daphnids were consequences of the different grazing regimes and were not linked to the cage transfer.

The presence of grazers affected algal performance negatively in most experiments. With the exception of experiment 5 algal growth was significantly higher in the control treatments than in any treatment containing daphnids. In experiment 5 relative algae growth in the ‘ordinary nocturnal grazing’ treatments was similar to the growth in the control treatments (Fig. 1).

In the experiments with *D. magna*, all algae declined over time under the ‘continuous grazing’ regime (Fig. 1, left column). Relative algal growth was significantly higher in the ‘enhanced nocturnal grazing’ than in the ‘continuous grazing’ treatments in 7 out of 10 cases (Table 1b). In *S. obliquus*, the negative algal growth in the ‘continuous grazing’ treatments was even converted into positive growth in the ‘enhanced nocturnal grazing’ treatments in two of the three experiments where this alga was used (Fig. 1, left column). Only *R. minuta* and *C. sphaeroides*, which was used in two experiments, did not show statistically significant differences between the ‘continuous grazing’ and ‘enhanced nocturnal grazing’ treatment (Table 1b). Considering the algal growth in the ‘continuous grazing’ and the ‘ordinary nocturnal grazing’ treatments algal growth was always higher in the ‘ordinary nocturnal’ grazing treatments. This was always statistically significant (all $p < 0.05$).

In the experiments with *D. hyalina*, again all algae declined over time under the ‘continuous grazing’ regime (Fig. 1, right column). Relative algal growth was higher in the ‘enhanced nocturnal grazing’ than in the ‘continuous grazing’ treatments in 5 out of 10 cases (Table 1c). Again there was one case where the negative algal growth in the ‘continuous grazing’ treatment was converted into positive growth in the ‘enhanced nocturnal grazing’ treatment (*C. pseudostelligaria*) (Fig. 1, right column). Considering the algal growth in the ‘continuous grazing’ and the ‘ordinary nocturnal grazing’ treatments algal growth was significantly higher in the ‘ordinary nocturnal grazing’ treatments in 7 out of 10 cases. No such difference was visible in *S. obliquus* (exp. 1), *C. sphaeroides* (exp. 4) and *S. acuminatus* (exp. 2). These were also the algae species where no differences could be seen between the ‘continuous grazing’ and ‘enhanced nocturnal grazing’ treatments.

In both experiments (with *D. magna* and with *D. hyalina*) relative algal growth in the ‘enhanced nocturnal grazing’ treatments was in most cases intermediate between the ‘continuous grazing’ and the ‘ordinary nocturnal grazing’ treatments.

Algal performance related to intrinsic growth rates

In the experiments with *D. magna* fast-growing algae benefited more from a discontinuous grazing regime than did slow-growing algae. The effect discontinuous vs. continuous grazing, calculated as $\ln(N_{EN}/N_C)$, was positively related to the intrinsic growth rate (Fig. 2; linear regression: $r^2 = 0.66$, $p < 0.05$, $n = 6$). In contrast no such relationship was found in the experiments with *D. hyalina* (linear regression: $r^2 = 0.20$, $p = 0.45$, $n = 6$). Unfortunately, the raw data from experiments 4 and 5 got lost before we had calculated the intrinsic growth rates of the algae, reducing the sample size in this analysis to n = 6.

Algal competitive performance ability

There was never any difference between the ratios in the two control treatments (t-test; experiment1: $t_6 = 0.35$, $p = 0.77$; experiment 2: $t_6 = -1.21$, $p = 0.27$; experiment 3: $t_5 = 1.53$, $p = 0.19$; experiment 4: $t_6 = -0.45$, $p = 0.67$). Therefore they were considered as one treatment in the following analysis.

The results of these analysis are very complex and are shown in Fig. 3. There were significant treatment effects in all experiments except for experiment 1 with *D magna* (Table 2). Within most experiments relative algal performance of at least one *Daphnia* treatment was different to relative algal performance in the control treatment. The only exception for this was experiment 1 with *D. magna.* Here there were no differences in any of the treatments. In most cases algal ratios of the *Daphnia*-treatments were different to algae ratios in the control treatments. However there were some cases where the algae ratio also differed between the *Daphnia* treatments (different grazing regimes). Within most experiments there was one algae species which performed better in all experiments with daphnids. However this was not the case in experiment 2 (with *D. magna*) as here *M. minutum* performed better in the 'ordinary nocturnal grazing' treatment but *S. acuminatus* did better in the 'enhanced nocturnal grazing' treatment. Unfortunately, the raw data of experiment 5 got lost before we had calculated the algal ratios, therefore no results can be shown for this experiment.

Discussion

The main focus of the analyses of the experimental data was put on the advantage algae have when being grazed upon in a discontinuous way with enhanced filtration rates during the night (here: ‘enhanced nocturnal grazing’ treatments) instead of being exposed to a ‘continuous grazing’ pattern. The community grazing rate experiment showed, that the ‘enhanced nocturnal grazing’ treatments can be assumed to simulate an epilimnion where zooplankton individuals migrate and double their filtration rate during the night.

In our experiments in 12 out of 20 cases the algae showed a significantly higher growth when grazing was nocturnal only (‘enhanced nocturnal grazing’) (Fig. 1). This gives strong evidence that algae can benefit from a nocturnal grazing pattern even if the daphnids’ filtration rate, averaged over a full diel cycle, is the same in both cases. This effect was even stronger when the daphnids did not alter their filtration rate while grazing at night. Here in 17 out of 20 experiments the algae species had higher growth rate (‘ordinary nocturnal grazing’). As the grazing pressure was actually halved (exp. 2-5) or even divided into thirds (exp.1) in the ‘ordinary nocturnal grazing’ treatments it was to some extent surprising that not all algae showed higher growth. A potential explanation might be that possibly existing slight differences were superimposed by the relatively high variance of these data (Fig. 1).

So far it seems that there is not a unique answer to the question whether zooplankton individuals increase their filtration rate during the night while grazing in the epilimnion (Haney 1973; Haney and Hall 1975; Duval and Geen 1976; Gulati et al. 1982; Haney 1985; Lampert and Taylor 1985; Geller et al. 1992). Most probably this depends not only on the zooplankton species (Haney 1973), but also on factors like temperature or food conditions in the hypolimnion during the day. However, the actual filtration rate seems to play an important role for the potential outcome for phytoplankton communities as in our experiments the phytoplankton seemed to have a bigger advantage when the grazing regime was ‘ordinary nocturnal’ only. Here in 5 cases a difference was evident between the ‘continuous grazing’ and ‘ordinary nocturnal grazing’ but not between the ‘continuous grazing’ and the ‘enhanced nocturnal grazing’ treatments.

The experiments showed very clearly that diel vertical migration could result in enhanced algae biomass. This was expressed by the higher algal growth in the nocturnal grazing treatments and was also supported by particulate organic carbon (POC) and chlorophyll *a* data (Reichwaldt, unpublished data). The results confirm the hypotheses of earlier models of McAllister (1969), Petipa and Makarova (1969) and Gabriel (in Lampert 1986, 1987), which predict a higher algal growth when grazing is nocturnal.

It is imaginable, that a negative algal growth under a continuous grazing regime can shift to a positive algal growth when migration starts and grazing occurs only during the night (Lampert 1987). This was obvious in 5 of our experiments (Fig. 1). Such a change can be of great ecological importance for phytoplankton communities as algae actually shift from a negative population development to a positive one.

Changes in the diel vertical migration pattern of daphnids can influence the composition of a phytoplankton community as not all algae species show the same reaction to a changed feeding regime. This influence would even be more conspicuous if algae with different growth rates had differently strong advantages as predicted by Gabriel (in Lampert 1986, 1987) and McAllister (1969). Indeed the latter was visible in our experiments with *D. magna*, however it was not apparent in the experiments with *D. hyalina*. Probably the grazing impact of the smaller *D. hyalina* was not strong enough under this experimental design to stress a small difference in advantage so much more that the growth rates of the algal species in our experiment did not vary extraordinarily.

Looking at Fig. 1 it becomes clear, that in all 20 cases (separated by zooplankton and phytoplankton species) the mean algal growth in the ‘enhanced nocturnal grazing’ treatments was higher than in the ‘continuous grazing’ treatments. The probability that this tendency is realized randomly in 20 cases is about $0.5^{20} = 9.5 \times 10^{-7}$, a very small probability.

The experiments do not answer the question of the mechanism that account for the advantage of algae during a nocturnal grazing regime. One relatively obvious reason considering the ‘ordinary nocturnal grazing’ pattern is that algae experience a grazing reduced by the time the zooplankton spends in the hypolimnion. The phytoplankton is therefore only grazed during part of a whole day. Considering the ‘enhanced nocturnal grazing’ pattern one possible explanation for the higher growth is that a time frame results in which algae can grow undisturbed before they are grazed again.

Two algae species were always put pairwise into one experiment to look whether different grazing regimes had different effects on the relative competitive performance of two algae. In almost all experiments there was a significant change in the relative competitive performance in at least some of the treatments. This indicates that not only the amount of grazing but also the pattern of grazing can decide on the dominance of an alga species. Most often the ratio of the two algae in the control treatments differed from the ratio in the *Daphnia* treatments. This supports the widely accepted concept, that grazers can cause shifts in phytoplankton communities. Naturally such a finding can result from different vulnerability of algae to grazers. As this was very improbable in our experiment as all algae were similar in size and probably also in digestibility there has to be another explanation for this result. In 6 cases there was also a significant difference in the ratio of the two algae between the *Daphnia* treatments. This supports the idea, that the grazing pattern itself can have a strong influence on the algal community. The competition of algae in a lake will therefore not only depend on the amount of grazers but also on the quality of the grazing pattern itself. Such influence is of great ecological relevance in respect of species composition in a limnetic ecosystem. All these results make clear that the effect of diel vertical migration of zooplankton on phytoplankton is not a simple one. Imagine a lake with a typical diversified composition of phytoplankton. If DVM occurs, larger zooplankton individuals or species tend to migrate more pronounced (Lampert 1992) and also have a higher filtering rate for larger algae (Burns 1968; Dini et al. 1987; Angeli et al. 1995). This results in a dominance of small cladocerans, ciliates and rotifers in the epilimnion during the day. Therefore the above mentioned advantage of fast-growing algae (usually small algae) can be leveled out to some extent as they actually receive a nearly continuous grazing in the epilimnion from the small zooplankton species. Thus small algae might not have an advantage over large (slow growing) algae in nature as could be predicted by our experiments and by the models of Gabriel (in Lampert 1986, 1987) and McAllister (1969), because they are only exposed to a weak nocturnal grazing pattern but a strong continuous grazing regime by small non-migrating grazers. Thus, continuous and nocturnal grazing do not represent alternatives but occur simultaneously in a natural lake system As algae which are in progress of division are larger than algae which are not, there might also be different grazing pressures within one and the same algae species.

Our experiments show, that the effect of DVM is not only a quantitative but also a qualitative one and this may influence the phytoplankton species composition in a lake profoundly.

Acknowledgements

We wish to thank Sebastian Diehl for valuable suggestions on the manuscript. During the experiments E. S. Reichwaldt was supported by a scholarship for Ph.D. students of the University of Munich (LMU).

References

Angeli N, Pinel-Alloul B, Balvay G, Ménard I (1995) Diel patterns of feeding and vertical migration in daphnids and diaptomids during the clear water phase in Lake Geneva (France). Hydrobiologia 301:163-184

Burns CW (1968) The relationship between body size of filter-feeding Cladocera and the maximum size of particles ingested. Limnol Oceanogr 13:675-678

Dawidowicz P, Loose CJ (1992) Metabolic costs during predator-induced diel vertical migration of *Daphnia.* Limnol Oceanogr 37:1589-1595

Diehl S, Cooper SD, Kratz KW, Nisbet RM, Roll SK, Wiseman SW, Jenkins TM (2000) Effects of multiple, predator-induced behaviors on short-term producer-grazer dynamics in open systems. American Naturalist 156:293-313

Diehl S, Eklöv P (1995) Effects of piscivore-mediated habitat use on resources, diet, and growth of perch. Ecology 76:1712-1726

Dini ML, O'Donnell J, Carpenter SR, Elser MM, Elser JJ, Bergquist AM (1987) Daphnia size structure, vertical migration, and phosphorus redistribution. Hydrobiologia 150:185-191

Duval WS, Geen GH (1976) Diel feeding and respiration rhythms in zooplankton. Limnol Oceanogr 21:823-829

Geller W, Pinto-Coelho R, Pauli H-R (1992) The vertical distribution of zooplankton (Crustacea, Rotatoria, Ciliata) and their grazing over the diurnal and seasonal cycles in Lake Constance. Arch Hydrobiol Beih Ergebn Limnol 35:79-85

Giebelhausen B, Lampert W (2001) Temperature reaction norms of Daphnia magna: the effect of food concentration. Freshwater Biology 46:281-289

Gliwicz ZM (1986) Predation and the evolution of vertical migration in zooplankton. Nature 320:746-748

Gulati RD, Siewertsen K, Postema G (1982) The zooplankton: its community structure, food and feeding, and role in the ecosystem of Lake Vechten. Hydrobiologia 95:127-163

Haney JF (1973) An in situ examination of the grazing activities of natural zooplankton communities. Arch Hydrobiol 72:87-132

Haney JF (1985) Regulation of cladoceran filtering rates in nature by body size, food concentration, and diel feeding patterns. Limnol Oceanogr 30:379-411

Haney JF, Hall DJ (1975) Diel vertical migration and filter-feeding activities of *Daphnia*. Arch Hydrobiol 75:413-441

Hedges LV, Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in experimental ecology. Ecology 80:1150-1156

Hülsmann S (2001) Reproductive potential of *Daphnia galeata* in relation to food conditions: implications of a changing size-structure of the population. Hydrobiologia 442:241-252

Lampert W (1987) Vertical migration of freshwater zooplankton: Indirect effects of vertebrate predators on algal communities. In: Kerfoot WC, Sih A (eds) Predation : direct and indirect impacts on aquatic communities. University press of England, New Hampshire, pp 291-298

Lampert W (1992) Zooplankton vertical migrations: Implications for phytoplankton-zooplankton interactions. Arch Hydrobiol Beih Ergebn Limnol 35:69-78

Lampert W (1993) Ultimate causes of diel vertical migration of zooplankton: New evidence for the predator-avoidance hypothesis. Arch Hydrobiol 39:79-88

Lampert W, Taylor BE (1985) Zooplankton grazing in a eutrophic lake: implication of vertical migration. Ecology 66:68-82

Loose C, Dawidowicz P (1994) Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. Ecology 75:2255-2263

Lund JWG, Kipling G, Le Creen ED (1958) The inverted microscope method of estimating algae numbers and the statistical basis of estimation by counting. Hydrobiologia 11:143-170

McAllister CD (1969) Aspects of estimating zooplankton production from phytoplankton production. Journal of the Fisheries Research Board of Canada 26:199-220

McIntosh AR, Townsend CR (1996) Interactions between fish, grazing invertebrates and algae in a New Zealand stream: A trophic cascade mediated by fish induced changes to grazer behaviour? Oecologia 108:174-181

McKee D, Ebert D (1996) The interactive effects of temperature, food level and maternal phenotype on offspring size in *Daphnia magna.* Oecologia 107:189-196

Neill WE (1990) Induced vertical migration in copepods as a defense against invertebrate predation. (Letters to) Nature 345:524-526

Orcutt JD, Porter KG (1984) The synergistic effects of temperature and food concentration on life history parameters of *Daphnia* . Oecologia 63:300-306

Persson L (1993) Predator-mediated competition in prey refuges - the importance of habitat dependent prey resources. Oikos 68:12-22

Petipa TS, Makarova NP (1969) Dependence of phytoplankton production on rhythm and rate of elimination. Mar Biol 3:191-195

Power ME, Dudley TL, Cooper SD (1989) Grazing catfish, fishing birds, and attached algae in a Panamanian stream. Environmental Biology of Fishes 26:285-294

Ringelberg J (1991) A mechanism of predator-mediated induction of diel vertical migration of *Daphnia hyalina*. J Plankton Res 13:83-89

Ringelberg J (1993) Phototaxis as a behavioural component of diel vertical migration in a pelagic *Daphnia*. Arch Hydrobiol Beih Ergebn Limnol 39:45-55

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

Schmitz OJ, Beckerman AP, O'Brien KM (1997) Behaviorally mediated trophic cascades: Effects of predation risk on food web interactions. Ecology 78:1388-1399

Stich H-B, Lampert W (1981) Predator evasion as an explanation of diurnal vertical migration by zooplankton. Nature 293:396-398

Stich H-B, Lampert W (1984) Growth and reproduction of migrating and non-migrating *Daphnia* species under simulated food and temperature conditions of diurnal vertical migration. Oecologia 61:192-196

Trussell GC, Ewanchuk PJ, Bertness MD (2003) Trait-mediated effects in rocky intertidal food chains: Predator risk cues alter prey feeding rates. Ecology 84:629-640

Turner AM, Mittelbach GG (1990) Predator avoidance and community structure - interactions among piscivores, planktivores, and plankton. Ecology 71:2241-2254

Turner AM, Fetterolf SA, Bernot RJ (1999) Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. Oecologia 118:242-247

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

Zaret TM, Suffern JS (1976) Vertical migration in zooplankton as a predator avoidance mechanism. Limnol Oceanogr 21:804-813

Table 1a: Results of one way ANOVA testing for treatment effects on relative growth of each algal species in experiment (=exp.) 1-5, with either *D.magna* or *D. hyalina* as the grazer.

		D. magna		*D. hyalina*	
exp.	algae species	*F*	*p*	*F*	*p*
1	*S. obliquus*	$F_{(4,15)}=33.38$	<0.001	$F_{(4,15)}=14.26$	<0.001
	M. minutum	$F_{(4,15)}=41.84$	<0.001	$F_{(4,15)}=9.31$	0.001
2	*M. minutum*	$F_{(4,15)}=197.54$	<0.001	$F_{(4,15)}=95.10$	<0.001
	S. acuminatus	$F_{(4,15)}=204.94$	<0.001	$F_{(4,15)}=70.82$	<0.001
3	*C. sphäeroides*	$F_{(4,14)}=86.28$	<0.001	$F_{(4,14)}=132.29$	<0.001
	R. minuta	$F_{(4,14)}=242.87$	<0.001	$F_{(4,14)}=95.59$	<0.001
4	*C. sphäeroides*	$F_{(4,15)}=33.63$	<0.001	$F_{(4,15)}=5.37$	0.01
	S. obliquus	$F_{(4,15)}=103.14$	<0.001	$F_{(4,15)}=32.02$	<0.001
5	*C. pseudostelligaria*	$F_{(4,15)}=149.43$	<0.001	$F_{(4,15)}=52.98$	<0.001
	S. obliquus	$F_{(4,15)}=133.70$	<0.001	$F_{(4,15)}=48.82$	<0.001

Table 1b: Results of contrasts between relative growth in the 'continuous' and the 'enhanced nocturnal grazing' treatments for experiment 1-5 with *D. magna*.

exp.	algae species	*t*	*p*
1	*S. obliquus*	$t_{(15)}=-4.32$	0.001
	M. minutum	$t_{(15)}=-4.70$	<0.001
2	*M. minutum*	$t_{(3.4)}=-4.04$	0.02
	S. acuminatus	$t_{(15)}=-6.96$	<0.001
3	*C. sphäeroides*	$t_{(14)}=-1.68$	0.12
	R. minuta	$t_{(14)}=-0.76$	0.46
4	*C. sphäeroides*	$t_{(15)}=-1.31$	0.21
	S. obliquus	$t_{(6.0)}=-7.67$	<0.001
5	*C. pseudostelligaria*	$t_{(15)}=-5.63$	<0.001
	S. obliquus	$t_{(5.7)}=-8.94$	<0.001

Table 1c: Results of contrasts between relative growth in the ‘continuous’ and the ‘enhanced nocturnal grazing’ treatments for experiment 1-5 with *D. hyalina*.

exp.	algae species	*t*	*p*
1	*S. obliquus*	$t_{(15)}$=-2.04	0.06
	M. minutum	$t_{(15)}$=-1.84	0.09
2	*M. minutum*	$t_{(15)}$=-0.44	0.67
	S. acuminatus	$t_{(4.1)}$=-1.30	0.26
3	*C. sphäeroides*	$t_{(5.8)}$=-5.87	0.001
	R. minuta	$t_{(14)}$=-2.39	0.03
4	*C. sphäeroides*	$t_{(15)}$=-1.64	0.12
	S. obliquus	$t_{(15)}$=-5.71	<0.001
5	*C. pseudostelligaria*	$t_{(15)}$=-5.81	<0.001
	S. obliquus	$t_{(15)}$=-9.71	<0.001

Table 2: Results of one way ANOVA testing for treatment effects on algal ratios in experiment 1-4. The ‘continuous’ and the ‘nocturnal’ control treatments are handeled as one treatment.

		D. magna		*D. hyalina*	
exp.	ratio of algae species	*F*	*p*	*F*	*p*
1	*M. minutum/S. obliquus*	$F_{(3,16)}$ = 2.02	0.15	$F_{(3,16)}$ = 11.46	<0.001
2	*M. minutum/S. acuminatus*	$F_{(3,16)}$ = 22.34	<0.001	$F_{(3,16)}$ = 6.55	0.004
3	*R. minuta/C. sphäeroides*	$F_{(3,15)}$ = 34.50	<0.001	$F_{(3,15)}$ = 10.01	0.001
4	*S. obliquus/C. sphäeroides*	$F_{(3,16)}$ = 28.64	<0.001	$F_{(3,16)}$ = 9.94	0.001

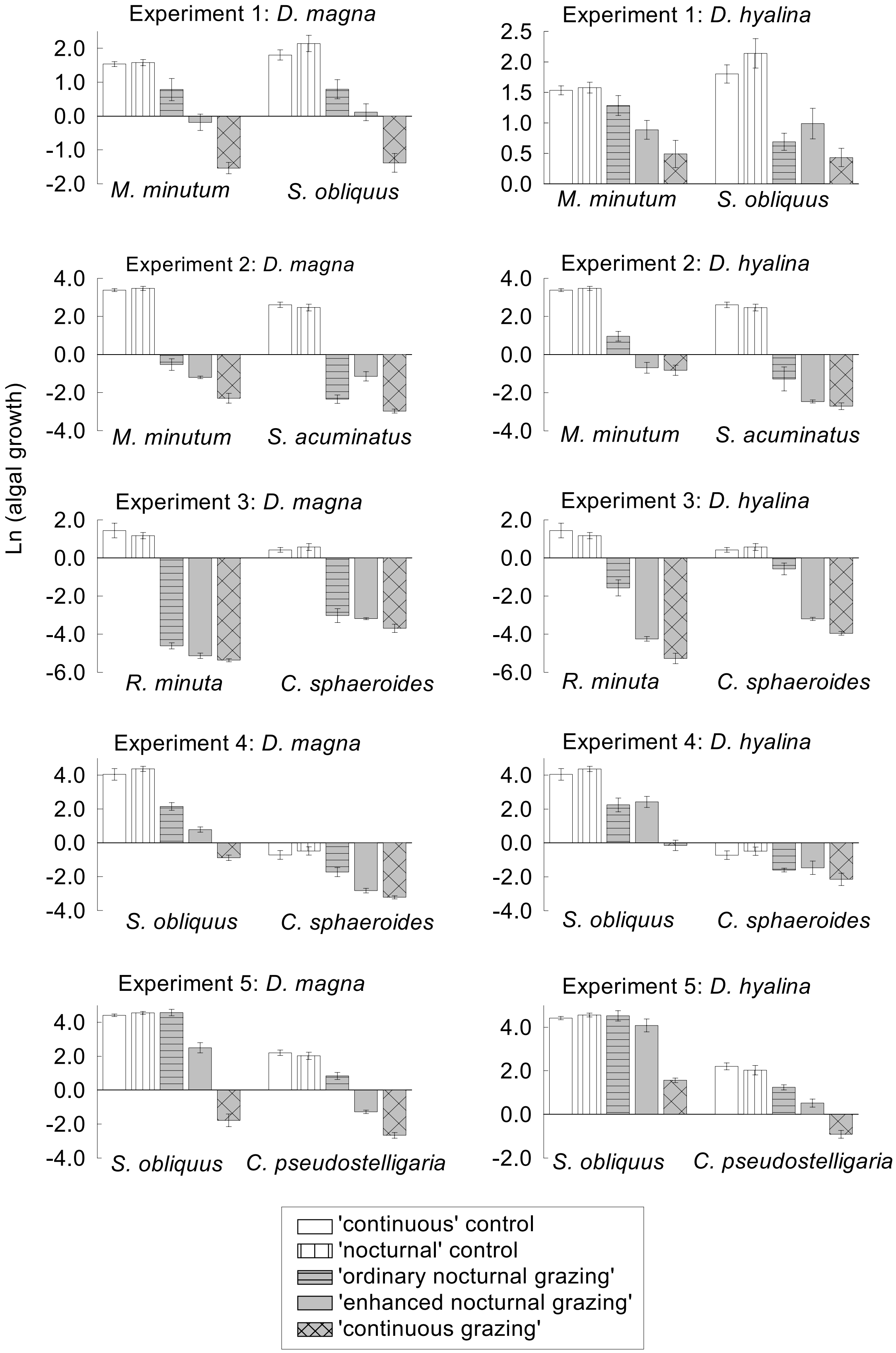


Fig. 1: Relative algal growth (equation 1) in experiments 1-5 with *D. magna* and *D. hyalina*, respectively. All bars represent means of 4 replicates ± 1 SE except for experiment 3, where n = 3 in the nocturnal control treatment.

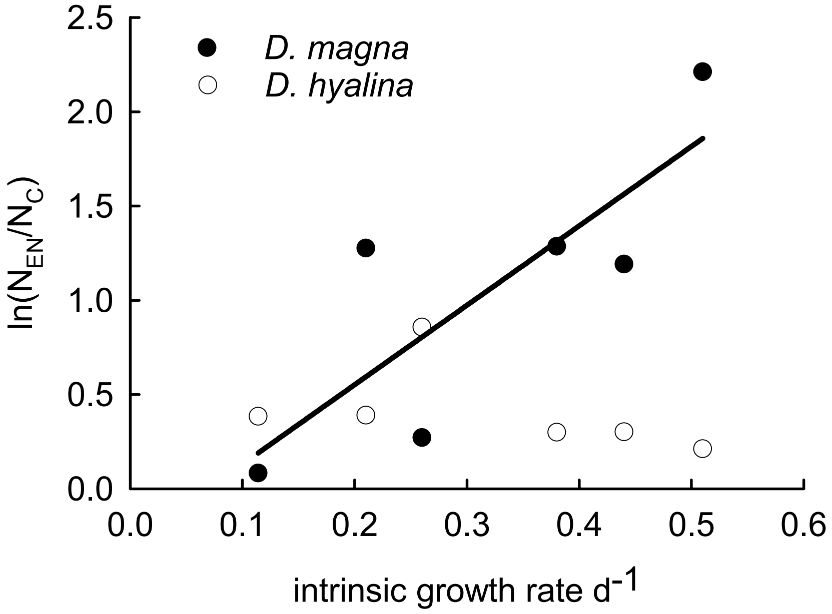


Fig. 2: Relative benefit of discontinuous vs. continuous grazing regime (expressed as $\ln(N_{EN}/N_C)$) vs. algal intrinsic growth rate in the experiments of *D. magna* and *D. hyalina*. Only the statistically significant linear regression for the *D. magna* experiments is shown ($y = -0.291 + 4.213 * x$; $r^2 = 0.660$). Data from experiment 1-3.

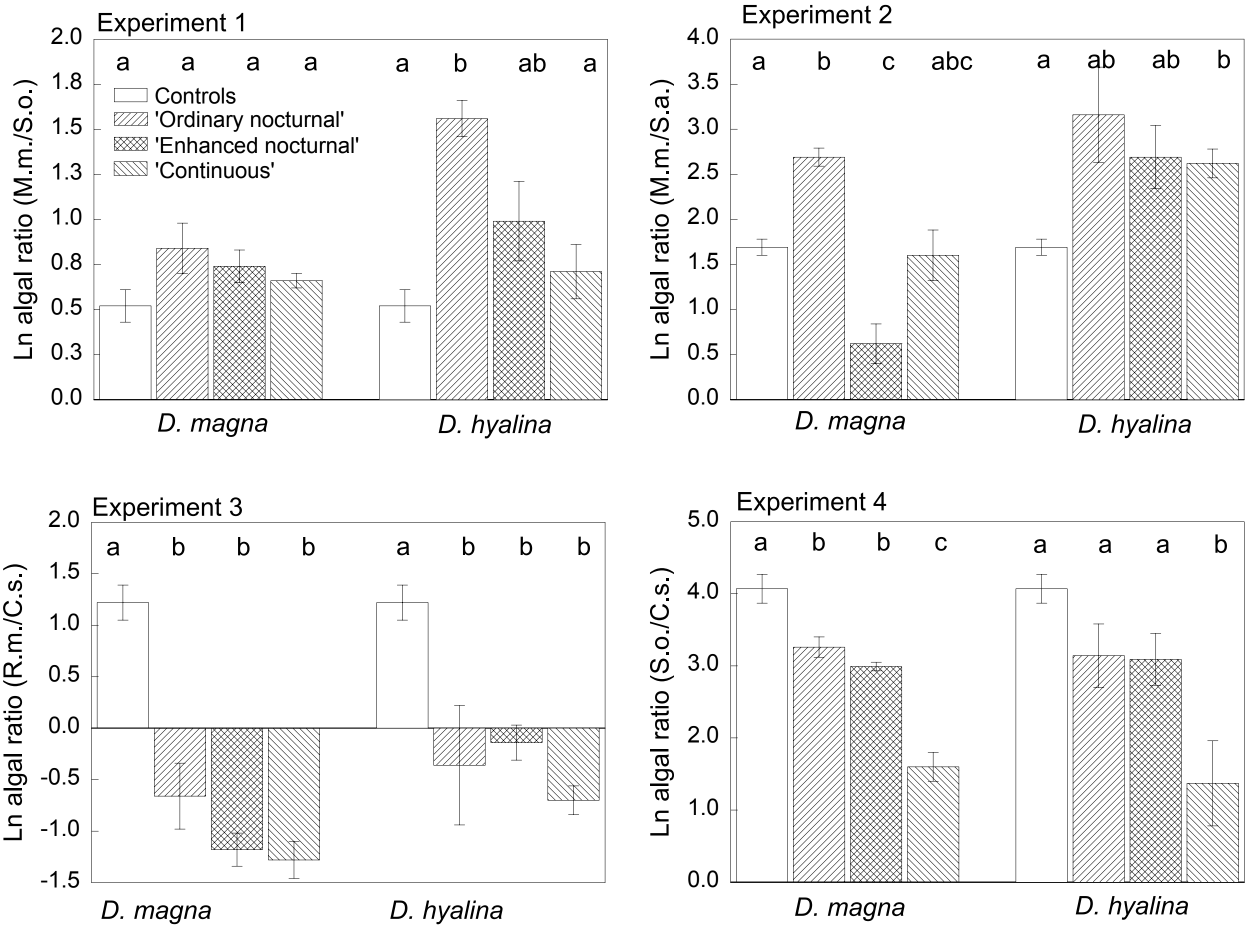


Fig. 3: Algal ratios in experiment 1-4. For clarity reasons, the continuous and the nocturnal control are combined into one bar. Bars are means +- 1 SE (n = 4 in the grazing treatments, n = 8 in the controls except for experiment 3, where n = 7) Abbreviations are as follows: M.m.=*Monoraphidium minutum,* S.o.=*Scenedesmus obliquus,* S.a.=*Scenedesmus acuminatus,* R.m.=*Rhodomonas minuta,* C.s.=*Chlamydomonas sphaeroides*. Bars that do not share a common letter are significantly different.

Paper 2

The impact of diel vertical migration of *Daphnia* on phytoplankton dynamics

(Reichwaldt and Stibor, submitted to Nature)

The impact of diel vertical migration of *Daphnia* on phytoplankton dynamics

Elke S. Reichwaldt * & Herwig Stibor *

* Ludwig-Maximilians Universität, Department Biology II, Aquatic Ecology, Karlstr. 25, D-80333 München, Germany

Diel vertical migration (DVM) of large zooplankton is an almost ubiquitous phenomenon in the pelagic zone of lakes and oceans, and the underlying mechanisms [1-4]and consequences for the zooplankton [5,6]are known well. As the zooplankton migrates downwards into lower and darker water strata by day and upwards into surface layers by night a huge amount of herbivorous biomass moves through the water column twice a day making it into one of the world's most massive synchronized animal migrations. This migration must have profound consequences for the zooplankton's food source (e.g. phytoplankton). However, these consequences were never investigated experimentally. It is generally assumed that migration supports an enhanced phytoplankton biomass and a change in the composition of the phytoplankton community towards smaller, edible algae [7,8]. We tested this for the first time in field experiments by comparing phytoplankton biomass and community assemblage in mesocosms with and without artificially migrating natural stocks of *Daphnia hyalina*. We show that DVM can enhance phytoplankton biomass in the upper layer of a lake (= epilimnion) and that it has a strong impact on the composition of a phytoplankton community.

DVM is induced by a chemical trigger substance (kairomone [6,9]) of fish and if no such cue is present the zooplankton stays in the epilimnion continuously. DVM therefore represents the result of a non-lethal interaction between zooplankton (prey) and fish (predator). Such interactions have gained more and more interest in the last years as they are numerous and diverse. Predators can for example influence morphology [10], behaviour [11-13] or life-history parameters [14,15] of the prey and these interactions are thus called trait-mediated interactions. It is generally known that these trait-mediated interactions can have substantial impacts on the prey's resource [12,16,17]. It is, however, surprising that research on a well-understood behaviour like DVM did not yet involve the study of the possible effects of fish-induced DVM on the phytoplankton in the epilimnion.

There are at least two mechanisms through which DVM of zooplankton in stratified lakes can affect phytoplankton dynamics. Firstly, the phytoplankton in the epilimnion is confronted with a reduced grazing pressure due to usually lower zooplankton densities of migrating populations. This is mainly due to the fact that migrating zooplankton populations experience lower temperatures in the lower layer of the lake (= hypolimnion) which implies high metabolic costs [5,6,18,19], and ultimately leads to a lower population growth. Secondly, zooplankton migration leads to a more or less grazing-free period during the day in the epilimnion resulting in an intermittent grazing pressure for the phytoplankton in this layer. Both mechanisms will lead to a reduced grazing pressure for the phytoplankton in the epilimnion if zooplankton migrate and it is generally assumed that both mechanisms can result in an enhanced phytoplankton biomass [7,8]. Additionally, both mechanisms might have direct positive effects on small, edible algae (which are often fast growing) because their faster growth rates might support a faster population growth during the grazing-free period, and there is also less grazing on these algae due to a lower zooplankton density. On the other hand, large inedible algae, which often have low growth rates, should have an advantage from permanent grazing, as there is then less competition with edible algae.

Difficulties in testing the effects of DVM on phytoplankton arise experimentally, as it is difficult to induce DVM. The kairomone is not exactly known and a described chemical which can induce DVM would have to be added in an amount that the carbon fixed in the kairomone would be higher than the carbon fixed in food [20]. On the other hand DVM could be induced by fish swimming in a cage or in a separate tank to avoid predation on zooplankton. However, the release of nitrogen and phosphorus from fish is high and will have direct effects on phytoplankton which makes it difficult to separate the effects of fish-induced DVM and fish-released nutrients on phytoplankton. We, therefore, developed a technique to mimic diel vertical migration without addition of chemical cues or the presence of predators by using a cage which enclosed the zooplankton and which was hauled up and down the water column every evening and morning, respectively. Proper control treatments showed that cages had no effect on phytoplankton dynamics.

We performed mesocosm experiments with either migrating or non-migrating *Daphnia* to test the hypotheses that phytoplankton quantity and quality in the epilimnion is influenced by the zooplankton's migration regime. Each mesocosm had a length of 10 m and enclosed a distinct epilimnion and hypolimnion with temperatures of about 18°C and 9°C, respectively. Statistical analyses were done with the calculated mean of the last two sample dates of the experiment of each mesocosm. However, for a better understanding of population development we additionally show development of important parameters over time. We only consider phytoplankton dynamics in the epilimnion as the hypolimnion never showed any differences between the treatments (data not shown).

There was no difference between the two types of control treatments in any of the measured parameters (chlorophyll-*a*: $t_{(6)} = 1.13$, $P = 0.30$; ratio 'biovolume <1000 μm^3/total biovolume': $t_{(6)} = -0.91$, $P = 0.40$; biomass development (*Cyclotella meneghiniana*): $t_{(6)} = -1.03$, $P = 0.34$; biomass development (*Monoraphidium minutum*): $t_{(6)} = -0.11$, $P = 0.31$; biomass development (pennate diatoms): $t_{(1.0)} = -0.32$, $P = 0.80$). Thus, the cages provided a good method to mimic diel vertical migration.

Daphnia density in the 'migration' treatment declined in the beginning of the experiment but levelled off at a low density at the end of the experiment. Differently, *Daphnia* density in the 'no-migration' treatment increased during the experiment and showed a high and more or less stable density at the end (Fig.1a). Thus, according to predictions, migration into cold deep water reduced population growth of *Daphnia* and there was a significant difference between the densities of *Daphnia* inside the cages in the 'migration' and 'no-migration' treatments at the end of the experiment ($t_{(4)} = 11.80$, $P < 0.001$) (Fig.1a).

Chlorophyll-*a* (as a measure for phytoplankton biomass) increased at the beginning of the experiment in all treatments but was then relatively stable over time with the highest values in the control treatments and the lowest values in the 'no-migration' *Daphnia* treatment (Fig.1b). Comparing the 'no-migration' *Daphnia* treatment and the 'migration' *Daphnia* treatment at the end of the experiment there was significantly more biomass in the 'migration' treatment ($t_{(6)} = -2.43$, $P = 0.05$) (Fig.1b).

Very similar results can be seen in the proportion of edible algal biovolume to total biovolume (measured as the ratio 'biovolume <1000 μm^3/total biovolume', as species <1000 μm^3 are favoured as food by daphnids [21]). Here the ratio increased first, but declined to different levels in the different treatments, the lowest ratio being in the 'no-migration' *Daphnia* treatment and the highest in the control treatments (Fig.1c). The proportion of edible algae biovolume to total biovolume was significantly higher in 'migration' treatment than in the 'no-migration' treatment at the end of the experiment ($t_{(6)} = -3.09$, $P = 0.02$) (Fig.1c).

We also examined biomass development of species or taxonomic groups which are known to be either good or poor food for daphnids. Examples for highly edible algae species are *Cyclotella meneghiniana*, which was the most common species in our experiment with a mean relative frequency of 30% of total biomass, and *Monoraphidium minutum*, which was the second most frequent edible algal species (1% of total biomass). Both species also constitute worldwide representatives for edible phytoplankton species in freshwater systems. Biomass development of both algae was significantly higher in the 'migration' treatment (*C. meneghiniana*: $t_{(6)} = -2.68$, $P = 0.04$; *M. minutum*: $t_{(6)} = -2.70$, $P = 0.04$) (Fig. 2a, b). On the other hand, biomass development of pennate diatoms (>60 µm in length as representatives for inedible phytoplankton species [22]) was significantly lower in the 'migration' treatment ($t_{(4)} = 3.91$, $P = 0.02$) (Fig.2c).

Our results indicate that DVM of zooplankton can have profound effects on phytoplankton dynamics of a lake. We showed that clear differences in phytoplankton biomass and composition could develop depending on the migration scheme of zooplankton. Migration led to a reduction in consumption time (intermittent grazing) and zooplankton abundance (due to temperature effects) which increased phytoplankton biomass in the epilimnion, and was advantageous for edible algae species. In this experiment we cannot distinguish whether the effects on phytoplankton dynamics in the 'migration' treatment were due to both a lower *Daphnia* density and intermittent grazing, or whether the density effect was stronger. However, previous laboratory experiments by Reichwaldt et al. (in revision) indicated that similar phytoplankton dynamics could be solely an effect of intermittent grazing. We thus think that the effects seen in the present study are at least partly also an effect of intermittent grazing.

We found large effects in our experiment in spite of the fact that the daphnids had only access to 11% of the volume of the epilimnion per enclosure. This emphasizes the strong impact of grazing on phytoplankton.

Our results suggest that in lakes predators (e.g. fish) can have an effect on the number of prey (e.g. zooplankton) without directly causing mortality because prey is searching for refuge which often includes worse growth conditions. This might also weaken the strong coupling between algal community dynamics and *Daphnia* density documented in a variety of studies [23-25], as suppression of *Daphnia* grazing pressure due to migration could create a grazing pressure characteristic for low *Daphnia* densities even though *Daphnia* density may be numerically high. Such considerations are of general importance if one takes into account that a large part of the world's plant production is bound into phytoplankton and that the above described phenomenon of DVM is also present in marine pelagic ecosystems [26].

Methods

Experimental design

A mesocosm experiment was conducted in the oligotrophic Lake Brunnsee (Germany) by enclosing the natural phytoplankton community in 10 cylindrical plastic bags which were attached to a raft. These mesocosms had an inner diameter of 95 cm, a depth of 10 m, and were open to the atmosphere. As the thermocline in this lake is usually between 5 to 7 m we thus enclosed a distinct hypolimnion and epilimnion divided by a steep temperature gradient. On the starting date we filled the mesocosms with surface water from the lake which was filtered through a 100-µm mesh in order to retain all mesozooplankton. Phytoplankton in Lake Brunnsee is generally P-limited in its growth. Therefore each mesocosm was enriched with 7µg P l^{-1} (P 10mM: $K_2HPO_4 \cdot 3H_2O$) to account for loss of nutrients by sedimentation.

Each of the 10 mesocosms contained a cage with walls of gauze with a mesh size of 250-µm. Each cage had a diameter of 70 cm and a length of 1 m. The cages were used to mimic diel vertical migration. Thus, in five mesocosms the cages stayed at a depth of 1.5 m (epilimnion) for the night but were lowered to a depth of 8.5 m (hypolimnion) for the day to imitate migration and thus an intermittent grazing regime in the epilimnion (day length from 6 a.m. to 10 p.m.). Three of these mesocosms contained daphnids in the cage (*Daphnia* 'migration' treatments) and the other two were control treatments without *Daphnia*. In the remaining five mesocosms we mimicked a 'no-migration' regime and the cages stayed at a depth of 1.5 m throughout 24 hours per day resulting in a continuous grazing pressure in the epilimnion. Again, three of the five mesocosms contained daphnids in the cage (*Daphnia* 'no-migration' treatments) and two of the five mesocosms were control treatments without *Daphnia*. The two distinct control treatments should discover any difference which derived from the mechanical influence due to cage handling.

The *Daphnia* species used was *Daphnia hyalina* which is the naturally occurring representative of the genus *Daphnia* in this lake and which is known to perform DVM. *Daphnia* was reared in advance and was released into the cages at the beginning of the experiment at a mean density of 32 Daphnia l^{-1}. The densities of *Daphnia* in our experiment were within the naturally occurring densities of this species in Lake Brunnsee.

Sampling procedure

Every fifth day a four-litre water sample was taken from a depth of 1.5 m and 8.5 m outside the *Daphnia* cage using a 2 l Ruttner sampler. Subsamples of it were used for determination of chlorophyll-*a* after Marker et al. [27] to analyse phytoplankton quantity response to 'migration' and 'no-migration' treatments. Further subsamples were fixed immediately with acid Lugol's iodine for subsequent determination of biovolume in a particle counter (Schärfe Systems GmbH) and for identification of phytoplankton in an inverted microscope (Wild). Particle counter results were used to analyse changes in the phytoplankton assemblage as we calculated the arc sine of the ratio 'biovolume of particles $<1000\mu m^3$/total biovolume'. Earlier studies showed that the amount of particles $<1000\mu m^3$ is a good measure for edible food available for daphnids [21]. For enumeration and identification of phytoplankton to species or genus, subsamples (10-30 ml) of the Lugol fixed samples were analysed with an inverse microscope at magnifications of 250 and 400 [28]. Additionally, the size of 25 individuals of each species was measured by using an inverse microscope and a computer measuring program (analySIS 2.11). Biomass of phytoplankton species was estimated as biovolume, which was calculated by converting size into biovolume using appropriate geometrical figures [29]. We then calculated biomass development for the most important phytoplankton species during the experiment by using the equation $\ln(\text{biomass}_t)-\ln(\text{biomass}_0)$, where biomass_t and biomass_0 are biomasses on day t and at the beginning, respectively. We calculated biomass development of the two most frequent edible algal species (*Cyclotella meneghiniana* and *Monoraphidium minutum*) and one taxonomic group of inedible species (pennate diatoms of >60 µm length). Existing pennate diatoms were *Asterionella formosa*, *Fragillaria crotonensis* and *Synedra spec*. (Bacillariophyceae).

For enumerating *Daphnia,* each cage was mixed with a secchi disc and subsequently one vertical net haul was taken inside of each cage every fifth day (diameter of net: 0.25 m; mesh width: 150-µm). These samples were preserved in 4% sucrose-formaldehyde solution [30] until enumeration.

The experiment lasted from May 29th to June 21st 2002.

Statistical analysis

For each parameter we calculated the mean of the last two sample dates of the experiment of each mesocosm. Analysis of *Daphnia* density was calculated with an independent sample t-test. Analyses of all measured phytoplankton parameters were calculated with an ANOVA with contrasts between the two control treatments and between the two *Daphnia* treatments using SPSS 11.0. We ln-transformed data where appropriate to meet assumptions of ANOVA. Biomass development of pennate diatoms was the only parameter that did not have homogenous variances.

Acknowledgements

We wish to thank Angelika Wild, Achim Weigert for help during the experiments and Sebastin Diehl and Ulrich Sommer for comments on earlier versions of the manuscript.

Correspondence and requests for materials should be addressed to E.S.R. (e-mail: ereichwaldt@web.de)

Reference List

1. Gliwicz, Z. M. Predation and the evolution of vertical migration in zooplankton. *Nature* **320**, 746-748 (1986).
2. Neill, W. E. Induced vertical migration in copepods as a defence against invertebrate predation. *(Letters to) Nature* **345**, 524-526 (1990).
3. Zaret, T. M. & Suffern, J. S. Vertical migration in zooplankton as a predator avoidance mechanism. *Limnol. Oceanogr.* **21**, 804-813 (1976).
4. Ringelberg, J. The photobehaviour of Daphnia spp. as a model to explain diel vertical migration in zooplankton. *Biological Reviews of the Cambridge Philosophical Society* **74**, 397-423 (1999).
5. Dawidowicz, P. & Loose, C. J. Metabolic costs during predator-induced diel vertical migration of *Daphnia. Limnol. Oceanogr.* **37**, 1589-1595 (1992).
6. Loose, C. & Dawidowicz, P. Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. *Ecology* **75**, 2255-2263 (1994).
7. Lampert, W. Limnologie: funktionelle Beziehungen in Ökosystemen; Kaskadeneffekte in See-Ökosystemen: die Bedeutung der tagesperiodischen Vertikalwanderung des Zooplankton. *Verh. Dtsch. Zool. Gesell.* **79**, 105-116 (1986).
8. Lampert,W. Predation: direct and indirect impacts on aquatic communities. Kerfoot,W.C. & Sih,A. (eds.), pp. 291-298 (University press of England, New Hampshire,1987).
9. Loose, C. *Daphnia* diel vertical migration behaviour: Response to vertebrate predator abundance. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* **39**, 29-36 (1993).
10. Raimondi, P. T., Forde, S. E., Delph, L. F. & Lively, C. M. Processes structuring communities: evidence for trait-mediated indirect effects through induced polymorphisms. *Oikos* **91**, 353-361 (2000).
11. Turner, A. M., Fetterolf, S. A. & Bernot, R. J. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. *Oecologia* **118**, 242-247 (1999).

12. Turner, A. M. & Mittelbach, G. G. Predator avoidance and community structure - interactions among piscivores, planktivores, and plankton. *Ecology* **71**, 2241-2254 (1990).
13. Trussell, G. C., Ewanchuk, P. J. & Bertness, M. D. Field evidence of trait-mediated indirect interactions in a rocky intertidal food web. *Ecol. Lett.* **5**, 241-245 (2002).
14. Stibor, H. & Lüning, J. Predator-induced phenotypic variation in the pattern of growth and reproduction in Daphnia-hyalina (Crustacea, Cladocera). *Funct. Ecol.* **8**, 97-101 (1994).
15. Tollrian,R. & Harvell,C.D. The ecology and evolution of inducible defenses. Princeton University Press, Princeton (1999).
16. McIntosh, A. R. & Townsend, C. R. Interactions between fish, grazing invertebrates and algae in a New Zealand stream: A trophic cascade mediated by fish induced changes to grazer behaviour? *Oecologia* **108**, 174-181 (1996).
17. Schmitz, O. J., Beckerman, A. P. & O'Brien, K. M. Behaviorally mediated trophic cascades: Effects of predation risk on food web interactions. *Ecology* **78**, 1388-1399 (1997).
18. Sakwinska, O. Plasticity of *Daphnia magna* life history traits in response to temperature and information about a predator. *Freshwater Biology* **39**, 681-687 (1998).
19. Giebelhausen, B. & Lampert, W. Temperature reaction norms of Daphnia magna: the effect of food concentration. *Freshwater Biology* **46**, 281-289 (2001).
20. Boriss, H., Boersma, M. & Wiltshire, K. H. Timehylamine induces migration of waterfleas. *Nature* **398**, 382-382 (1999).
21. Sommer, U. *et al.* Complementary impact of copepods and cladocerans on phytoplankton. *Ecol. Lett.* **4**, 545-550 (2001).
22. Tessier, A. J. & Woodruff, P. Cryptic trophic cascade along a gradient of lake size. *Ecology* **83**, 1263-1270 (2002).
23. Sarnelle, O. Nonlinear effects of an aquatic consumer: Causes and consequences. *Am. Nat.* **161**, 478-496 (2003).
24. Svensson, J.-E. & Stenson, J. A. E. Herbivoran impact on phytoplankton community structure. *Hydrobiologia* **226**, 71-80 (1991).
25. Kagami, M., Yoshida, T., Gurung, T. B. & Urabe, J. Direct and indirect effects of zooplankton on algal composition in situ grazing experiments. *Oecologia* **133**, 356-363 (2002).

26. Hays, G. C., Kennedy, H. & Frost, B. W. Individual variability in diel vertical migration of a marine copepod: Why some individuals remain at depth when others migrate? *Limnol. Oceanogr.* **46**, 2050-2054 (2001).
27. Marker, A. E., Nusch, E. A., Rai, H. & Riemann, B. The measurement of photosynthetic pigments in freshwaters and standardization of methods: conclusions and recommendations. *Arch. Hydrobiol.* **14**, 91-106 (1980).
28. Utermöhl, H. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. Int. Ver. Limnol.* **9**, 1-38 (1958).
29. Hillebrand, H., Dürselen, D., Kirschtel, D., Pollingher, U. & Zohary, T. Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.* **35**, 403-424 (1999).
30. Haney, J. F. & Hall, D. J. Sugar coated *Daphnia*: a preservation technique for Cladocera. *Limnol. Oceanogr.* **18**, 331-333 (1973).

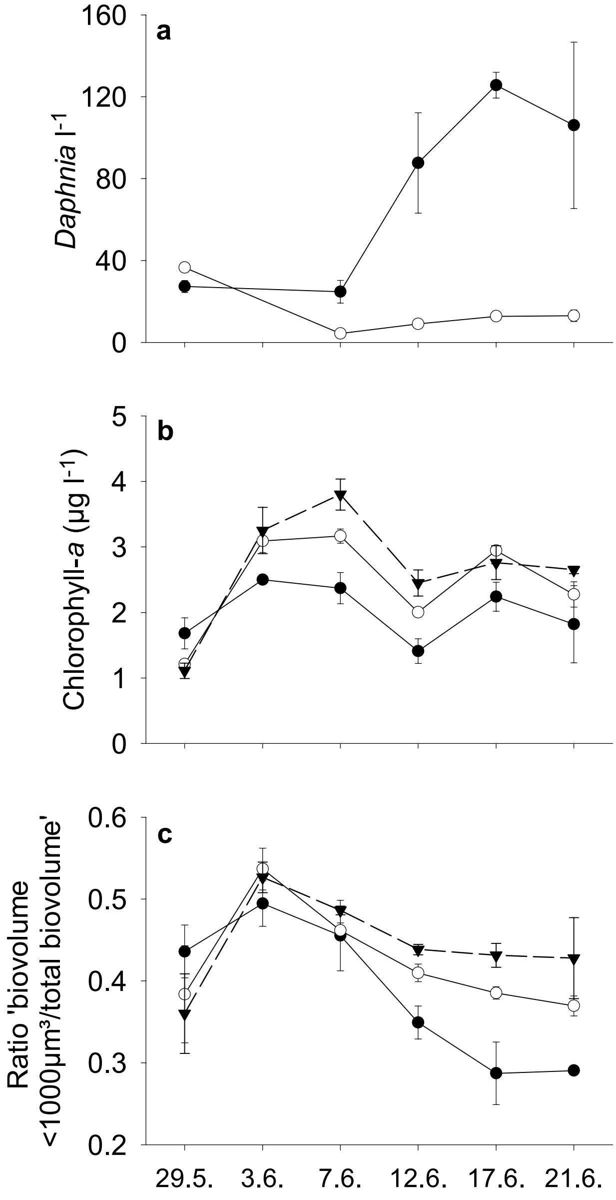


Figure 1 Time response of *Daphnia* l^{-1} (**a**), chlorophyll-*a* (µm l^{-1}) (**b**), and ratio 'biovolume <1000µm³/total biovolume' (**c**) in the four treatments. For reasons of clarity the two control treatments are represented as one line, as they never differed significantly (▾ = control treatments, • = 'no-migration' *Daphnia* treatment, ○ = 'migration' *Daphnia* treatment). Error bars represent ± 1 SE.

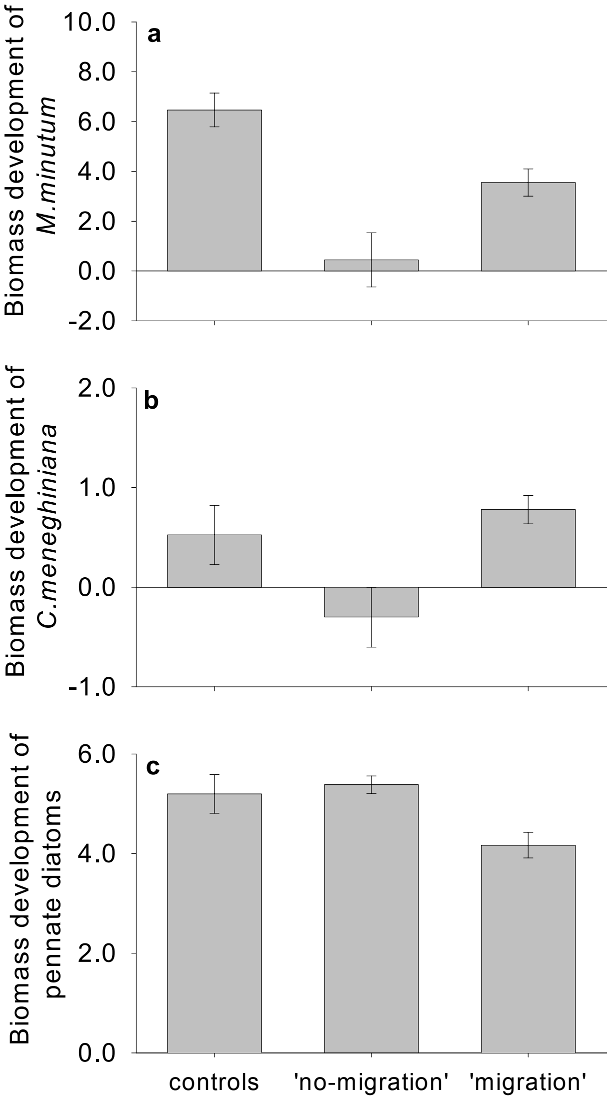


Figure 2 Mean values of algal development in the control treatments, ‘no-migration’ *Daphnia* treatment, and ‘migration’ *Daphnia* treatment calculated from the last two sample dates of the experiment (**a** = Biomass development of *Cyclotella meneghiniana*, **b** = Biomass development of *Monoraphidium minutum*, **c** = Biomass development of pennate diatoms of >60 µm in length). For reasons of clarity the two control treatments are represented in one bar, as they never differed significantly. Error bars represent ± 1 SE.

Paper 3

The Effects of *Daphnia* Diel Vertical Migration on Phytoplankton Dynamics and their implications for *Daphnia* life history Parameters

(Reichwaldt, Wolf and Stibor, submitted to Limnology and Oceanography)

The Effects of *Daphnia* Diel Vertical Migration on Phytoplankton Dynamics and their implications for *Daphnia* life history Parameters

Elke S. Reichwaldt [1]*, Isabelle D. Wolf [1] & Herwig Stibor[1]

[1] Ludwig-Maximilians Universität, Department Biologie II, Aquatische Ökologie, Karlstr. 25, D-80333 München, Germany

* Correspondence author

Address: Ludwig-Maximilians Universität, Department Biologie II, Abteilung Aquatische
Ökologie, Karlstr. 25, D-80333 München, Germany

E-mail: ereichwaldt@web.de

Running headline: DVM and *Daphnia*-Phytoplankton interaction

Acknowledgements

We wish to thank Sebastian Diehl for valuable suggestions on the manuscript. During the experiments E.S. Reichwaldt was supported by a scholarship for PhD students from the University of Munich (LMU) and I.D. Wolf was supported by a scholarship from Studienstiftung des Deutschen Volkes. We wish to thank Angelika Wild, Achim Weigert and Maren Striebel for help during the experiment.

Abstract

Diel vertical migration (DVM) of large zooplankton is a wide-spread and well-known behaviour in freshwater and marine ecosystems. Although the underlying mechanisms of DVM are well understood we lack experimental studies on the consequences of this behaviour for phytoplankton communities. We conducted a mesocosm experiment in which *Daphnia hyalina* either migrated diurnally in the water column (‘migration’ regime) or stayed permanently in the epilimnion (‘no-migration’ regime) to assess the effects of two different migration regimes on phytoplankton dynamics. In our experiment the presence of a migrating *D. hyalina* population resulted in an enhanced phytoplankton biomass in the epilimnion. Phytoplankton community composition also depended strongly on the migration pattern, as different algae groups benefited differently from the two migration regimes. For example migration of *D. hyalina* was advantageous for edible algae species. Phytoplankton diversity was lower when migration took place, probably due to the dominance of *Cyclotella meneghiniana* in the ‘migration’ treatment. We ran a laboratory experiment parallel to the mesocosm experiment to assess the feedback effects of the different phytoplankton communities (caused by the two migration regimes in the mesocosms) on *D. hyalina* life history parameters. We showed that somatic growth rate and number of offspring was higher, and age until first reproduction was lower when daphnids were fed with food mimicking the conditions of a migrating population. This shows that food conditions were better for migrating daphnids than for non-migrating daphnids.

Our results indicate the strong effects of DVM of *Daphnia* on phytoplankton dynamics and the clear feedback effects of these changes in the phytoplankton community on zooplankton life history parameters.

Introduction

Diel vertical migration (DVM) of mesozooplankton is a well-known behaviour in the pelagic zones of freshwater and marine ecosystems. It is very widespread and represents one of the world's most massive animal migrations. In the presence of hazards like planktivorous fish, large zooplankton individuals or species migrate into the dark, cold hypolimnion during the day to escape predation by optically orientated fish (Zaret and Suffern 1976; Stich and Lampert 1981; Lampert 1993). During the night the zooplankton stays in the upper, warmer epilimnion often crossing the thermocline of stratified lakes during their migration upwards and downwards. Previous research on DVM mainly focused on direct effects of migration on the zooplankton itself. For example, many studies showed that lower temperatures in the hypolimnion of stratified lakes have negative effects on life history parameters of migrating zooplankton due to higher metabolic costs at lower temperatures (Dawidowicz and Loose 1992; Loose and Dawidowicz 1994). These costs lead to a reduced zooplankton population growth which might ultimately lead to lower densities of migrating zooplankton populations compared to non-migrating populations.

DVM is induced by chemical cues, i.e. kairomones released by planktivorous fish, and if no such cue is present the zooplankton stays in the epilimnion continuously (Dodson 1988; Loose and Dawidowicz 1994). DVM is therefore the result of a non-lethal interaction between fish (predator) and zooplankton (prey) induced by the fish-kairomone. During migration large amounts of zooplankton biomass move up and down the water column twice a day and this must have profound consequences for the zooplankton's food (e.g. phytoplankton). Thus the phenomenon of DVM represents a good possibility to study indirect effects of predators on their prey's resource. Such interactions have gained more and more interest in the last years as they are numerous and diverse. Several other studies have already shown such effects in a variety of different ecosystems, such as streams (McIntosh and Townsend 1996), grasslands

(Schmitz et al. 1997), lakes (Turner and Mittelbach 1990), and in rocky intertidal zones (Trussell et al. 2002), emphasizing the broad relevance of these interactions. However, so far, we lack experimental studies on the effect of DVM on phytoplankton.

In most lakes the phytoplankton occurs mainly in the epilimnion and the absence or presence of migration leads to different conditions for the phytoplankton in this layer. In the absence of migration, phytoplankton experiences a continuous grazing pressure in the epilimnion. On the other hand, the presence of DVM leads to a period without grazing by large daphnids during the day in the epilimnion resulting in an intermittent grazing pressure for phytoplankton. Additional to less grazing due to intermittent grazing, the phytoplankton in the epilimnion is also confronted with a reduced grazing pressure during the night due to usually lower zooplankton densities of migrating populations (because of a lower temperature experienced by the zooplankton in the hypolimnion during the day).

It is generally assumed that after initiation of DVM in lakes in spring and early summer both intermittent grazing and a lower zooplankton density (due to direct predation of fish and lower experienced mean temperatures) can result in enhanced phytoplankton biomass development in the epilimnion. Additionally, fast-growing algae should benefit more from DVM than slow-growing algae because their faster growth rates might support a faster population growth during the grazing-free period and there is also less grazing on these algae due to a lower zooplankton density (Lampert 1986; Lampert 1987). As the interaction between zooplankton and phytoplankton is mutual, direct effects of zooplankton on phytoplankton by grazing should also have feedback effects on the zooplankton itself.

We here conducted a mesocosm experiment to investigate the effects of DVM of *Daphnia hyalina* on a lake’s phytoplankton assemblage. To separate the effects of zooplankton density on phytoplankton dynamics from the effects of intermittent grazing of zooplankton on phytoplankton dynamics, temperature was held constant throughout the water column as much as possible. In addition we conducted a laboratory experiment which ran parallel to the mesocosm experiment and which assessed the feedback effects of DVM-related changes in seston composition in the mesocosms on life history parameters of *Daphnia hyalina*.

Experimental induction of DVM poses some problems for several reasons. For one, the kairomone is not exactly known yet. A described chemical which can induce DVM would have to be added in an amount that the carbon fixed in the kairomone would be higher than the carbon fixed in food (Boriss et al. 1999). Moreover, DVM could be induced by fish swimming in a cage or in a separate tank to avoid predation on zooplankton. However, the release of nitrogen and phosphorus from fish is high and will have direct effects on phytoplankton which makes it difficult to separate the effects of fish-induced DVM and fish-released nutrients on phytoplankton. We, therefore, developed a technique to mimic diel vertical migration without addition of chemical cues or the presence of predators by using a cage which enclosed the zooplankton and which was hauled up and down the water column every evening and morning, respectively.

Materials and Methods

The mesocosm experiment

A mesocosm experiment was conducted in the mesotrophic Lake Brunnsee (southern Bavaria, Germany) by enclosing the natural phytoplankton community in cylindrical plastic bags, which were attached to a raft. Each mesocosm had an inner diameter of 95 cm, a depth of 10 m and was open only to the atmosphere. The experiment consisted of 10 mesocosms. We achieved an almost homogenous temperature over the entire water column of our mesocosms by enclosing them in the water with a huge plastic bag which was closed at the bottom but open to the atmosphere. Air was pumped into this bag to mix the water within this plastic bag (every 20 min. for 3 min.). However, temperature was not exactly the same in epilimnia and hypolimnia of the mesocosms but the mean temperature difference between the two layers never exceeded 2.7°C.

On the starting date we filled the mesocosms with surface water from the lake, which was filtered through a 100-µm mesh in order to retain all mesozooplankton. Phytoplankton in Lake Brunnsee is generally P-limited in its growth. Therefore each mesocosm was enriched with 7µg P L^{-1} (P 10mM: $K_2HPO_4 \cdot 3H_2O$) to account for loss of nutrients by sedimentation.

Each mesocosm contained a cage with walls of gauze with a mesh size of 250-µm. Each cage had a diameter of 70 cm and a length of 1 m. The cages were used to mimic diel vertical migration. Thus, the cages of five mesocosms stayed in the epilimnion at a depth of 1.5 m (middle of the cage) for the night but were lowered into the hypolimnion to a depth of 8.5 m for the day to mimic migration, i.e. an intermittent grazing regime in the epilimnion (day length: 6 a.m. to 10 p.m.). Three of these mesocosms contained daphnids in the cage (*Daphnia* ‘migration’ treatment), the other two were control-treatments without *Daphnia*. In the remaining five mesocosms we mimicked a ‘no-migration’ regime and the cages stayed at a depth of 1.5 m throughout 24 h per day resulting in a continuous grazing regime in the epilimnion. Again, three of the five mesocosms contained daphnids in the cage (*Daphnia* ‘no-migration’ treatment) the two remaining cages were control-treatments without *Daphnia*. The two control treatments should discover any difference which derived from mechanical influence due to cage handling.

The *Daphnia* species used was *Daphnia hyalina*, which is the naturally occurring representative of the genus *Daphnia* in this lake and which is known to perform DVM. The daphnids were reared in advance and released into the cages at a mean density of 25 *Daphnia* L^{-1} (± 3) at the beginning of the experiment.

Every fifth day a four-litre sample was taken from the depth of 1.5 m using a 2 L Ruttner sampler. Subsamples of it were used for determination of chlorophyll-*a* after Marker et al.

(1980) to analyse quantitative phytoplankton response to ‘migration’ and ‘no-migration’ treatments. Further subsamples were fixed immediately with acid Lugol’s iodine for subsequent determination of biovolume in a particle counter (Schärfe Systems GmbH) and for identification of phytoplankton in an inverted microscope (Wild). Particle counter results were used to analyse changes in the phytoplankton assemblage as we calculated the arc sine of the ratio ‘biovolume of particles <1000µm³/total biovolume’. Earlier studies showed that the amount of particles <1000µm³ is a good measure for edible food available for daphnids (Sommer et al. 2001). For enumeration and identification of phytoplankton to species or genus we analysed subsamples (10-30 ml) of the Lugol-fixed samples with an inverse microscope at magnifications of 250 and 400 (Utermöhl 1958). Additionally, the size of 25 individuals of each phytoplankton species was measured by using an inverse microscope and a computer-measuring program (analySIS 2.11). Biomass of phytoplankton species was estimated as biovolume, which was calculated from size using appropriate geometrical figures (Hillebrand et al. 1999). We then calculated biomass development of *Cyclotella meneghiniana* (most common edible phytoplankton species) and pennate diatoms of >60 µm length (most common inedible taxonomic phytoplankton group) by using the equation ln (biomass_t)-ln (biomass_0), where biomass_t and biomass_0 are the biomasses on day t and at the beginning. Existing pennate diatoms of >60 µm length were *Asterionella formosa*, *Fragillaria crotonensis* and *Synedra* spec. We additionally described species diversity with the Shannon-Wiener index

$$H' = -\sum p_i \ln p_i ,$$

where p_i is the proportion of each species.

For enumerating *Daphnia* each cage was mixed with a secchi disc and subsequently one vertical net haul was taken inside of each cage every fifth day (diameter of net: 0.25m; mesh width 150µm). These samples were preserved in 4% sucrose-formaldehyde solution until enumeration (Haney and Hall 1973). Measured *Daphnia* population parameters were *Daphnia* L^{-1}, population growth rate d^{-1}, number of eggs per *Daphnia*, number of eggs per egg-bearing *Daphnia,* and instantaneous birth rate. Although several models have been proposed for the estimation of instantaneous birth rate (b), the formula from Paloheimo (1974), $b=\ln(E+1)/D$, where E = eggs per *Daphnia* and D = egg development time, has proved to be most robust against violations of the underlying assumptions (e.g. uniform age distribution) and is easy to use (Gabriel et al. 1987). Temperature-dependent egg development times were calculated after Bottrell (1975) by using the temperatures from the mesocosms.

For statistical analyses we calculated means of the last two sample dates of the experiment. Analyses of seston parameters were calculated with ANOVA with contrasts between the two control treatments and between the two *Daphnia* treatments. If appropriate data were ln transformed to meet assumptions of ANOVA. *Daphnia* parameters were tested with independent sample t-tests. Given data represent mean ±1SE.

The experiment lasted from 29 May to 21 June 2002.

The laboratory experiment

We performed a laboratory experiment with juvenile *Daphnia hyalina* parallel to the mesocosm experiment described above to test whether the effects of the two migration regimes (‘migration’ and ‘no-migration’) on the seston in the mesocosm experiment had different feedback effects on *Daphnia* life history parameters. As the experiment was performed in a climatic chamber with a constant temperature of 19 °C and constant dim light, the only differing factor between the treatments was food quantity and quality.

Experiments were carried out in 100 ml glass vessels. We performed three different treatments in the laboratory experiment with 25 replicates each. In the first treatment daphnids were fed water from the epilimnion of the ‘no-migration’ *Daphnia* mesocosm treatment (NM-

E = No-Migration-Epilimnion). In the other two treatments daphnids received water from the 'migration' *Daphnia* mesocosms: one treatment was conducted with water from the epilimnion (M-E = Migration-Epilimnion), the other with water from the epilimnion and the hypolimnion in an alternating way every 12 hours (M-A = Migration Alternating). Comparison of parameters in the NM-E and M-A treatments describe the difference in life history parameters between migrating and non-migrating daphnids. Comparison of parameters in the NM-E and M-E treatments reflect the effect of migration on the seston in the epilimnion and its feedback on the daphnids. The M-E treatment would also reflect a situation in which a *Daphnia* feed continuously in the epilimnion although the rest of the population migrates (e.g. juvenile daphnids do not migrate). The water was taken from two of the three replicates of each *Daphnia* mesocosm treatment every morning. It was mixed in equal parts, filtered over 250-µm gauze to retain all mesozooplankton, and taken into the laboratory. From the 'no-migration' *Daphnia* treatment water was taken from the depth of 1.5 m only, whereas water was taken from 1.5 m and 8.5 m from the 'migration' *Daphnia* treatment.

At the beginning of the experiment each vessel received one *D. hyalina* not older than 12 hours. During the experiment each *Daphnia* was transferred to a similar vessel containing freshly fetched water from the mesocosm once a day in the morning. Daphnids in the M-A treatment were transferred to new water twice a day (morning and evening in 12 h intervals). However, because filtration rate of daphnids of this size is only approximately 4 ml per day (Wetzel 1983) the different water exchange rate should not have resulted in different food depletion.

At the beginning of the experiment the lengths of 25 neonates were measured under a dissecting microscope (Wild) to obtain a mean initial body length (mm). Due to shortage in neonates we had to use these daphnids in the experiment and distributed them evenly across all treatments. Length was again measured on day 6. The measured lengths (in mm) were converted into specific weights (ω) using the following equation:

D. hyalina: $\omega = 5.59 \text{ x length}^{2.21}$ (Stibor and Lüning 1994)

The somatic juvenile growth rate (g) was then calculated as

$$g = \frac{(\ln \omega_{\text{adult}} - \ln \omega_{\text{neonate}})}{t}$$

where ω_{adult} and ω_{neonate} are the specific weights of *D. hyalina* as adults and neonates, and t is the time in days. In addition, we measured age at first reproduction (AFR) (in days), eggs per *Daphnia*, and egg development time (in days). AFR was defined as the time from birth until the day when eggs were visible in the brood chamber for the first time. Egg development time was defined as the time from when eggs became visible in the brood chamber until neonates were born. We additionally calculated instantaneous birth rates (b) after Paloheimo (1974) whose formula was already described in the methods section for the mesocosm experiment. On the one hand, we calculated b here with the parameters directly measured in the laboratory (eggs per *Daphnia*, egg development time) to compare population development in the treatments of the laboratory experiment. Differences in b would then reflect different population growths due to food quality and quantity effects only. On the other hand, we calculated temperature-corrected b by using temperatures from the mesocosm experiments instead of the constant temperature in the laboratory (temperature for non-migrating daphnids: 15.2°C; temperature for migrating daphnids: 14.4°C). Thus, we here used egg development times calculated after Bottrell (1975). We looked at temperature-corrected instantaneous birth rates to decide whether small temperature differences in the mesocosm experiment had any effect on population development.

To quantify food availability in the laboratory experiment, chlorophyll-*a* [μg L^{-1}] was measured after Marker et al. (1980), and particulate organic carbon (POC) [mg L^{-1}] and particulate phosphorus (PP) [μg L^{-1}] were measured to calculate the C:P ratio as a measure for food quality. For determination of POC water samples were filtered on glass fibre filters (Schleicher & Schuell) and filters were dried for at least 24 h at a temperature of 60°C. POC was then determined by combustion and infrared detection of CO_2 with a C-mat 500 (Stroehleins Instruments). For determination of PP water samples were first filtered on membrane filters (Schleicher & Schuell). After chemical disintegration of PP to dissolved phosphorus, the amount of PP was then determined by photometry. Chemical parameters were measured three times during the experiment and for statistical analysis we used the calculated mean of these. Additionally, we measured the ratio 'biovolume <1000μm^3/total biovolume' as described for the mesocosm experiment.

Each individual *D. hyalina* was removed from the experiment after it had released the first brood.

We tested differences of seston and life history parameters between NM-E/M-A and NM-E/M-E using one-way ANOVA with contrasts. Given data represent the mean ±1SE.

Results

The mesocosm experiment

Daphnia parameters

Contrary to predictions migration in water columns with almost constant temperatures reduced population growth of *Daphnia*. Thus, densities of *Daphnia* inside the cages were 123.9 (±28.3) and 16.9 (±2.4) *Daphnia* L^{-1} in the 'no-migration' and 'migration' treatments, respectively (t=7.61, df=4, *p*=0.002). If calculated for the entire water column of the mesocosm this would match a density of 6.7 (±1.5) and 0.9 (±0.1) *Daphnia* L^{-1} in the 'no-migration' and 'migration' treatment, respectively. *Daphnia* growth rates therefore differed significantly between the 'no-migration' and 'migration' treatments (t=4.46, df=4, *p*=0.011) with a mean growth rate of 0.08 (±0.02) and -0.02 (±0.01) in the 'no-migration' and 'migration' treatments, respectively. Thus, the phytoplankton in the 'migration' and 'no-migration' treatments experienced differences in grazing pressure not only due to grazing time (intermittent vs. continuous grazing) but also due to the density of the grazers. Number of eggs per egg-bearing *Daphnia* was also different between the treatments (t=-4.56, df=2.1, *p*=0.039) being 3.6 (±0.1) and 7.1 (±1.0) in the 'no-migration' and 'migration' treatment, respectively. However, mean number of eggs per *Daphnia* did not show a difference (t=-2.36, df=4, *p*=0.077) (0.19 ±0.04 and 0.5 ±0.13 eggs *Daphnia*$^{-1}$ in the 'no-migration' and 'migration' treatment, respectively). Instantaneous birth rates (b) in the mesocosm experiment were calculated with temperatures measured in the mesocosms (14.4°C for migrating and 15.2°C for non-migrating *Daphnia*) to receive a measure for the possible population developments in the two treatments. Instantaneous birth rates were not significantly different between migrating and non-migrating daphnids (t=-1.09, df=4, *p*=0.339), and were 0.052 (±0.003) and 0.064 (±0.011) in the 'no-migration' and 'migration' treatment, respectively.

Seston parameters

Comparing seston parameters in the 'no-migration' and the 'migration' *Daphnia* treatment there was significantly more biomass in terms of chlorophyll-*a* in the epilimnion of the 'migration' treatment than in the treatment without migration (t=-2.46, df=6, *p*=0.049) (Fig.1). The proportion of edible algae biovolume (measured as the ratio 'biovolume <1000 µm^3/total biovolume') was also significantly higher in the 'migration' treatment than in the 'no-migration' treatment (t=-3.41, df=6, *p*=0.014) (Fig. 1). We also examined biomass development of *Cyclotella meneghiniana* (Bacillariophyceae), which is known to be consumed by daphnids. *C. meneghiniana* was the most common phytoplankton species in our mesocosms and its biomass ranged between 31% and 57% of total biomass throughout the experiment. Biomass of *C. meneghiniana* was significantly enhanced in the 'migration' treatments (t=-5.85, df=2.6, *p*=0.015) (Fig. 1). Biomass development of pennate diatoms (>60 µm) – as the most common taxonomic group of inedible algae – was not different between the treatments (t=2.30, df=3.1, *p*=0.102). We also compared phytoplankton diversity between the treatments using the Shannon-Wiener function. Diversity was significantly higher in the 'no-migration' treatment (t=-3.26, df=3.6, *p*=0.017). The index was 2.89 (±0.014) and 2.54 (±0.125) in 'no-migration' and 'migration' treatments, respectively.

With the exception of chlorophyll-*a* no differences occurred between the two control treatments (chlorophyll-*a*: t=-3.99, df=6, *p*=0.007; ratio 'biovolume <1000µm^3/total biovolume': t=1.19, df=6, *p*=0.279; biomass development (*C. meneghiniana*): t=-0.18, df=1, *p*=0.886; biomass development (pennate diatoms): t=1.82, df=1, *p*=0.32; Shannon-Wiener: t=-1.20, df=6, *p*=0.275). However, chlorophyll-*a* was higher in the 'no-migration' control treatment, which is opposite to the result achieved in the two *Daphnia* treatments in which we found higher values of chlorophyll-*a* in the 'migration' treatment.

The laboratory experiment

Seston parameters

Seston parameters differed significantly between the treatments of the experiment. There was a significant difference in chlorophyll-*a* ($F_{2,3}$=46.42, *p*=0.006) and in the ratio 'biovolume <1000µm^3/total biovolume' between the treatments ($F_{2,3}$=11.18, *p*=0.041) (Fig. 2). Contrasts showed that both chlorophyll-*a* and the proportion of edible algae were significantly lower in the NM-E treatment than in the other two treatments. The ratio of carbon to phosphorus (C:P) of the seston was not different between the treatments (C:P: $F_{2,3}$=0.64, *p*=0.587) (mean ratio of C:P was 286 ±7).

Zooplankton parameters

There was never any difference in egg development time in the experiment ($F_{2,41}$=0.14, p=0.874), and mean egg development time was 4.3 days (±0.1). All other zooplankton life history parameters differed significantly in the experiment (AFR: $F_{2,41}$=6.28, p=0.004; eggs per *Daphnia*: $F_{2,41}$=29.45, p<0.001; somatic growth rate: $F_{2,41}$=5.00, p=0.011) (Fig. 3). Daphnids fed with food from the 'no-migration' treatment had the lowest number of eggs per *Daphnia*, the slowest somatic growth rate and needed longest until first reproduction. Comparing instantaneous birth rate in the laboratory experiment (constant temperature: 19°C) there was a significant difference between the treatments of the experiment ($F_{2,41}$=14.96, p<0.001) (Fig.4 A). Again daphnids fed with food from the 'no-migration' treatment had the lowest instantaneous birth rates. This was also evident from analyses of temperature-corrected instantaneous birth rates, which were also significantly different in the treatments ($F_{2,43}$=28.17, p<0.001) (Fig.4 B).

Discussion

Naturally, one of the strongest consequences of migration for zooplankton arises due to a lower temperature in the hypolimnion of lakes (Dawidowicz and Loose 1992; Loose and Dawidowicz 1994). To minimize the effects of a different grazing pressure on phytoplankton due to different zooplankton densities in the 'migration' and 'no-migration' regime, we kept temperature constant over the water column as much as possible. However, there were still small temperature differences between the epilimnion and the hypolimnion (max. 2.7°C). Nevertheless, differences in observed growth rates of *D. hyalina* between the 'migration' and 'no-migration' treatments were not due to the existing difference in temperature because instantaneous birth rates were equal in both mesocosm *Daphnia* treatments (probably due to the fact that the number of eggs per *Daphnia* were equal). As there were more eggs per egg-bearing *Daphnia* in the ‘migration’ treatment but also mainly adult daphnids in this treatment, we think that a high mortality rate in juvenile daphnids in the ‘migration’ treatment was responsible for the lower population growth in this treatment. Migration in our experiment therefore not only led to a difference in the grazing pattern (intermittent vs. continuous) but also in zooplankton abundance, which might also be true in lakes and, thus, does not weaken the validity of our results concerning phytoplankton dynamics.

All our results showed that clear differences in phytoplankton biomass and composition can result from different migration patterns of zooplankton. Migration led to increased phytoplankton biomass (chlorophyll-*a*) and was advantageous for edible algae (*C. meneghiniana*, ratio ’biovolume <1000µm^3/total biovolume) which might be due to both intermittent grazing, which leads to a grazing-free period during the day in which the algae can grow undisturbed, and a lower zooplankton density. These results are concordant with results achieved in a similar experiment with a natural temperature gradient (Reichwaldt and Stibor, unpubl.). We also showed a difference in algal diversity depending on the migration scheme of the daphnids: diversity was lower in the ‘migration’ *Daphnia* treatment than in the ‘no-migration’ treatment. In our experiment a lower grazing rate apparently favoured one edible algae species (*Cyclotella meneghiniana*) which then dominated the phytoplankton community in the migration treatment. Consequently this reduced diversity.

In our experiment there was no possibility to separate the effects that were caused by intermittent grazing from the effects that were caused by a lower zooplankton density. However, previous laboratory experiments by Reichwaldt et al. (unpubl.) indicated that similar phytoplankton dynamics can be caused by intermittent grazing only. We therefore think that our results here are at least partly also an effect of intermittent grazing and not only due to the lower zooplankton density in the ‘migration’ treatment.

The results of this experiment give strong indirect evidence that fish can have an effect on phytoplankton dynamics by changing the zooplankton's behaviour (which was done artificially in our experiment) and thus also its grazing pattern. Such effects of predators on the density of their prey's resource were already shown in a variety of other ecosystems (reviewed in Schmitz et al. 2004) emphasizing that this effect is of broad relevance. Tessier and Woodruff (2002) have shown that predators can also have a more cryptic effect on the prey's resource by changing the community structure of the resource but not the total biomass. In our experiments we could show that DVM of *Daphnia* led to both effects simultaneously.

Although we only had low densities of *Daphnia* in the 'migration' treatment there was always a clear grazing effect in our experiment. This can be seen by the fact that chlorophyll-*a* in the 'migration' treatment was only 60% of the amount of chlorophyll-*a* in the control treatments (Fig. 1A), the ratio of edible algae was reduced to 87% of the value in the control treatments (Fig.1B), and the biomass change of *C. meneghiniana* was only 63% of the biomass change in the control treatments (Fig.1C). This indicates that grazing was still high in the 'migration' treatment despite the low *Daphnia* densities.

Application of continuous and nocturnal grazing as alternatives as was done in our experiment to study the effects of different grazing regimes on phytoplankton, is artificial as in lakes both types of grazers might be present simultaneously. The possibly most prominent example for this is Lake Constance in which non-migrating *Daphnia galeata* populations coexist with migrating *Daphnia hyalina* populations (Stich and Lampert 1981). But even in lakes with only migrating *Daphnia* species there are still many other grazers that usually do not migrate (e.g. ciliates, rotifer, nauplia, juvenile *Daphnia*). It seems likely that the strength of the effects of DVM on phytoplankton might depend on the diversity of the zooplankton fauna in a lake because the species and their developmental stages that constitute the zooplankton community may use different size ranges of food articles and may exhibit different migration patterns. Consequently, the effects of DVM might be less pronounced in lakes with a highly diverse zooplankton fauna and might also vary seasonally.

The laboratory experiment was designed to study feedback effects of different seston compositions (due to the different grazing regimes in the mesocosms) on juvenile daphnids. The results showed clearly that different grazing regimes in the mesocosms generated water bodies of different seston quantity and quality (more chlorophyll-*a* / higher proportion of edible algae in the 'migration' treatment), and that zooplankton parameters were highly affected by this. Daphnids fed with seston from the epilimnion of mesocosms with migration (M-E) had a lower AFR, more offspring, and higher somatic growth rates than daphnids fed with seston from the epilimnion from mesocosms without migration (NM-E). This emphasizes that seston composition (chlorophyll-*a*, proportion of edible algae) was changed by migration in a way that it was advantageous for those daphnids which stayed in the epilimnion whereas the main part of the population migrated. This would for example not only lead to favourable conditions for juvenile daphnids which often stay in the epilimnion as long as they are small (Lampert 1992), but also for other zooplankton species which do not migrate diurnally (e.g. ciliates, rotifers). Especially ciliates and rotifers can then benefit from these food conditions as they prefer small algae as food. Comparing migrating and non-migrating daphnids (NM-E vs. M-A), migrating daphnids had a lower AFR, more offspring, and a higher somatic growth rate in our laboratory experiment. Apparently, conditions faced by migrating daphnids were advantageous due to a higher mean chlorophyll-*a* content and a higher mean proportion of edible algae. In our experiment the higher mean chlorophyll-*a* content experienced by migrating daphnids was not only due to the impact of 'migration' in the mesocosms but also due to the fact that we had a higher algal biomass in the hypolimnion (data not shown). This is not unusual as lakes can also have deep-chlorophyll-maxima (DCM) under certain circumstances (Fee 1976; Brooks and Torke 1977; Coon et al. 1987). Our results support earlier studies of Williamson et al. (1996) and Winder et al. (2003) which showed that food conditions are not necessarily worse for migrating daphnids if a deep-chlorophyll-maximum is present in a lake.

Egg development time in our laboratory experiment never differed between the treatments. This is in accordance to predictions as egg development time only depends on temperature (Bottrell 1975), and temperature was constant in our laboratory experiment. A negative relationship between the C:P ratio and lake depth seems to be a common feature and several studies have related the variability of C:P ratio of seston to the light supply per unit nutrient (Urabe and Sterner 1996; Sterner et al. 1997). We did not find different C:P ratios in the epilimnion and hypolimnion of our mesocosms which might have been due to the fact that we had high visibility and a very deep compensation level that was reaching down to the bottom of the lake (data not shown).

Comparison of instantaneous birth rates (b) of *D. hyalina* in the laboratory experiment showed that b was significantly higher in the 'migration' treatments (M-E/M-A) than in the 'no-migration' treatment. This is a result of the higher number of eggs per *Daphnia* in the 'migration' treatment which in turn might be have been due to better and/or more food as already mentioned above. These differences did not disappear after temperature-correction of b emphasizing that small differences in temperature (2.7°C) did not have an influence on population growth and that in our experiment food quality and quantity were the most important factors affecting population growth. In our experiments the results of life history parameters and instantaneous birth rates both indicate that migration can lead to favourable food conditions for the daphnids.

Our experiments emphasize the strong effects of a migrating *Daphnia* population on the phytoplankton community in the epilimnion of a lake. This is of general interest because

DVM is also present in marin ecosystems (Enright and Hamner 1967; Hays et al. 2001) and pelagic phytoplankton represent the major primary producers on two thirds of the earth's surface. The experiments also indicate that DVM-related effects on phytoplankton can have a positive impact on life history parameters of the daphnids themselves. This positive impact was also due to a higher mean amount of food experienced by migrating daphnids because of a deep-chlorophyll-maxima in our experiment which shows that food is apparently not always worse in the hypolimnion.

Our experiments show that this indirect interaction between fish, zooplankton and phytoplankton can have substantial effects on both the phytoplankton community and the zooplankton populations by creating functional variations in grazer-resource coupling. We think that our results are a good example for a cascading effect from a high trophic level (fish) down to a lower trophic level (phytoplankton) that is mediated through a behavioural change of the intermediate trophic level (zooplankton).

Reference List

Boriss, H., M. Boersma, and K. H. Wiltshire. 1999. Timehylamine induces migration of waterfleas. Nature **398:** 382-382.

Bottrell, H. H. 1975. The relationship between temperature and duration of egg development in some epiphytic Cladocera and Copepoda from the river Thames, Reading, with a discussion of temperature functions. Oecologia **18:** 63-84.

Brooks, A. S. and B. G. Torke. 1977. Vertical and seasonal distribution of chlorophyll *a* in Lake Michigan. J. Fish. Res. Bd. Can. **34:** 2280-2287.

Coon, T. G., M. M. Lopez, P. J. Richerson, T. M. Powell, and C. R. Goldman. 1987. Summer dynamics of the deep chlorophyll maximum in Lake Tahoe. J. Plankton Res. **9:** 327-344.

Dawidowicz, P. and C. J. Loose. 1992. Metabolic costs during predator-induced diel vertical migration of *Daphnia.* Limnol. Oceanogr. **37:** 1589-1595.

Dodson, S. 1988. The ecological role of chemical stimuli for the zooplankton: Predator-avoidance behavior in *Daphnia*. Limnol. Oceanogr. **33:** 1431-1439.

Enright, J. T. and W. M. Hamner. 1967. Vertical diurnal migration and endogenous rhythmicity. Science **157:** 937-941.

Fee, E. J. 1976. The vertical and seasonal distribution of chlorophyll in lakes of the experimental Lake Area, northwest Ontario: implications for primary production estimates. Limnol. Oceanogr. **21:** 767-783.

Gabriel, W., B. E. Taylor, and S. Kirsch-Prokosch. 1987. Cladoceran birth and death rates estimates: experimental comparison of egg-ratio methods. Freshwater Biology **18:** 361-372.

Haney, J. F. and D. J. Hall. 1973. Sugar coated *Daphnia*: a preservation technique for Cladocera. Limnol. Oceanogr. **18:** 331-333.

Hays, G. C., H. Kennedy, and B. W. Frost. 2001. Individual variability in diel vertical migration of a marine copepod: Why some individuals remain at depth when others migrate? Limnol. Oceanogr. **46:** 2050-2054.

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

Lampert, W. 1986. Limnologie: funktionelle Beziehungen in Ökosystemen; Kaskadeneffekte in See-Ökosystemen: die Bedeutung der tagesperiodischen Vertikalwanderung des Zooplankton. Verh. Dtsch. Zool. Gesell. **79:** 105-116.

Lampert,W. 1987. Vertical migration of freshwater zooplankton: Indirect effects of vertebrate predators on algal communities., p. 291-298. *In* [eds.], W.C.Kerfoot and A.Sih, Predation: direct and indirect impacts on aquatic communities. University press of England.

Lampert, W. 1992. Zooplankton vertical migrations: Implications for phytoplankton-zooplankton interactions. Arch. Hydrobiol. Beih. Ergebn. Limnol. **35:** 69-78.

Lampert, W. 1993. Ultimate causes of diel vertical migration of zooplankton: New evidence for the predator-avoidance hypothesis. Arch. Hydrobiol. **39:** 79-88.

Loose, C. and P. Dawidowicz. 1994. Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. Ecology **75:** 2255-2263.

Marker, A. E., E. A. Nusch, H. Rai, and B. Riemann. 1980. The measurement of photosynthetic pigments in freshwaters and standardization of methods: conclusions and recommendations. Arch. Hydrobiol. **14:** 91-106.

McIntosh, A. R. and C. R. Townsend. 1996. Interactions between fish, grazing invertebrates and algae in a New Zealand stream: A trophic cascade mediated by fish induced changes to grazer behaviour? Oecologia **108:** 174-181.

Paloheimo, J. E. 1974. Calculation of instantaneous birth rate. Limnol. Oceanogr. **19:** 692-694.

Schmitz, O. J., A. P. Beckerman, and K. M. O'Brien. 1997. Behaviorally mediated trophic cascades: Effects of predation risk on food web interactions. Ecology **78:** 1388-1399.

Schmitz, O. J., P. A. Hambäck, and A. P. Beckerman. 2000. Trophic cascades in terrestrial systems: A review of the effects of carnivore removals on plants. Am. Nat. **155:** 141-153.

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

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

Stibor, H. and J. Lüning. 1994. Predator-induced phenotypic variation in the pattern of growth and reproduction in Daphnia-hyalina (Crustacea, Cladocera). Funct. Ecol. **8:** 97-101.

Stich, H.-B. and W. Lampert. 1981. Predator evasion as an explanation of diurnal vertical migration by zooplankton. Nature **293:** 396-398.

Tessier, A. J. and P. Woodruff. 2002. Cryptic trophic cascade along a gradient of lake size. Ecology **83:** 1263-1270.

Trussell, G. C., P. J. Ewanchuk, and M. D. Bertness. 2002. Field evidence of trait-mediated indirect interactions in a rocky intertidal food web. Ecol. Lett. **5:** 241-245.

Turner, A. M. and G. G. Mittelbach. 1990. Predator avoidance and community structure - interactions among piscivores, planktivores, and plankton. Ecology **71:** 2241-2254.

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

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

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

Williamson, C. E., R. W. Sanders, R. E. Moeller, and P. L. Stutzman. 1996. Utilization of subsurface food resources for zooplankton reproduction: Implications for diel vertical migration theory. Limnol. Oceanogr. **41:** 224-233.

Winder, M., M. Boersma, and P. Spaak. 2003. On the cost of vertical migration: are feeding conditions really worse at greater depths? Freshwater Biology **48:** 383-393.

Zaret, T. M. and J. S. Suffern. 1976. Vertical migration in zooplankton as a predator avoidance mechanism. Limnol. Oceanogr. **21:** 804-813.

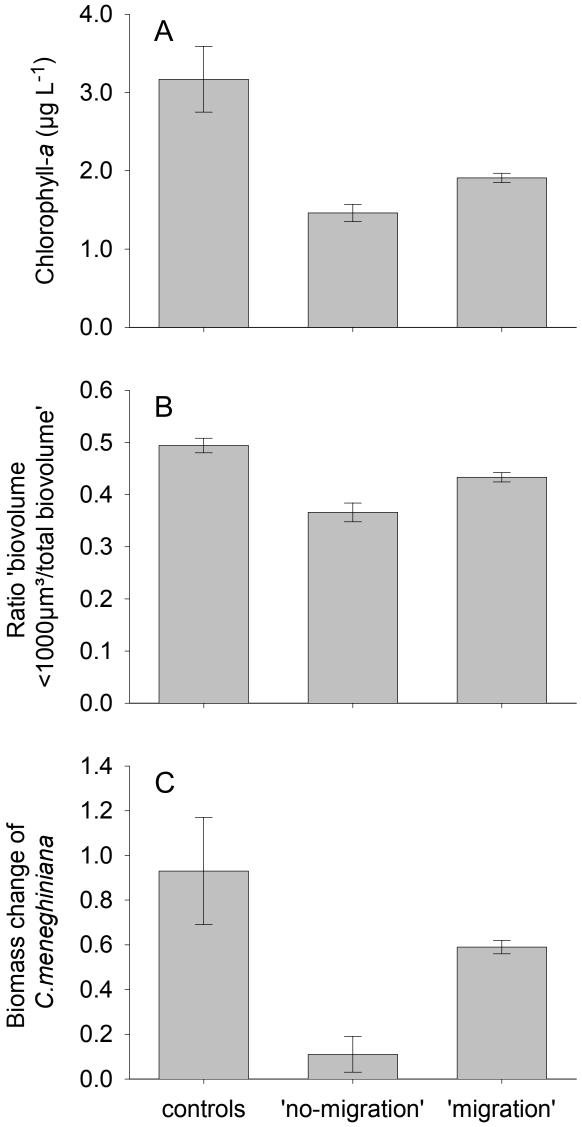


Fig. 1: Phytoplankton quantity and composition in the treatments of the mesocosm experiment. For reasons of clarity the two control treatments are represented in one bar. A: Chlorophyll-*a*, B: Ratio ‘biovolume <1000µm³/total biovolume’, C: Biomass change of *C. meneghiniana*. Error bars represent ± 1 SE.

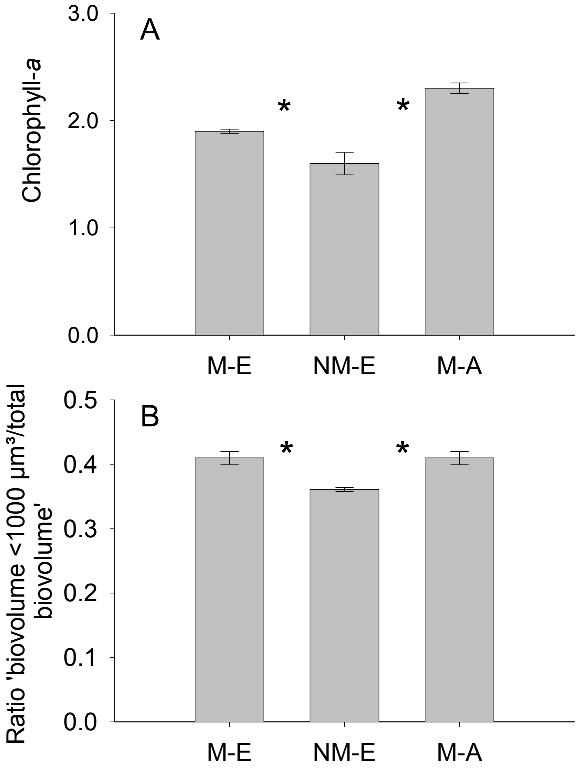


Fig. 2: Phytoplankton quantity and composition in the treatments of the laboratory experiment. A: Chlorophyll-*a*, B: Ratio ‘biovolume <1000µm³/total biovolume’. Error bars represent ± 1 SE. Stars represent significant differences calculated with contrasts between the corresponding treatments.

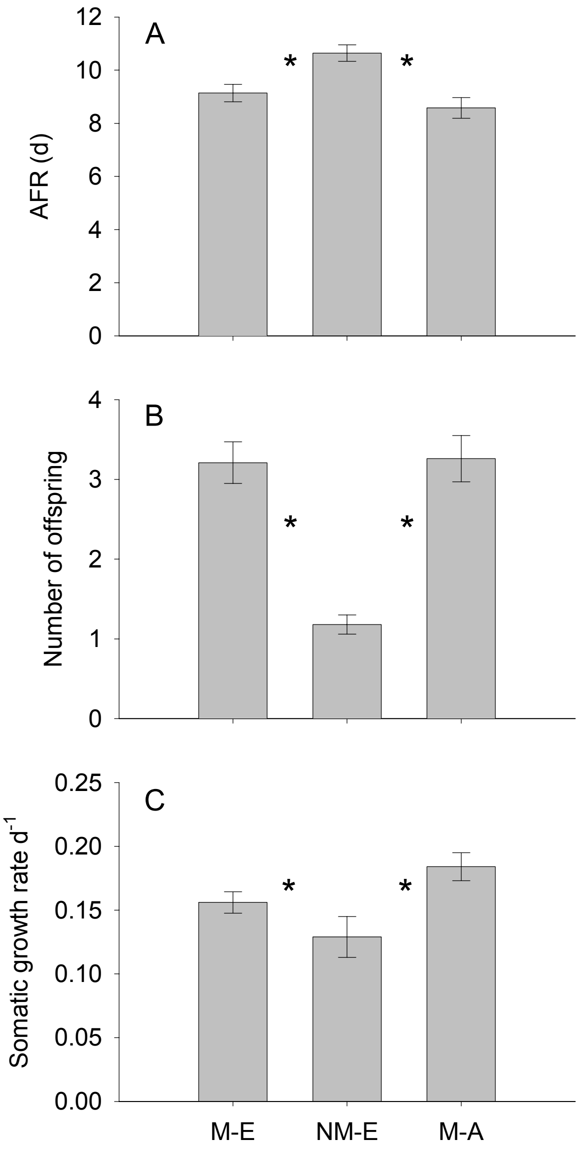


Fig. 3: Life history parameters in the treatments of the laboratory experiment reflecting conditions as experienced by their counterparts in nature when either ‘migrating’ (M-A), ‘non-migrating’ (NM-E), or staying in the epilimnion although the rest of the population migrates (M-E). A: AFR, B: Egg per *Daphnia*, C: Somatic growth rate d^{-1}. Error bars represent ± 1 SE. Stars represent significant differences calculated with contrasts between the corresponding treatments.

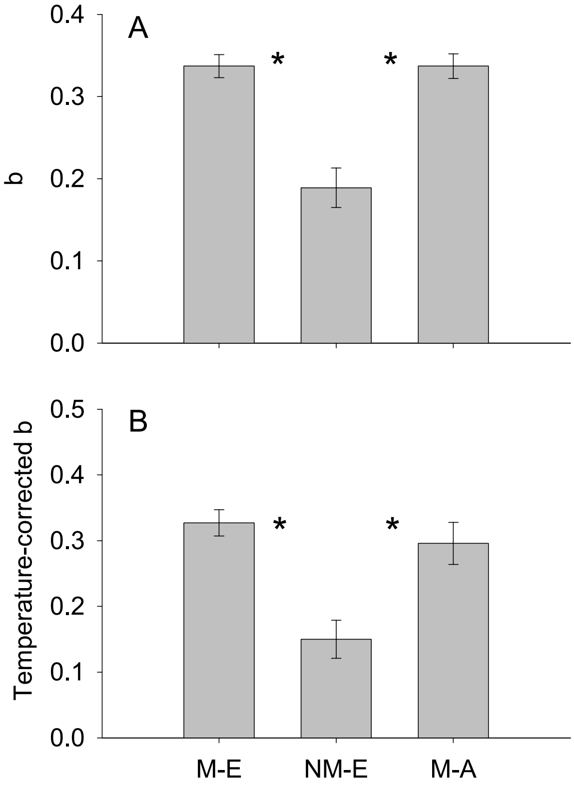


Fig. 4: Instantaneous birth rate (b) in the treatments of the laboratory experiment without (A) and with (B) temperature correction. Error bars represent ± 1 SE. Stars represent significant differences calculated with contrasts between the corresponding treatments.

Paper 4

Effects of a fluctuating temperature regime experienced by *Daphnia* during diel vertical migration on *Daphnia* life history parameters

(Reichwaldt, Wolf and Stibor, submitted to Hydrobiologia)

Effects of a fluctuating temperature regime experienced by *Daphnia* during diel vertical migration on *Daphnia* life history parameters

Elke S. Reichwaldt [1]*, Isabelle D. Wolf [1] & Herwig Stibor [1]

[1] Ludwig-Maximilians Universität, Department Biologie II, Aquatische Ökologie,
Karlstr. 25, D-80333 München, Germany

* Correspondence author
address: Ludwig-Maximilians Universität, Department Biologie II, Abteilung
Aquatische Ökologie, Karlstr. 25, D-80333 München, Germany
e-mail: ereichwaldt@web.de

Keywords: Cladocera, AFR, somatic growth rate, number of offspring

This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to *Hydrobiologia*.

Abstract

Many freshwater zooplankton species perform a diel vertical migration and spend the day within the lower, colder hypolimnion of stratified lakes. Trade-offs that arise from this migration have already attracted much attention and the cold temperature in the hypolimnion is thought to be responsible for the main costs of this behaviour. Until now laboratory experiments have almost exclusively investigated the situation where zooplankton stayed in either permanently cold water, permanently warm water, or in water with a constant intermediate temperature. Here, a fourth approach was realised in which two *Daphnia* species were exposed to a fluctuating temperature regime. In our experiment *Daphnia hyalina* and *Daphnia magna* either spent 24 hours in constant warm water (19°C), 24 hours in constant cold water (12°C), or spent 12 hours in warm water and 12 hours in cold water in an alternating way (fluctuating temperature regime). We expected the values of the life history parameters of *Daphnia* in the fluctuating temperature regime to be exactly halfway between the values of the life history parameters in the warm and cold treatments because the daphnids spent exactly half of the time in warm water, and half of the time in cold water. Results showed that age at first reproduction and egg development time were reduced at higher temperatures. In the fluctuating temperature regime the values of both parameters were exactly halfway between the values at permanently warm and cold temperature regimes. In contrast, somatic growth was increased at higher temperatures but was lower in the fluctuating temperature regime than expected from the mean somatic growth rate. This suggests that a fluctuating temperature regime experienced by migrating daphnids in stratified lakes involves additional costs for the daphnids.

Introduction

Diel vertical migration (DVM) of large zooplankton species or individuals is a wide-spread behaviour in the pelagic zone of freshwater and marine ecosystems. Individuals which perform DVM spend the day in the hypolimnion to reduce the probability of an attack by optically orientated predators but migrate upwards at sunset to feed within the upper epilimnion during the night (Lampert 1993). Stratified lakes of the temperate climatic zones have a warm epilimnion and a cold hypolimnion separated by a steep cline in temperature in the intermediate metalimnion during summer. This strong vertical variation in temperature has important effects on zooplankton individuals that migrate between the layers as they regularly experience large differences in temperature between day and night (Zaret & Suffern 1976; Stich & Lampert 1981; Gliwicz 1986; Neill 1990; Ringelberg 1991). Numerous studies have dealt with the influence of temperature on zooplankton growth and reproduction because temperature is, along with food quality and quantity, considered to be the major factor regulating zooplankton production (Bottrell 1975; Mitchell & Lampert 2000; Giebelhausen & Lampert 2001). All earlier experiments have shown, that daphnids had disadvantages when being reared in colder water and that these disadvantages were mainly caused by a higher age at first reproduction (Orcutt & Porter 1983; Orcutt & Porter 1984) and a slower growth rate (Orcutt et al. 1983; Orcutt et al. 1984; Sakwinska 1998; Mitchell et al. 2000; Giebelhausen et al. 2001). Data on the effect of temperature on the clutch size are not as clear. Orcutt & Porter (1983; 1984) found the biggest clutch sizes at intermediate temperatures, whereas Giebelhausen & Lampert (2001) found a decreasing clutch size with increasing temperature, but concluded that generally food concentration has a stronger effect on clutch size than temperature.

Based on these previous studies it is obvious that staying in the cold hypolimnion entails physiological disadvantages for the zooplankton. For example reduced population growths caused by lower temperatures in deep water are thought to be the main costs for migrating zooplankton (Dawidowicz & Loose 1992; Loose & Dawidowicz 1994). Thus, diel vertical migration can be explained as a possible solution to trade-offs between growth and predation risk at different depths. However, migrating zooplankton not only experience a low temperature during the day, but also a diurnal, regular change in temperature due to their migration in stratified lakes. In this context, the question then arises whether migrating zooplankton have additional costs because of this regular change in temperature two times a day (e.g. costs for regularly re-adjusting metabolic rates). To our knowledge there are only two studies in which *Daphnia* performance has been studied under a fluctuating temperature regime (Orcutt et al. 1983; Stich & Lampert 1984). Stich & Lampert (1984) varied temperature simultaneously with the food concentration so that simple effects of temperature on life history parameters can not be determined. Orcutt & Porter (1983) compared life history parameters in a fluctuating temperature regime with life history parameters at an intermediate temperature. However it depends on the relationship between the life history parameter and temperature whether the value of a parameter at an intermediate temperature is equal to the value of the parameter in the fluctuating temperature regime. This would only be the case if the parameters follow a linear relationship to temperature. As most parameters have a negative exponential correlation with temperature, the values at intermediate temperatures are generally lower than at a fluctuating temperature. However, migrating daphnids do not experience an intermediate temperature but experience warm and cold temperatures in an alternating way. Therefore if we want to assess the costs or benefits of a fluctuating temperature regime experienced by migrating zooplankton, we must compare the values of the life history parameters in the fluctuating temperature regime with the calculated mean of the values of life history parameters in warm and cold temperature regimes (and not with the value at intermediate temperature). For example we expect the values of the life history parameters in the fluctuating temperature regime to be exactly halfway between the values in the warm and the cold temperature regimes, if *Daphnia* spends half of the time in warm, and half of the time in cold water, and if a fluctuating temperature regime does not imply any costs.

The laboratory experiment described here was performed parallel to extensive studies on the effects of DVM on *Daphnia*-phytoplankton interactions in Lake Brunnsee (Germany). It was conducted to investigate the influence of a diurnally fluctuating temperature regime (as experienced by *Daphnia* during migration) on the performance of *Daphnia*. The applied temperatures in the laboratory experiment were similar to the temperatures experienced by *Daphnia* Lake Brunnsee in our field experiment (epilimnion 19°C, hypolimnion 12°C). Although the lake experiment was only performed with *Daphnia hyalina* Leydig which is the naturally occurring *Daphnia* species in Lake Brunnsee, the laboratory experiment described here was conducted with with *D. hyalina* and *Daphnia magna* Straus to obtain a broader data basis. We experimentally exposed the two *Daphnia* species to a permanently cold, a permanently warm, and a fluctuating temperature regime. Measured life history parameters were somatic growth rate, age at first reproduction, egg development time, and number of first offspring

Materials and Methods

Two experiments were performed to quantify developmental differences in *Daphnia* caused by different temperature regimes as experienced during diel vertical migration. One experiment was conducted with *D. magna*, the other with *D. hyalina*. The basic design was identical in both experiments.

The experiments took place in a climatic chamber at a 12h light : 12h dark cycle. The daphnids were exposed to three different temperature regimes: permanently warm water (19°C), permanently cold water (12°C), or a cold/warm fluctuation regime (12°C/19°C), where the temperature changed every 12 hours from 12°C to 19°C or vice versa. This temperature range simulated the typical temperature conditions experienced by migrating daphnids in Lake Brunnsee during summer. The experimental design consisted of 100 ml-jars filled with sterile-filtered water from mesotrophic Lake Langbürgener See (Bavaria, Germany). The jars were sealed with plastic film against evaporation and were immersed in water baths that maintained temperatures within ±0.5°C of the desired temperatures. Water bath temperature was controlled via a flow heater. In the fluctuating temperature regime transition to either temperature took 30 min.

One juvenile *Daphnia* was held separately in each jar. The daphnids used in the experiments were individuals of the second clutch of synchronised females. They were put into the experiments within 12 hours after their birth. Both *Daphnia* species had been cultured in the laboratory for more than two years and were reared under the experimental light condition at a constant temperature of 19°C prior to the experiments. Individual daphnids in the experiments were exposed to a warm, a cold, or a fluctuating temperature regime throughout the experiment. Every second day *Scenedesmus obliquus* Turpin (Chlorophyceae) was given as food for the daphnids in a concentration equivalent to 1 mgC l^{-1}. This amount of algae represents food in excess for daphnids so that the experiments ran under a high food supply. In both experiments each treatment was replicated 40 times.

At the beginning of the experiments the body lengths of 16 *D. hyalina* and 18 *D. magna* siblings of the synchronised neonate daphnids were measured to the nearest 0.01 mm under a dissecting microscope using an image analysis system (analySIS 2.11) to calculate mean initial body lengths (mm). These individuals were not used in the experiment. After four days body lengths of all individuals in the experiment was determined as described above. At that time individuals did not yet carry any eggs in the gonads or brood chambers. Length was always measured from the top of the eye to the basis of the spine. All measured lengths (in mm) were converted into individual masses (ω) using the following equations:

(1) *D. magna*: $\omega = 12.583 \times \text{length}^{2.41}$ (Stibor 2002)

(2) *D. hyalina*: $\omega = 5.59 \times \text{length}^{2.21}$ (Stibor & Lüning 1994)

The somatic growth rate d^{-1} (g) was then calculated as

$$(3) \qquad g = \frac{\ln \omega_{adult} - \ln \omega_{neonate}}{t}$$

where ω_{adult} and ω_{neonate} are the masses of adult and neonate *D. magna* and *D. hyalina*, respectively, and t is time in days. All individuals were returned to their experimental jars immediately after being measured.

The experiments ran for 21 days. During this time the following additional parameters were measured: age at first reproduction (in days), egg development time of the first brood (in days), and number of offspring at first reproduction. Age at first reproduction was defined as the time from birth until the day when first eggs were visible in the brood chamber. Egg development time was considered as the time from when eggs became visible in the brood chamber until neonates were born.

The experiments with the two *Daphnia* species were analysed separately. Treatment effects on response variables were analysed statistically with a one-way ANOVA. All tests were calculated with SPSS for Windows 11.5.0. To assess costs or benefits for a migrating population that experience a fluctuating temperature regime we calculated the mean of each parameter in the fluctuating temperature regime and compared it to the calculated mean of the same parameter averaged across the cold and warm temperature regimes. Calculative the values of the life history parameters in the fluctuating temperature regime are expected to be exactly halfway between because daphnids experienced each temperature (warm and cold) for 12 hours per day. The statistical significance of these comparisons was evaluated with independent sample t-tests (SPSS for Windows 11.5.0).

Results

Mortality was high in the experiment with *D. hyalina*. Approximately 50 % of all *D. hyalina* individuals died within the first 4 days, most deaths occurring in the fluctuating temperature regime (33% of all deaths). In *D. magna* only 4 % of all individuals died during this experiment.

In both *D. magna* and *D. hyalina*, age at first reproduction decreased with increasing mean temperature (Fig. 1). *D. magna* required on average 12.0, 10.0 and 7.5 days until their first reproduction in the cold, fluctuating, and warm temperature regimes, respectively. The corresponding figures for *D. hyalina* were 12.2, 9.1 and 6.1 days. In both species these differences were statistically significant (*D. magna*: $F_{(2, 95)} = 336.12$; $p<0.001$; *D. hyalina*: $F_{(2, 60)} = 128.37$; $p<0.001$).

Somatic growth rate increased with increasing mean temperature in the experiments with *D. magna* (Fig. 1). The somatic growth rates were 0.12 d^{-1}, 0.16 d^{-1} and 0.27 d^{-1} (cold, fluctuating, warm temperature regime) and these differences were always statistically significant ($F_{(2, 99)} = 164.34$, $p<0.001$). In the experiments with *D. hyalina* the average somatic growth rate was also affected by the treatment ($F_{(2, 66)} = 90.96$, $p<0.001$), but here statistically significant differences were only found between the warm temperature regime and the two remaining treatments, which, in turn were similar (Fig. 1). The average somatic growth rates were 0.09 d^{-1}, 0.11 d^{-1}, and 0.24 d^{-1} in cold, fluctuating, and warm temperature regimes.

Egg development time decreased with increasing mean temperature (Fig. 1). Differences among treatments were always statistically significant (*D. magna*: $F_{(2, 95)} = 267.12$, $p<0.001$; *D. hyalina*: $F_{(2, 60)} = 48.61$, $p<0.001$). The eggs of *D. magna* required on average 5.1, 3.2 and 2.0 days for development in the cold, fluctuating, and warm temperature regimes. The corresponding egg development times of *D. hyalina* were 3.6, 2.5 and 1.7 days.

In both *Daphnia* species the mean number of offspring per female at first reproduction was similar in all three treatments. *D. magna* had on average 9.7 offspring per female (SE = 0.30, n = 98) while *D. hyalina* had a mean of 3.4 offspring per female (SE = 0.15, n = 63) (*D. magna*: $F_{(2, 95)} = 0.21$, $p = 0.880$; *D. hyalina*: $F_{(2, 60)} = 0.17$, $p = 0.840$).

Comparing life history parameters in the fluctuating temperature regime with the calculated mean of the parameters averaged across the cold and warm temperature regimes, somatic growth rate in both *Daphnia* species was lower in the fluctuating temperature regime than expected (Fig. 1; *D. magna*: $t_{(98.555)} = 2.799$, $p = 0.006$; *D. hyalina*: $t_{(57.759)} = 3.575$, $p = 0.001$). All other parameters (age at first reproduction, egg development time, and number of offspring at first reproduction) had similar values in the fluctuating regime compared to the calculated mean of the warm and cold temperature regimes and did not show a significant difference between the two means (all $p > 0.05$).

Discussion

In our experiments, temperature had striking effects on various life history parameters of daphnids such as age at first reproduction, somatic growth rate, and egg development time. Higher temperatures generated a younger age at first reproduction, higher somatic growth rates, and shorter egg development times. These results support earlier studies (e.g. Bottrell 1975; Orcutt et al. 1983; Orcutt et al. 1984; Stich et al. 1984; Giebelhausen et al. 2001).

Comparing the mean number of eggs per female there was no significant difference between the three temperature treatments in our experiments. Similarly, in the study of Giebelhausen & Lampert (2001) the mean number of eggs per female of *D. magna* was equal in the experiment with two similarly different temperature regimes (15°C and 20°C). This contrasts with other studies in which clutch size was highest at an intermediate temperature regime (15°C) (Orcutt et al. 1983; Orcutt et al. 1984).

Our experiments were designed to test for costs due to a fluctuating temperature regime corresponding to the thermal environment experienced by individuals that perform DVM. Therefore, we compared the calculated mean of the values of life history parameters in warm and cold temperature regimes with the values of the life history parameters in the fluctuating temperature regime. We expected values of life history parameters in the fluctuating temperature regime to be exactly halfway between the values in the warm and the cold temperature regimes because *Daphnia* species spent half of the time in warm, and half of the time in cold water. While age at first reproduction and egg development time in the fluctuating temperature regime conformed to a mean value of the parameters in the warm and the cold temperature regimes, somatic growth rate of both *Daphnia* species was lower in the fluctuating temperature regime than expected. This means that both *Daphnia* species had a bigger disadvantage from being in a fluctuating temperature regime than was expected from the average of the permanently warm and permanently cold temperature regimes. Somatic growth rate of *D. hyalina* under a fluctuating temperature regime was even as low as at constant cold temperature which points to high costs of a fluctuating temperature. A possible explanation for these costs is that the process of re-adjusting metabolic rates to a prevailing temperature needs some time in which metabolism might only be sub-optimal for the daphnids. Migrating daphnids would then experience sub-optimal metabolism every time they cross the thermocline.

The results showed that a regular change in temperature as experienced by migrating *Daphnia* implied high costs for the daphnids due to the fact that somatic growth rates were much lower than expected in our experiments. Somatic growth rate is an important factor for population fitness as it directly affects population development for example by an earlier start of *Daphnia* reproduction, or a larger size of adult *Daphnia* which in turn involves a larger maximum brood size (Hülsmann 2001).

As a consequence of these costs due to a fluctuating temperature regime, vertically migrating *Daphnia* should not only minimise the time they spend within the hypolimnion but should migrate downwards as little as necessary. This is due to the fact that values of life history parameters under a fluctuating temperature regime can be similar to the values at the lowest temperature they experience as seen in our experiment. On the other hand, *Daphnia* should migrate from the hypolimnion into the warmer epilimnion even though the somatic growth rate of the individuals staying permanently in the cold water was similar to that of the migrating individuals in our experiment, because of a shorter egg development time when daphnids migrated.

The regular change in temperature experienced by migrating daphnids is not an isolated case as almost all organisms have to face diurnally changing temperatures. Consequently, we think that the costs demonstrated in our experiments are very wide-spread and can have a strong influence on population dynamics.

Acknowledgements

We wish to thank Sebastian Diehl for valuable suggestions on the manuscript. During the experiments E.S. Reichwaldt was supported by a scholarship for PhD students from the University of Munich (LMU) and I.D. Wolf was supported by a scholarship from Studienstiftung des Deutschen Volkes.

Reference List

Bottrell, H. H., 1975. The relationship between temperature and duration of egg development in some epiphytic Cladocera and Copepoda from the river Thames, Reading, with a discussion of temperature functions. Oecologia 18: 63-84.

Dawidowicz, P. & C. J. Loose, 1992. Metabolic costs during predator-induced diel vertical migration of *Daphnia.* Limnology and Oceanography 37: 1589-1595.

Giebelhausen, B. & W. Lampert, 2001. Temperature reaction norms of Daphnia magna: the effect of food concentration. Freshwater Biology 46: 281-289.

Gliwicz, Z. M., 1986. Predation and the evolution of vertical migration in zooplankton. Nature 320: 746-748.

Hülsmann, S., 2001. Reproductive potential of *Daphnia galeata* in relation to food conditions: implications of a changing size-structure of the population. Hydrobiologia 442: 241-252.

Lampert, W., 1993. Ultimate causes of diel vertical migration of zooplankton: New evidence for the predator-avoidance hypothesis. Archiv für Hydrobiologie 39: 79-88.

Loose, C. & P. Dawidowicz, 1994. Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. Ecology 75: 2255-2263.

Mitchell, S. E. & W. Lampert, 2000. Temperature adaptation in a geographically widespread zooplankter, *Daphnia magna.* Journal of Evolutionary Biology 13: 371-382.

Neill, W. E., 1990. Induced vertical migration in copepods as a defence against invertebrate predation. Nature 345: 524-526.

Orcutt, J. D. & K. G. Porter, 1983. Diel vertical migration by zooplankton: Constant and fluctuating temperature effects on life history parameters of *Daphnia*. Limnology and Oceanography 28: 720-730.

Orcutt, J. D. & K. G. Porter, 1984. The synergistic effects of temperature and food concentration on life history parameters of *Daphnia*. Oecologia 63: 300-306.

Ringelberg, J., 1991. A mechanism of predator-mediated induction of diel vertical migration of *Daphnia hyalina*. Journal of Plankton Research 13: 83-89.

Sakwinska, O., 1998. Plasticity of *Daphnia magna* life history traits in response to temperature and information about a predator. Freshwater Biology 39: 681-687.

Stibor, H., 2002. The role of yolk protein dynamics and predator kairomones for the life history of *Daphnia magna*. Ecology 83: 362-369.

Stibor, H. & J. Lüning, 1994. Predator-induced phenotypic variation in the pattern of growth and reproduction in Daphnia-hyalina (Crustacea, Cladocera). Functional Ecology 8: 97-101.

Stich, H.-B. & W. Lampert, 1981. Predator evasion as an explanation of diurnal vertical migration by zooplankton. Nature 293: 396-398.

Stich, H.-B. & W. Lampert, 1984. Growth and reproduction of migrating and non-migrating *Daphnia* species under simulated food and temperature conditions of diurnal vertical migration. Oecologia 61: 192-196.

Zaret, T. M. & J. S. Suffern, 1976. Vertical migration in zooplankton as a predator avoidance mechanism. Limnology and Oceanography 21: 804-813.

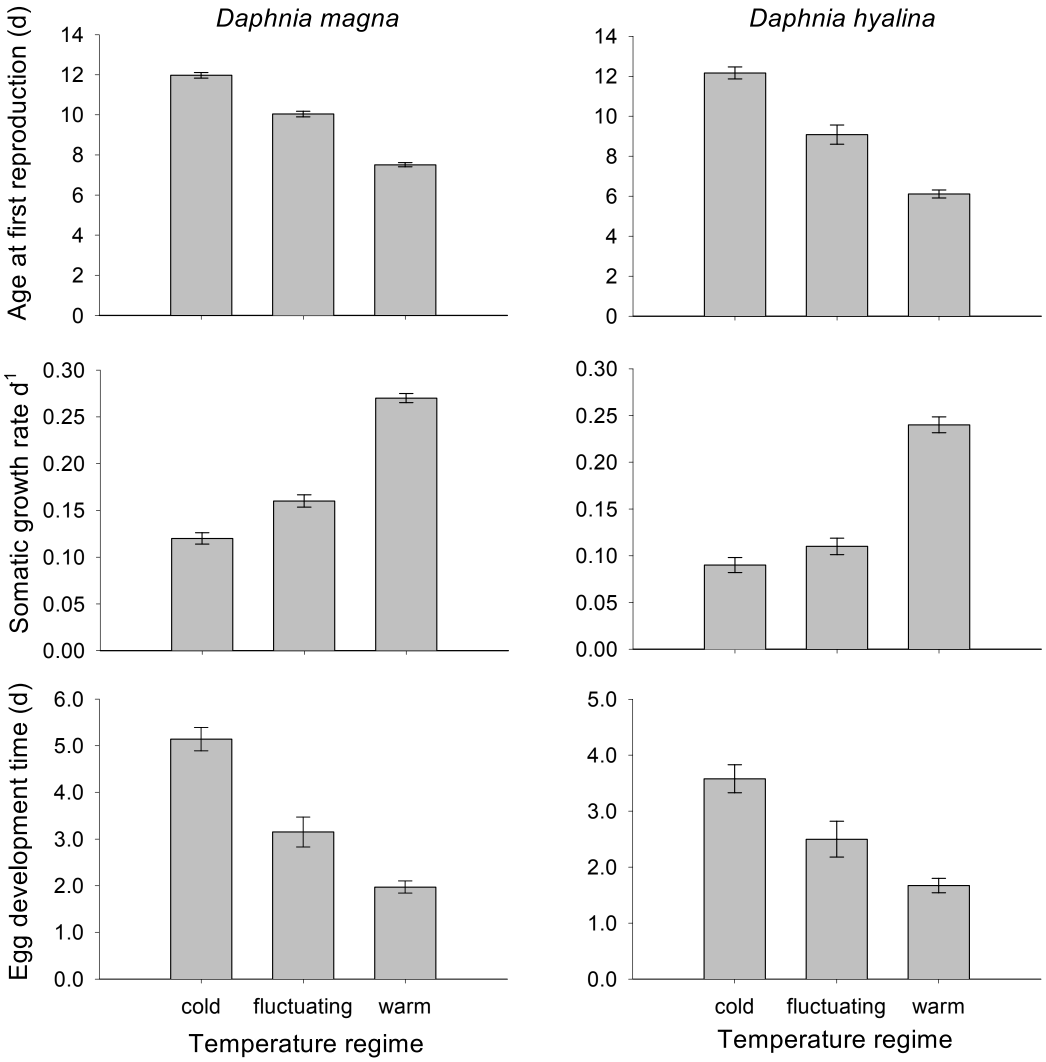


Fig. 1: Mean age at first reproduction (d), mean somatic growth rate d^{-1}, and mean egg development time in the three temperature regimes for *Daphnia magna* and *Daphnia hyalina*. Error bars represent ± 1SE.

Acknowledgements

Primarily, I want to thank PD Dr. Herwig Stibor for his amicable support during the project. He always had plenty of time for inspiring discussions and his never-ending ideas greatly enriched this study.

My very special thanks goes to Prof. Dr. Sebastian Diehl for his endless support during the 3 ½-year period. I also want to thank him for his countless helpful comments on all manuscripts.

I especially want to thank Isabelle D. Wolf who conducted several experiments for me during her diploma thesis.

Angelika Wild for any technical support in the laboratory and for the very welcome daily coffee break during the field experiments.

Achim Weigert who was always willing to get out to the lake at 6 a.m. to help me sampling the mesocosms. He was also a great help in the laboratory doing chemical analyses.

All students who helped me with my field experiments. I especially want to thank Maren Striebel whose help was almost endlessly and who took over the part of Angelika Wild whenever she was not there by preparing coffee and tea for me.

Well, there are still many others I have to thank. Pia Gabriel, Michael Effenberger, Maren Striebel, Gabi Sigl for giving comments on earlier versions of the manuscript. Pia Gabriel, Hugh Boyd, Renate Boyd, Martin Seidel, and Markus Knapek for linguistic improvements.

Mechthild Kredler for tending my *Daphnia* clones.

Karlheinz Seidel who provided me with material for the field experiment.

Thanks to everybody in the Department of Aquatic Ecology. It was always enjoyable and stimulating working in the Karlstrasse!

I want to thank my parents for their steadfast support and understanding, and my brother, who was always available for help and advice.

Finally, I want to thank my long-time companion Martin Seidel for giving me strength and inspiring me.

To my grandparents

Curriculum vitae

Name	Elke Susanne Reichwaldt
Date of birth	27.04.1974
Abitur 1993	Feodor-Lynen Gymnasium, Planegg, Germany; mark: 2.0
Studies in Biology 1993-1999	Ludwigs-Maximilians-Universität München, Germany
Studies in life-sciences 9/1996-3/1997	University College Galway, Ireland
Diplom 1999	Subjects: Ecology, Zoology, Botany and Neurobiology; mark:1.1
Travelling abroad 8/1999-3/2000	Travelling South America, New Zealand and Australia to broaden the personal horizon
Work experience 8/2000 - 9/2000	Full-time student job with the purchasing Department of MAN Technologie AG, München, Germany
Post-graduate student 11/2000 - 2004	Ludwigs-Maximilians-Universität München, Department Biologie II, Aquatic Ecology Group, Germany

Additional scientific qualifications

Part-time research assistant at the Zoological Collection of the State of Bavaria (Zoologische Staatssammlung) in München, Germany (November 2000, February to May 2001)

Co-supervisor of Isabelle D. Wolf by helping her to complete her diploma thesis successfully (February 2001 to January 2002)

Part-time project research assistant and co-supervisor of a field experiment of the Department of Aquatic Ecology (Ludwigs-Maximilians-Universität München, Germany) including co-supervision of Maren Striebel, Pia Gabriel und Florian Haupt by helping them to complete their diploma theses successfully (May 2003 to March 2004)

Member of the ‘Verband deutscher Biologen’ (Vdbiol) since 2001

Member of the ‘International Association of Theoretical and Applied Limnology’ (SIL) since 2002

Member of the ‘American Society of Limnology and Oceanography’ (ASLO) since 2004

Participation in meetings

’VI. Internationale Naturschutztagung’, organised by ‘Arbeitsgruppe Artenschutz Thüringen e.V.’, in Bad Blankenburg, Germany, 1997

Annual meeting of the ‘Deutsche Gesellschaft für Limnologie’ (DGL) and of the German section of ‘Societas Internationalis Limnologiae’ (SIL) in Braunschweig, Germany, 2002

‘Third Symposium for European Freshwater Sciences’ (SEFS3) in Edinburgh, U.K., 2003

Annual meeting of the ‚Deutsche Gesellschaft für Limnologie’ (DGL) and of the German section of ‘Societas Internationalis Limnologiae’ (SIL) in Cologne, Germany, 2003

Submitted paper to the ’ ASLO Summer meeting 2004’ (American Society of Limnology and Oceanography) in Savannah, USA, 2004

Hiermit bestätige ich, dass Frau Dipl. Biol. Elke Reichwaldt ihre Doktorarbeit mit dem Titel „The effects of diel vertical migration of *Daphnia* on zooplankton-phytoplankton interactions: laboratory and field experiments“ einreichen darf.

PD Dr. Herwig Stibor

München, 16.März 2004