Vertical distribution of *Daphnia* in a trade-off between food and temperature

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Summary

In a thermally stratified water column with a deep-water chlorophyll maximum (DCM), *Daphnia* face a trade-off between optimum temperature and food availability. The high temperatures in the surface layer (epilimnion) accelerate development, whereas the high food availability in the deep layer (hypolimnion) increases fecundity. I investigated the influence of this trade-off on the fitness and on the vertical distribution of *Daphnia*. I tested (1) whether *Daphnia* actively choose the best vertical habitat, implying that they can assess their fitness in the different layers, and (2) whether their distribution is “dynamic”, i.e., individuals frequently migrate between different habitats and allocate the time they spent there according to the fitness value.

I measured the vertical distribution of a *Daphnia* population in 11 m high indoor mesocosms, in which I established a hypolimnetic DCM in different temperature gradients. The zooplankton samples obtained from this experiment were counted and sized with the bench-top version of an optical plankton counter, which gave very accurate measurements of the total number and the size composition of *Daphnia* in each depth. In parallel to the vertical distribution, I measured the fitness that *Daphnia* can achieve in the different layers of the mesocosms. I transferred water from the surface and from the hypolimnion into flow-through systems, in which I assessed the juvenile growth rate of *Daphnia* as a proxy for fitness.

Both the relative fitness and the proportion of *Daphnia* in the hypolimnion increased with the hypolimnetic temperature (lower costs). However, ontogenetic stages differed in their vertical habitat choice. Large *Daphnia* avoided light, resulting in diel vertical migration. This was most likely a “residual” response of *Daphnia* to the potential threat of being preyed upon by visually hunting fish. Small *Daphnia* that are less conspicuous did not show such a response and mostly dwelled near the surface, because new-born *Daphnia* still dispose of egg-yolk reserves, which makes feeding in the cold hypolimnion less important.

Most *Daphnia* selected the vertical habitat where they achieved the highest fitness. However, a certain proportion of the population was always found in the less profitable habitat. Using a system of vertical perspex tubes (with a DCM and various temperature gradients), I demonstrated that individual egg-bearing females asynchronously move up and down the water column in intervals of about 5 hours, leading to a dynamic distribution of the population. These frequent migrations might be a mechanism to take advantage of both, high temperature in the epilimnion and high food availability in the hypolimnion. The time they spent feeding in the colder hypolimnion increased with the hypolimnetic temperature. Neonates with egg-yolk reserves, which did not experience the trade-off, continuously stayed in the warm epilimnion, regardless of the temperature gradient.

In summary, *Daphnia* apply several complementary behavioural strategies to optimise their fitness in the vertical trade-off between food and temperature. Additionally, other vertical gradients (e.g., of oxygen or predation) should similarly influence the fitness and hence the vertical distribution of *Daphnia* in the field.

Zusammenfassung

In einem durch Temperatur geschichteten See mit einem Tiefen-Chlorophyll-Maximum („deep-water chlorophyll maximum“, DCM) müssen Daphnien zwischen hohen Temperaturen und hoher Futterverfügbarkeit abwägen (Trade-off). Die hohen Temperaturen an der Oberfläche (Epilimnion) beschleunigen ihr Wachstum, während die hohe Futtermenge in der tiefen, kälteren Schicht (Hypolimnion) ihre Eiproduktion steigert. In meiner Arbeit habe ich den Einfluß dieses Trade-offs auf die Fitness und die vertikale Verteilung von *Daphnia* untersucht. Dazu habe ich erstens getestet, ob Daphnien das profitabelste vertikale Habitat aufsuchen, was voraussetzt, daß sie ihre Fitness in den unterschiedlichen Tiefen abschätzen können. Zweitens habe ich untersucht, ob die Verteilung der Daphnienpopulation dynamisch ist, d.h., ob die Individuen kontinuierlich zwischen den Habitaten wechseln und ihre Zeit zwischen diesen Habitaten entsprechend der jeweiligen Profitabilität aufteilen.

Ich habe die Vertikalverteilung einer Daphnienpopulation in 11 m hohen Mesokosmen gemessen, in deren Wassersäule ich ein DCM und verschiedene Temperaturgradienten etabliert habe. Die Zooplanktonproben aus diesem Versuch wurden in einem optischen Planktonzählgerät gezählt und vermessen, welches die Gesamtanzahl und die Größenverteilung der Daphnien in den Proben aus verschiedenen Tiefen sehr genau wiedergab. Parallel zur Vertikalverteilung habe ich die Fitness gemessen, die Daphnien in den jeweiligen Schichten der Mesokosmen erreichen können. Dazu habe ich Wasser aus dem Epilimnion und dem Hypolimnion in Durchfluss-Systeme überführt, um darin die juvenile Wachstumsrate von Daphnien als Annäherung an deren Fitness zu bestimmen.

Sowohl die relative Fitness als auch der Anteil der Daphnienpopulation im Hypolimnion erhöhte sich mit der Temperatur im Hypolimnion (geringere Kosten). Dennoch bevorzugten die verschiedenen Stadien von *Daphnia* unterschiedliche vertikale Habitate. Große Daphnien vermieden Licht, so dass sie tagesperiodische Vertikalwanderungen vollzogen. Diese Reaktion war vermutlich eine Restreaktion der Daphnien auf die mögliche Bedrohung durch optisch orientierte, planktivore Fische. Kleine Daphnien sind unauffälliger und wanderten daher nicht. Darüber hinaus verfügen Neugeborene meist noch über Eidotter, so dass sie nicht unbedingt im kalten Hypolimnion zu fressen brauchen und statt dessen im warmen Epilimnion bleiben können.

Demzufolge wählten die meisten Daphnien das vertikale Habitat, in dem sie die höchste Fitness erzielten. Trotzdem hielt sich immer ein gewisser Anteil der Population im weniger profitablen Habitat auf. In einem System aus durchsichtigen vertikalen Röhren mit einem DCM und verschiedenen Temperaturgradienten konnte ich zeigen, dass eitragende Weibchen in der Wassersäule in Abständen von etwa fünf Stunden auf und ab schwammen, was zu einer dynamischen Verteilung der Population führte. Diese kurzzeitigen Wanderungen könnten ein Mechanismus der Daphnien sein, sowohl von der Temperatur im Epilimnion als auch von der hohen Futterkonzentration im Hypolimnion zu profitieren. Die Zeit, die die eitragenden Weibchen zum Fressen im Hypolimnion verbrachten, stieg mit der Temperatur in dieser Schicht. Neugeborene waren aufgrund ihrer Eidotterreserven keinem Trade-off ausgesetzt und blieben daher in allen Temperaturgradienten an der Oberfläche.

Demnach verfügen Daphnien über mehrere sich ergänzende Strategien, um ihre Fitneß im vertikalen Trade-off von Futter und Temperatur zu optimieren. In einem See beeinflussen darüber hinaus auch noch andere Gradienten (z.B der Sauerstoffkonzentration oder des Prädationsrisikos) die Fitnessverteilung, was sich ebenso in der Vertikalverteilung einer Daphnienpopulation widerspiegeln sollte.

Introduction

Introduction

1. *Daphnia* in a heterogeneous environment

Lakes are heterogeneous environments, which harbour many vertical gradients. During summer, a temperature gradient stratifies the water column and divides it into three vertical habitats, a warm surface layer, the epilimnion, a cool layer at the bottom, the hypolimnion, and an intermediate layer, called the metalimnion (Lampert & Sommer 1997). Another gradient originates from the attenuation of light in the water column (e.g., Fee 1976). Furthermore, stratified lakes contain vertical gradients of chemicals such as nutrients (e.g., Fee 1976, Caraco *et al.* 1989, Watanabe 1992), or oxygen (Williamson *et al.* 1996, Winder *et al.* 2003a).

Due to these gradients, each of the vertical habitats is characterised by a different temperature, light intensity, and chemical conditions, which jointly influence growth and survival of planktonic organisms. These organisms, such as bacteria, heterotrophic nanoflagellates, protozoans, phytoplankton and zooplankton, prefer different environmental conditions and therefore choose different vertical habitats, resulting in different vertical distributions. Additionally, the vertical distribution of organisms is influenced by competition and predation (e.g., Leibold 1991, Leibold & Tessier 1997).

Many studies have investigated the influences of environmental cues and gradients on the vertical distribution of the zooplankter *Daphnia* spp. (Crustacea, Cladocera) (e.g., Loose 1993, Williamson *et al.* 1996, Leech & Williamson 2001, Lampert *et al.* 2003). *Daphnia* are of high interest to freshwater research, because they play a central role in the pelagic food web: On the one hand, they are efficient filter feeders, grazing on bacteria, heterotrophic flagellates, protozoans, and phytoplankton (e.g., Lampert 1987). On the other hand, they are an important food source for planktivorous fish (e.g., Ringelberg *et al.* 1991, Lemke *et al.* 2003).

The vertical distribution of a *Daphnia* population results from the habitat choice of its individuals. Although *Daphnia* are plankton organisms, they perform impressive diel vertical migrations with amplitudes up to 60 m (Hutchinson 1967), clearly indicating their capability of directed and efficient swimming. Therefore, it is likely that *Daphnia* actively move into their preferred vertical habitat (Leibold & Tessier 1997).

Figure 1: *Daphnia hyalina x galeata*

The habitat choice of *Daphnia* should depend on the vertical “fitness distribution”, that is the fitness *Daphnia* can achieve in each layer. The fitness distribution is determined by the joint action of abiotic and biotic environmental factors such as temperature, light, oxygen, food availability, competition, and predation (e.g., De Robertis 2002, Lampert *et al.* 2003). Of particular importance are temperature and light.

Daphnia respond to all of these factors in a variety of ways: They avoid low temperatures (Calaban & Makarewicz 1982, Haney 1993, Lampert *et al.* 2003), which decelerates growth (Orcutt & Porter 1984, Giebelhausen & Lampert 2001) and egg-development (Venkataraman & Job 1980, Kerfoot 1985).

The influence of light on their habitat selection is complex: When exposed to UV-radiation or to visually oriented, planktivorous fish, *Daphnia* choose different vertical habitats during day and at night, resulting in a diel vertical migration (Ringelberg *et al.* 1991, Ringelberg & Van Gool 2003). During day, *Daphnia* avoid light and migrate down into the dark hypolimnion, where the threat of UV-radiation or fish predation is low. At night, when mortality caused by UV radiation respectively fish predation is less important, *Daphnia* return into the warm and food rich epilimnion. Additionally, light effects the vertical distribution of photoautotrophic bacteria and algae (e.g., Fee 1976), which serve as food for *Daphnia* (e.g., Lampert 1987), and which produce oxygen. Both, food (e.g., Cuddington & McCauley 1994, Larsson 1997) and oxygen concentration (e.g., Lass *et al.* 2000, Winder *et al.* 2003a) again influence the habitat choice of *Daphnia.*

Even though *Daphnia* actively select a preferred vertical habitat, the individuals undertake short forays into other vertical habitats with different environmental conditions. These migrations occur on a shorter time scale than diel vertical migration (Haney 1988, Lampert *et al.* 2003). Such a behaviour can be interpreted as (1) food search behaviour, or (2) a mechanism of time allocation between spatially separated habitats which are advantageous with respect to different environmental factors (trade-off).

(1) *Daphnia* are capable of locating newly created food patches in large water bodies (Haney 1993, Plath 1998) in a very short time, but the underlying mechanism is unclear. Chemical stimuli have been suggested, and *Daphnia* were attracted by the “smell” of edible algae in some studies (Van Gool & Ringelberg 1996, Lauren-Määttä *et al.* 1997), but not in others (Roozen & Lürling 2001). Alternatively, *Daphnia* might perform food search migrations and find food patches coincidentally (Haney 1993, Plath 1998). In this case, *Daphnia* would leave a chosen habitat in certain time intervals to probe other habitats.

(2) The vertical distribution of *Daphnia* is not effected by one environmental factor alone, but by the joint influence of temperature, light, oxygen, food, competition, and predation. If the optimal depth with respect to one environmental factor does not coincide with the optimal depth for another, *Daphnia* are exposed to a trade-off. For example, in lakes with a surface algal maximum and high UV-radiation, *Daphnia* are exposed to the trade-off between foraging in the epilimnion and hiding from damaging UV-radiation (Haney 1988). Haney (1988) suggested that individuals undertake short forays between the food rich epilimnion and the safe hypolimnion, which means, on average these *Daphnia* have a low exposure to UV-radiation and a moderate availability of food. As there is no trigger to synchronise these short term migrations, the individuals move randomly between epilimnion and hypolimnion. From the vertical distributions of populations alone it is impossible to deduce the migration patterns of individuals (Pearré, 1979). The population seems to dwell statically, i.e., the distribution does not change, even though the individuals within the population may frequently migrate up and down. Such a distribution of a population has been termed “dynamic distribution” (Lampert & Grey 2003, Lampert *et al.* 2003).

The majority of studies on habitat choice and vertical migration of *Daphnia* have focused on the influence of fish predation (e.g., Flik & Vijverberg 2003, Primicerio 2003). However, fish are not always present, and in their absence or at night, mortality caused by visual hunters and UV-radiation does not play a role. In such a situation, the fitness and habitat choice of *Daphnia* is primarily determined by food and temperature.

2. The deep-water algal maximum or deep-water chlorophyll maximum (DCM)

The temperature gradient is similar in all thermally stratified lakes, with the temperatures being highest in the epilimnion and lowest in the hypolimnion. In contrast, the vertical distribution of phytoplankton varies between lakes and seasons (Fee 1976, Williamson *et al.* 1996), as it depends on nutrient availability and light. In nutrient rich lakes and early in the year, the highest phytoplankton biomass is found in the epilimnion, where the algae take advantage of the high nutrient availability and high light intensity. However, in some lakes during late summer, nutrients become depleted in the epilimnion and no longer support algal growth (Fee 1976). Then, nutrients re-suspended from the sediment may allow for algal growth in the metalimnion or in the hypolimnion, provided that light penetrates deep enough into the water column (Fee 1976). Additionally, a maximum of phytoplankton biomass in deep strata may be reinforced by heavy zooplankton grazing in the epilimnion (Pilati & Wurtsbaugh 2003). The resulting subsurface algal maximum is called "deep-water algal maximum" or "deep-water chlorophyll maximum" (DCM).

DCM are often dominated by a single species of cyanobacteria, cryptophytes, euglenids, dinophytes, chysophytes, chlorophytes, euglenids, prasinophytes, prymnesiophytes or diatoms (Lindholm 1992). These species are adapted to low light intensities and thus capable to grow deep in the water column (Fee 1976, Lindholm 1992).

DCM are common (Padisak *et al.* 1997, Barbiero & Tuchman 2001) in oligotrophic, mesotrophic and even eutrophic lakes in late summer (Fee 1976, Williamson *et al.* 1996). Williamson *et al.* (1996) sampled three lakes in Pennsylvania over five years, and they found a metalimnetic DCM in over 60 % of the samplings in a mesotrophic and a eutrophic lake, and a hypolimnetic DCM in 75 % of the samplings in an oligotrophic lake.

3. The trade-off between food and temperature

In a water column with a deep-water algal maximum, *Daphnia* basically experience two different vertical habitats with reciprocal food and temperature conditions: In the epilimnion, the temperature is high, but food availability is low, whereas in the hypolimnion, water is colder, but food is abundant (Lampert *et al.* 2003). Consequently, in the epilimnion, the low food concentration decreases somatic growth and fecundity (Williamson *et al.* 1996, Lampert *et al.* 2003, Winder *et al.* 2003a), although the high water temperatures in this layer accelerate growth and egg development (e.g., Vijverberg 1980, Kerfoot 1985).

In the DCM, though food availability is high, the lower temperature decelerates growth and egg-development (Venkataraman & Job 1980, Kerfoot 1985).

Therefore, the benefit of exploiting the DCM depends on the costs associated with foraging caused by the low temperatures (Lampert *et al.* 2003), and on the food quality of the algae or bacteria developing the subsurface bloom (Williamson *et al.* 1996, Cole *et al.* 2002). Algal groups differ in their nutritional value (Lampert 1987, Vanni & Lampert 1992, DeMott 1998), and in their edibility (Kasprzak *et al.* 2000, Barbiero & Tuchman 2001). For example, filamentous cyanobacteria and chlorophytes (Kasprzak *et al.* 2000, Barbiero and Tuchman 2001) hamper filtration and ingestion (Burns 1968). Some algae or bacteria are toxic and reduce growth and survival of *Daphnia* (Lampert 1981, DeMott 1999). In this case, there is no advantage in exploiting the DCM. However, the food quality of hypolimnetic seston can be sufficient for growth (Boersma *et al.* 2001), and growth and reproduction of *Daphnia* may be even higher in water originating from the DCM than in epilimnetic water (Williamson *et al.* 1996, Winder *et al.* 2003a). In this case, it is beneficial for *Daphnia* to feed in this layer, but they face the trade-off between food availability and temperature.

4. How do *Daphnia* optimise their fitness in the trade-off between food and temperature?

The goal of my Ph.D. thesis was to investigate the mechanisms *Daphnia* apply to optimise their fitness in a vertical trade-off between food and temperature. I hypothesised that individual *Daphnia* (1) actively select their vertical habitat and (2) migrate between vertical habitats in relatively short time intervals, thereby optimising their time allocation and taking advantage of both, high temperatures and high food availability.

(1) Active habitat selection means that individual *Daphnia* actively move into the vertical habitat allowing for the highest fitness. Thus, most individuals should dwell where the fitness is highest, which would be reflected in the vertical distribution of the population. Lampert *et al.* (2003) investigated the dependence of the vertical distribution of a *Daphnia* population on the corresponding fitness distribution. They exposed a *Daphnia* population to a trade-off between food and temperature and found a good agreement between the vertical distribution and the corresponding fitness distribution as predicted by a theoretical model. With increasing temperature in the hypolimnion, the proportion of *Daphnia* in the hypolimnion increased with the relative fitness in this layer.

(2) In the study by Lampert *et al.* (2003), the proportion of *Daphnia* found in a particular vertical habitat was approximately equal to the relative fitness estimated for this habitat. However, if *Daphnia* simply choose the habitat offering the highest fitness, all *Daphnia*, not only a proportion of the population, should dwell there. This unexpected observation by Lampert *et al.* (2003) suggests that there must be an additional mechanism of fitness optimization. Lampert *et al.* (2003) suggested that *Daphnia* distribute dynamically, which means, the individuals of the population move up and down the water column and allocate proportions of their time to different vertical habitats. The more beneficial such a habitat is, the more time they should spend there. Lampert and Grey (2003) could indeed demonstrate a dynamic distribution in a *Daphnia* population exposed to the trade-off between food and temperature.

5. Thesis outline

In the present thesis, I present experiments on the fitness optimization of *Daphnia hyalina x galeata* (Figure 1) facing the trade-off between food and temperature in a thermally stratified water column with a DCM. A prerequisite for these experiments was to develop a method to count large and measure the large numbers of zooplankton samples I would obtain during my experiments.

The thesis consists of four chapters:

I Set-up and calibration of an Optical Plankton Counter

II Comparison between the vertical distribution of a population with measurements of the corresponding fitness distribution

III Vertical habitat choice of different size classes of a *Daphnia* population

IV Dynamic distribution and time allocation of individual neonate and egg-bearing *Daphnia*

In the following, I will give a short introduction to each chapter:

(I) Most of the experiments were performed in large indoor mesocosms, the plankton towers. These experiments yielded large numbers of zooplankton samples, which needed to be counted and sized. In order to accelerate the processing of these samples, I set up the bench-top version of an Optical Plankton Counter OPC (OPC-1L, Focal Technologies, Canada), which counts and sizes zooplankton samples automatically. As Focal Technologies only provides the mere counting device, I developed a circulation system to insert the zooplankton sample into the counter. Further, the OPC had to be calibrated for the clone of *Daphnia hyalina x galeata* I used in the present thesis. Both, circulation system and calibration will be described in the first chapter.

(II) In the second chapter, I will compare the vertical distribution of a *Daphnia* population with the corresponding fitness distribution in a thermally stratified water column and a DCM. The experiment was performed in 11 m high indoor mesocosms, the Plön plankton towers (Lampert & Loose 1992). In these towers, I monitored the vertical distribution of a *Daphnia* population in the food and temperature trade-off (Figure 2). In parallel, I transferred water from the epilimnion and the hypolimnion of the plankton towers into two flow-through systems (Figure 2), where I measured the fitness of *Daphnia* as approximated by the juvenile growth rate. The juvenile growth rate is a proxy for fitness, because it is closely related to the intrinsic rate of increase of a population (Lampert & Trubetskova 1996).

With this design, I directly compared the vertical distribution of a population with the corresponding fitness distribution. This experiment is based on a similar study by Lampert *et al.* (2003). They compared the vertical distribution of a *Daphnia* population with the corresponding fitness distribution predicted by a model. However, the fitness estimates of Lampert *et al.* (2003) where somewhat inaccurate, because their model did not consider differences in food *quality* between the vertical habitats.

Therefore, I directly measured the fitness distribution which reflects the influence of temperature, food quantity *and* food quality on the fitness of *Daphnia*. A match of the measured fitness distribution and the monitored vertical distribution would indicate that *Daphnia* individuals indeed choose the optimal vertical habitat, but also shift between the different layers.

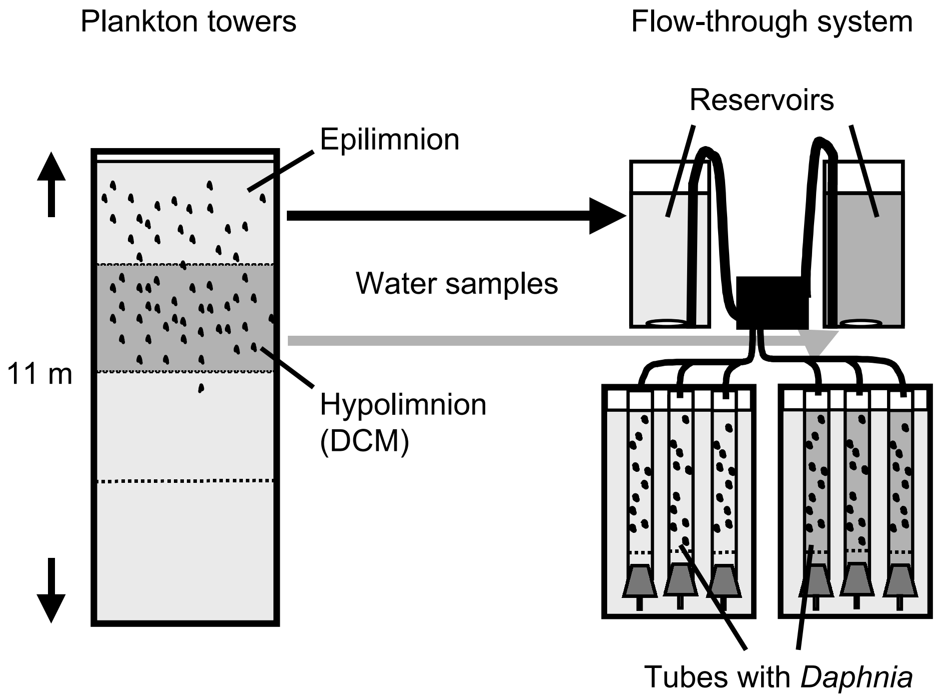


Figure 2*: Scheme of the set-up described in chapter II. The vertical distribution of the* Daphnia *population was measured in the plankton towers (left), whereas the juvenile growth rate of* Daphnia *in water taken from the plankton towers was measured in flow-through systems (right).*

(III) In the third chapter, I will compare the vertical distribution of different size classes of *Daphnia* in a thermally stratified water column with a DCM. The size and age structure of a population is known to influence the shape of the vertical distribution of a population (Boronat & Miracle 1997). Small juveniles and large, especially egg-bearing females (Guisande *et al.* 1991, Leibold *et al.* 1994, Primicerio 2003) are known to choose different vertical habitats. For example, in the presence of light and visually hunting, planktivorous fish, large and conspicuous individuals dwell deeper in the water column and move up into the epilimnion only at night (Dini & Carpenter 1988, Stirling *et al.* 1990, De Meester 1996, De Robertis 2002), resulting in diel vertical migration. However, even in the absence of fish, *Daphnia* may perform weak diel vertical migration (Ringelberg 1999), which may be interpreted as a constitutive response of large *Daphnia* to light or visually hunting predators. As small and large *Daphnia* choose different vertical habitats, the vertical distribution of a population highly depends on its size and age distribution. Therefore, it is important to know and to compare the age and size structure of a *Daphnia* population in order to interpret its vertical distribution.

(IV) In chapter four, I will present an experiment on the migration behaviour and time allocation of individual *Daphnia* in a thermally stratified water column with a DCM. Although Lampert and Grey (2003) already demonstrated the dynamic distribution of a *Daphnia* population in such a trade-off, their study does neither give any information on the time *Daphnia* allocate to different vertical habitats, nor on the frequency of migrations between them. These migration patterns can be observed only on individuals (Pearré 1979, Lampert *et al.* 2003). Therefore, I monitored the behaviour of individual *Daphnia* in a system of vertical Perspex tubes with a thermally stratified water column and a DCM (Figure 3).

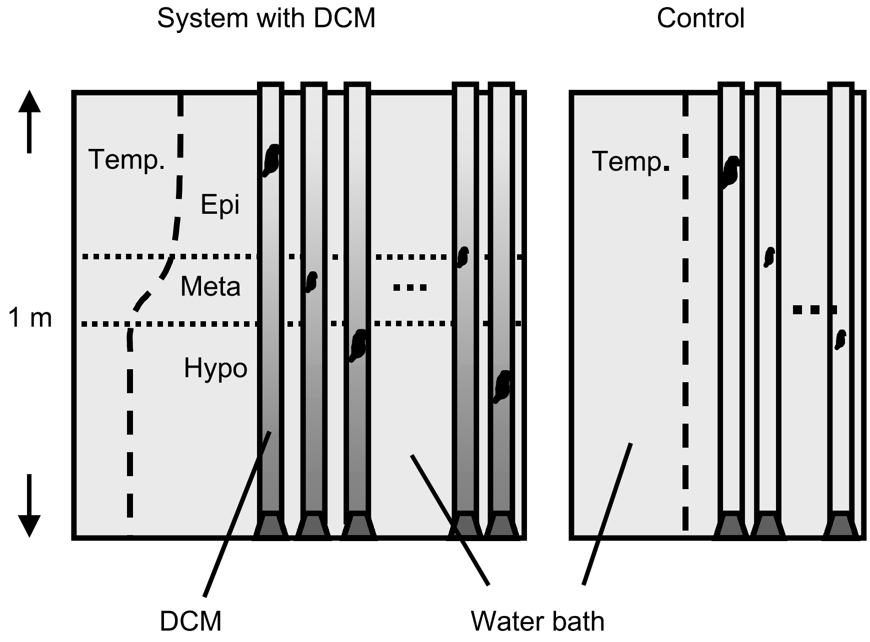
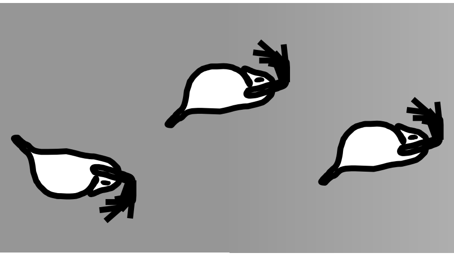


Figure 3: *System of vertical perspex tubes used to monitor the vertical migration patterns of individual neonate and egg-bearing* Daphnia*. The dashed lines indicates the temperature gradient. Epi, Meta and Hypo represent epilimnion, metalimnion, and hypolimnion, respectively.*

If shifting between the vertical habitats is a mechanism of *Daphnia* to optimise their fitness, *Daphnia* that are not exposed to the trade-off should constantly reside in one vertical habitat. For example, neonates carrying egg-yolk reserves do not need to feed during the first few hours after their release (Tessier & Consolatti 1989), and consequently they do not experience a food-temperature trade-off. Therefore, I compared the migration behaviour of neonates and egg-bearing females in order to study the influence of the food and temperature trade-off on their time allocation and the frequency of shifts between different vertical habitats.

Chapter I



Counting and sizing preserved *Daphnia* with the Optical Plankton Counter

Abstract:

Monospecific samples of preserved *Daphnia hyalina* were counted and sized by the bench-top version of the Optical Plankton Counter (OPC) and the results were compared to manual counts and size measurements. OPC and manual counts were in excellent agreement. Equivalent spherical diameters (ESD) provided by the OPC were slightly smaller than those calculated from microscopic measurements, but a constant correction of the ESD(OPC) by adding 80 µm resulted in good agreement between the sample means of ESD and biovolume for both methods. The OPC provides reliable counts and size distributions, which saves considerable time and effort in analysing monospecific zooplankton samples.

Introduction

The Optical Plankton Counter (OPC, Focal Technologies Inc., Dartmouth, NS, Canada) has opened new possibilities for processing large zooplankton samples in situ and in the laboratory (Herman 1992, Sprules *et al.* 1992). The improved version and its calibration with zooplankton was described by Herman (1992). The application in situ as a towed instrument was a particularly promising application and, thus, the OPC has been tested frequently in marine field surveys. Huntley *et al.* (1995) found good agreement between counts of small particles by the OPC and zooplankton caught by simultaneously towed Bongo nets. They concluded that the loss of taxonomic information was significantly compensated by the gain in horizontal and vertical resolution of measurements. Recent years have seen other successful marine large-scale surveys (Gallienne & Robins 1998), and more comparative estimates of zooplankton abundance, biomass and size distribution by Longhurst-Hardy Plankton Recorder and OPC have been reported (e.g., Grant *et al.* 2000, Halliday *et al.* 2001). The bench-top model of the OPC was mostly used for calibration purposes and the determination of correction factors (Zhang *et al.* 2000), but also to count preserved zooplankton samples (Beaulieu *et al.* 1999). Applications in fresh waters are less frequently reported (Stockwell & Sprules 1995), although Sprules *et al.* (1998) performed a very thorough study on the performance of both models of the OPC and provided evidence for their ability to accurately measure freshwater zooplankton size. Sprules *et al.* (1998) found particularly good agreement between OPC and microscopic measurements for monospecific samples of *Daphnia magna*, which encouraged us to apply the bench-top model OPC–1L to our counting and sizing problem.

In measuring the depth distribution of *Daphnia* in our Plankton Tower system (Lampert and Loose 1992), we generate large numbers of *Daphnia* samples ranging from a few to several thousand specimens per sample. These samples need to be counted and sized microscopically, which is very labour intensive even with the help of a computer assisted measuring system. As we usually work with monospecific populations, taxonomic distribution is not a problem. We tested the OPC in order to reduce the time and effort for analysing the preserved samples.

Methods

Daphnia samples were preserved in 4 % sucrose formaldehyde (Haney & Hall 1973) and stored in 100-mL polyethylene bottles until they were processed (several weeks). We used *D. hyalina* (0.6 –2 mm body length), a species much smaller than *D. magna*. The minimum size of particles to be counted by the machine according to the specifications is approximately 0.25 mm.

We used the OPC-1L model connected to a slightly modified circulation system (Figure I 1). Clean water from a reservoir was circulated through the optical chamber at a rate of 0.35 L/s. A small bypass carried water to a funnel at the top of the system used to deliver the preserved zooplankton to the optical system. The water stream caused a current in the funnel to keep zooplankton in suspension. The zooplankton sample was added to the funnel in small portions and the contents of the funnel were then continuously fed to the main water stream before the optics, avoiding bubbles. Depending on the density of daphniids in the sample, the flow of water from the funnel was regulated, aiming not to exceed 10 zooplankton per second passing the light beam. This number has proved to be sufficiently low to avoid errors due to coincidence of particles (Woodd-Walker *et al.* 2000). The lower end of the optical channel drained into a tube submerged in the reservoir that was covered at the end with 100 mm plankton mesh to retain the zooplankton. This tube could easily be removed to recover the zooplankton after counting with the OPC. Complete counting of a sample required 2 to 15 minutes depending on the number of zooplankton. For calibration purposes zooplankton were counted and sized under a dissecting microscope equipped with a video camera and an SIS 3.0 image analysis system (Soft-Imaging Software GmbH, Münster, Germany). Subsamples were counted when the numbers were too high. Subsequently the whole sample was recounted and sized by the OPC. To make microscopic measurements compatible with the output of the OPC, we measured the body length and estimated depth and width of the individual *Daphnia*. Body depth and width were measured for 100 individuals and a regression with length was used to estimate these parameters for all measured daphniids. We applied the formula of an ellipsiod to calculate the approximate volume (V) of each individual according to $V = p/6 \cdot L \cdot D \cdot W$, where L, D and W represent body length, depth and width, respectively. We then calculated the diameter of a sphere having the same volume. This parameter is called Equivalent Spherical Diameter (ESD) and it corresponds to the ESD values calculated from digital size units generated by the OPC (Herman 1992).

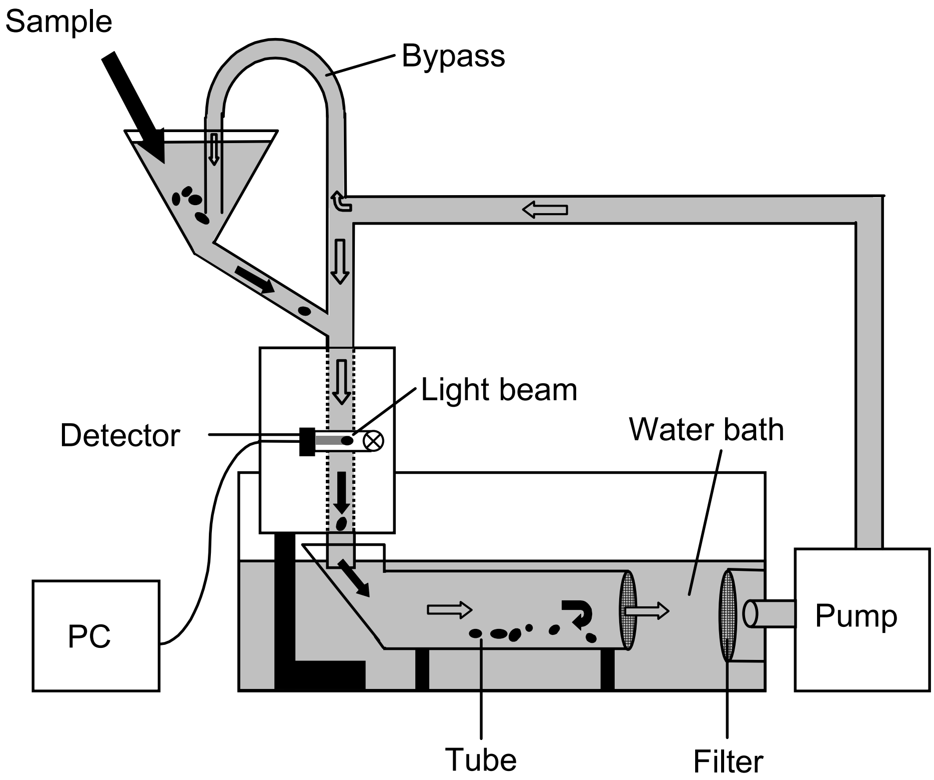


Figure I 1. *Schematic drawing of the Optical Plankton Counter bench-top model as used in this study.*

Results and discussion

Manual and OPC counts are in good agreement (Figure I 2) despite many manual counts being affected by an additional subsampling error. Evidently coincidence was not important in the OPC counts. A linear regression of OPC counts (C_O) vs. manual counts (C_m) has the equation $C_O = 1.03\ C_m - 29.5$ ($n = 17$, $r^2 = 0.986$). The intercept is not significant and the regression coefficient is not significantly different from 1.

Size distributions obtained by the OPC and measured microscopically in 17 *Daphnia* samples collected at different occasions are compared in Figure I 3. Counts were grouped in size classes of 0.1 mm ESD. The distribution of OPC measurements is shifted slightly to smaller sizes compared to the manual measurements. This is probably either a consequence of the daphniids being partly translucent, hence shading off less light than a completely dense particle of equal size, or different orientations of the *Daphnia* when they are passing the light beam. Both will result in smaller ESD estimates. Thus, we must apply a correction to the OPC measurements. A shift of the OPC data by one size class (0.1 mm) results in a very good consistency between the curves, although, due to the large sample sizes (26,000 and 4000, respectively), a Kolmogorov- Smirnov test on the original counts still detects a significant difference.

We calculated the mean ESDs and volumes for the 17 samples, ranging in sample size from n = 200 to n = 5000 (cf. Figure I 2), and compared the results by a paired t-test for OPC and microscopic measurements. The mean (± 1 S.D.) ESD(OPC) was 639 ± 57.8 µm compared to the ESD(manual) of 720.9 ± 66.3 µm, and the difference was highly significant (t = 13.5, *p* <0.0001). Applying a constant correction of + 80 µm to the ESD(OPC), there was no longer a significant difference between the OPC and the manual measurements.

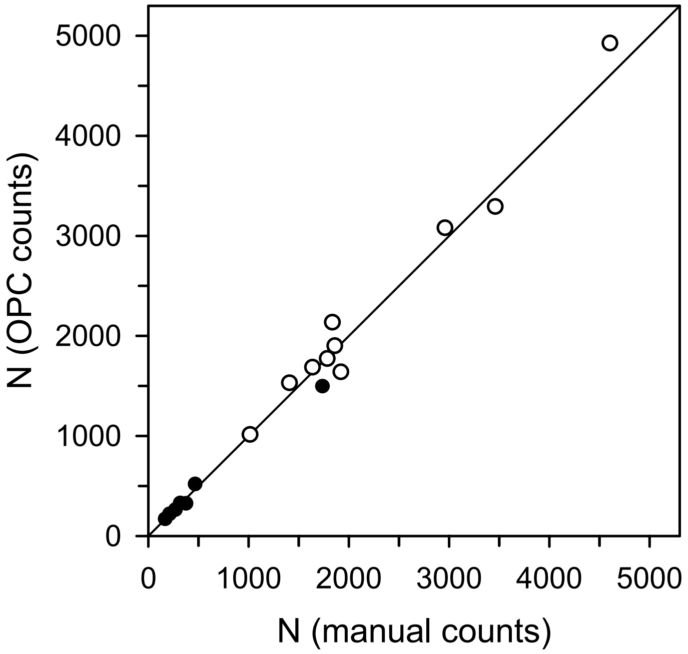


Figure I 2. *Comparison of manual and OPC counts of 17 samples of preserved* D. hyalina *(1: 1 line indicated). Full circles: manual counts of total sample; open circles: manual counts based on subsamples.*

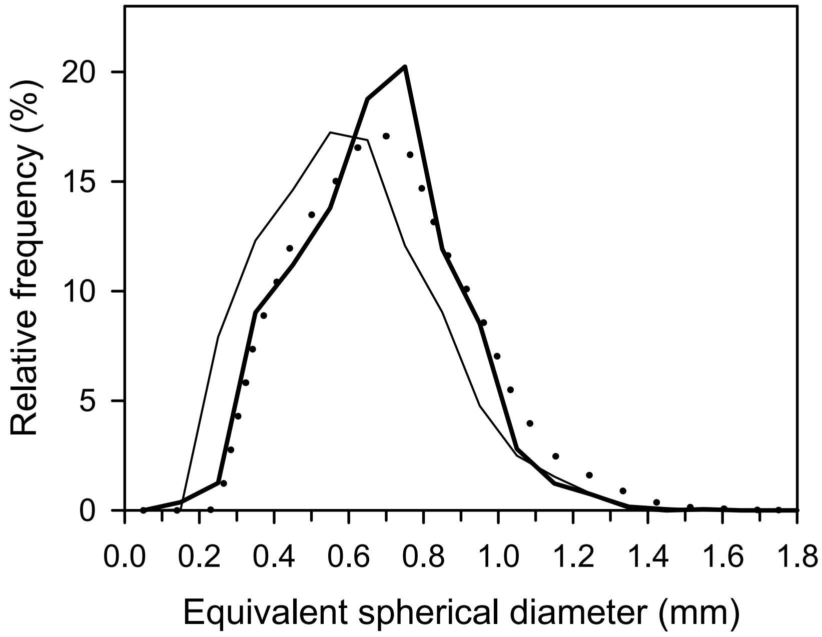


Figure I 3. *Size distributions in size classes of 0.1 mm of* D. hyalina *measured manually (thick solid line) and by the OPC (thin solid line) combined from 17 samples. The dotted line represents the OPC data shifted by one size class.*

We also tested the effect of an ESD correction by + 80 µm on mean estimated volumes. Based on uncorrected ESD(OPC), the mean volume was 192.8 (± 40.8) · 10^6 µm^3 compared to 246.9 (± 56.4) · 10^6 µm^3 for the manual estimates, i.e., the OPC underestimates the volume significantly (t = 9.1, *p* <0.0001). Using the corrected ESD(OPC), the mean volume was 257.5 (± 50.6) · 10^6 µm^3, and the difference at best marginally significant (t = 2.07, *p* = 0.055). Thus, adding a constant 80 µm to the ESD determined by the OPC resulted in reasonable agreement between manual and automatic size estimates, both in terms of ESD and volume. In a similar study, using the towed OPC, Hopcroft (2001) found a slight overestimation of the true size of copepods that could be corrected by an empirically derived factor.

The OPC returns biovolumes of an idealised shape. To estimate field biomasses, one would rather like to convert the OPC volumes to dry or carbon mass. Biovolume is most similar to "wet weight", which is notoriously difficult to determine precisely (Bottrell *et al.*

1976). However, we have shown that ESD(OPC) corrected by 80 µm and ESD(manual) are in good agreement. ESD(manual) has been calculated using body length (L), depth (D) and width (W) assuming a constant ratio of L: D: W. Consequently, there is a linear relationship between body length and ESD(manual). We plotted body length and ESD for a sample of 320 manual measurements and obtained a conversion factor of 0.62 (i.e., L can be estimated from L = ESD/0.62). The estimated L can then be applied to a length-weight or length-carbon relationship as found frequently in the literature (e.g., Bottrell *et al.* 1976). Length-weight relationships are power functions with exponents varying mostly between 2.5 and 3 depending on the nutritional situation (Geller & Müller 1985). Plotting the calculated biovolume vs. L results in an exponent of 3.23, which is higher than the majority of exponents for length-mass relationships that have been measured for the same species (Bottrell *et al.* 1976). This would indicate a negative relationship between size and biomass : biovolume ratio as observed for some zooplankton (Bottrell *et al.* 1976).

Conclusion

Due to the monospecific samples and the relatively small size range, compared to mixed field samples, our OPC counts are very reliable and they reflect the true size distribution accurately. Other attempts to calibrate OPC with microscopic measurements regressing counts, ESDs or biovolumes usually resulted in larger scatter and lower regression coefficients (e.g., Sprules *et al.* 1998, Beaulieu *et al.* 1999, Woodd-Walker *et al.* 2000). *Daphnia* may be particularly suitable for counting by the OPC as already observed by Sprules *et al.* (1998). Size distributions obtained by the OPC are not broader than those from microscopic counts, and we did not observe separate peaks in the OPC size spectrum caused by the different orientation of zooplankton in the light beam (see Sprules *et al.* (1998) for *Diaptomus*). Possibly, due to their hydrodynamic features *Daphnia* are oriented in the flow along the streamlines, which would cause the good agreement between OPC and microscopic size measurements. Quantitative recovery of a sample after OPC counting provides the opportunity to dry and weigh the complete sample and estimate a conversion factor of OPC biovolume to true biomass.

An example for the application of the OPC to measure the vertical distribution of *Daphnia* in our tower system is given in Figure I 4. We present 3 of 11 samples taken at different depths. The size distributions clearly show the difference in habitat choice of various size classes of *Daphnia*, small individuals dwelling in the epilimnion, while large adults stay mostly below the thermocline.

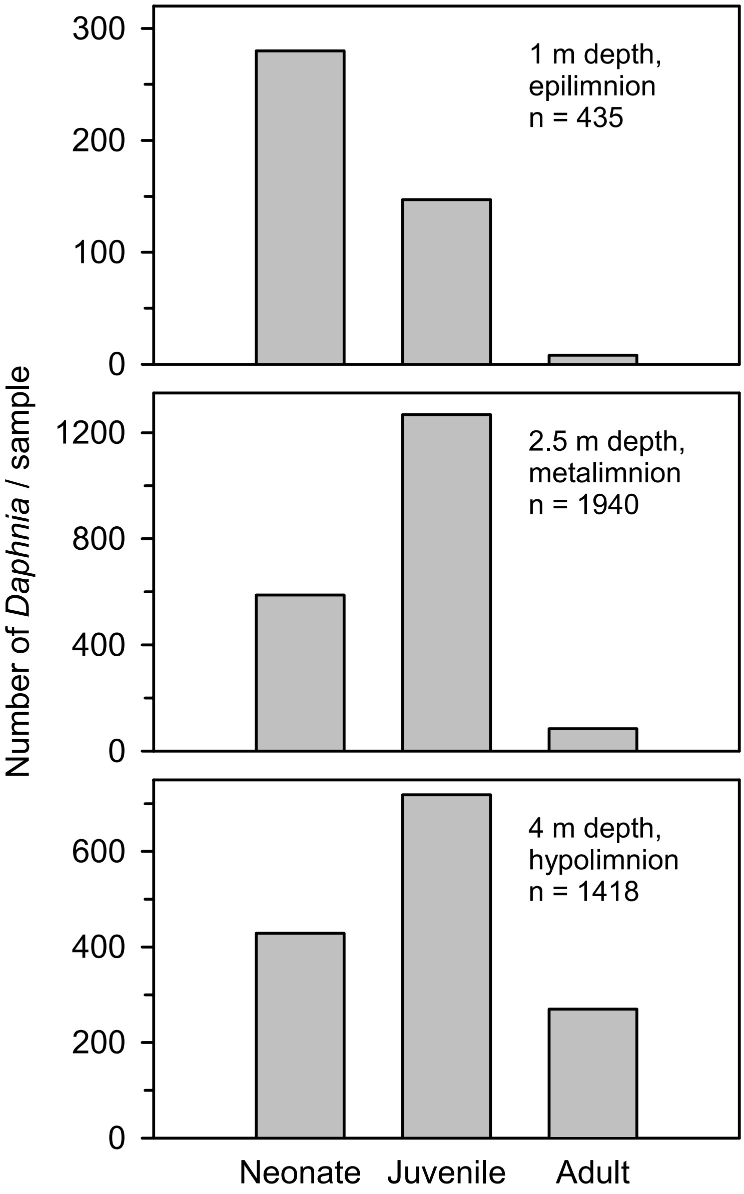


Figure I 4. *Analysis of numbers and size distributions of* D. hyalina *in different depths of the Plankton Tower system with an artificially created hypolimnetic chlorophyll maximum.*

In summary, our results show that the OPC is suitable for counting preserved *Daphnia*. The correction factor for the ESD must eventually be checked for different *Daphnia* species, which may have a different transparency. The automatic counting can save considerable time and effort when large numbers of samples are to be processed. This may be relevant not only for experimental ecology and population biology, but also in applied science using *Daphnia* as test organism (e.g., ecotoxicology).

Chapter II

Fitness optimisation of *Daphnia* in a trade-off between food and temperature

Abstract

In thermally stratified lakes with a deep chlorophyll maximum (DCM), *Daphnia* face a trade-off between food availability and optimum development temperatures. I hypothesise that *Daphnia* optimise their fitness by allocating the time spent in the different vertical habitats depending on the distribution of algal resources and the temperature gradient. I used the plankton towers (large indoor mesocosms) to study the vertical distribution of a population of *Daphnia hyalina x galeata* in three different temperature gradients with a DCM. Additionally, I determined the fitness of *Daphnia* in the epilimnion- and hypolimnion by transferring water from these layers into flow-through systems where I raised *Daphnia* and assessed their juvenile growth rate as a measure of fitness. The fitness distribution was proportional to the vertical distribution. The vertical distribution most likely reflected the proportions of time *Daphnia* allocated to dwelling in the two vertical habitats.

Introduction

In thermally stratified lakes, *Daphnia* experience a variety of vertical gradients, e.g., gradients in abiotic factors like temperature (Watanabe 1992, Haney 1993, Winder *et al.* 2003a), oxygen (Hanazato & Ooi 1992, Lass *et al.* 2000, Winder *et al.* 2003a), and light (Watanabe 1992), including UV-radiation (e.g. Leech and Williamson 2001, Rhode *et al.* 2001). Additionally, *Daphnia* are affected by biotic gradients in predation risk (Gliwicz & Pijanowska 1988, De Meester *et al.* 1995), competitors (Leibold 1991, Winder *et al.* 2003b), and food. Food can vary in both quantity (Gliwicz & Pijanowska 1988, Adrian & Schipolowski 2003) and quality (Kasprzak *et al.* 2000, Cole *et al.* 2002). In nature, all of these gradients act on *Daphnia* jointly*,* which means that *Daphnia* have to optimise their fitness under various trade-offs.

In lakes with a deep chlorophyll maximum, the main algal biomass is situated in the meta- or hypolimnion. Deep chlorophyll maxima (DCM) are common (Fee 1976, Williamson *et al.* 1996, Padisak *et al.* 1997). In thermally stratified lakes with a DCM (and without the interference of light dependent mortality), *Daphnia* are exposed to a trade-off between food availability and temperature. In the epilimnion, the water temperature is high, which allows for fast growth and egg development (Kerfoot 1985, Dawidowicz & Loose 1992), but food is scarce which reduces growth and fecundity (Gliwicz 1990, Kilham *et al.* 1997). In contrast, food is abundant in the hypolimnion but the water temperature is low.

In recent years, the vertical distribution of *Daphnia* in lakes with a DCM has received increasing attention. Several studies combined observations of the vertical distributions of *Daphnia* populations in lakes containing a DCM with bioassays on the food quality of the algae in the different layers (Williamson *et al.* 1996, Cole *et al.* 2002, Winder *et al.* 2003a). Williamson *et al.* (1996) and Winder *et al.* (2003a) showed that reproductive rates and growth rates of *Daphnia* were higher when they were raised with water from the DCM than with water taken from the surface layer. However, as they did not raise *Daphnia* in the same temperature as in the respective layer, they did not investigate the trade-off between food and temperature as such. In contrast, Cole *et al.* (2002) measured *Daphnia* growth and survival in water taken from the surface and from the DCM at the corresponding and reciprocal temperatures, but as *Daphnia* growth was higher in the epilimnion, the daphniids in this lake were not exposed to a trade-off between food and temperature. Lampert *et al.* (2003) performed a laboratory experiment in large indoor mesocosms, the Plön plankton towers (Lampert and Loose 1992), where they measured the vertical distribution of *Daphnia* in a water column with a DCM and different temperature gradients. They calculated the fitness distribution of *Daphnia* in the different food and temperature gradients by incorporating the empirical food and temperature measurements into a physiological model. They found a quite good agreement between the vertical distribution and the modelled fitness distribution, but the fitness estimates in the epilimnion were too high compared to the proportion of *Daphnia* found in this layer. The most likely reason for this discrepancy was, that Lampert *et al.* based their calculations of fitness on empirical measurements of food particle concentration in the plankton towers, and these measurements did not take food quality into account (Lampert *et al.* 2003). Consequently, they overestimated the fitness in the epilimnion where food was of poorer quality than in the hypolimnion.

In the present study, I also aimed to compare the fitness and the vertical distribution of *Daphnia* in a thermally stratified water column with a DCM, but I improved the estimation of fitness compared to Lampert *et al.* (2003). I directly measured the fitness *Daphnia* achieve in the different vertical habitats by raising them in a flow-through system in water taken from the towers. Thus, I assessed the joint influence of temperature, food quantity and food quality on the fitness distribution which was only partially considered in the model of Lampert *et al.* (2003). Simultaneously, I monitored the vertical distribution of a *Daphnia* population in such a water column. I attempted to demonstrate the trade-off between food and temperature and to test the hypothesis that the vertical distribution of *Daphnia* reflects the fitness distribution.

Material and Methods

Organisms: The experiments were performed with a single clone of *Daphnia hyalina x galeata*. *Scenedesmus obliquus* served as food. The mass cultures of *Daphnia* and of *Scenedesmus* were established as in Lampert *et al.* (2003).

Plankton towers: The vertical distribution of *Daphnia* was monitored in large indoor mesocosms, the plankton towers (Lampert & Loose 1992, Lampert *et al.* 2003). I applied a design similar to Lampert *et al.* (2003): The plankton towers were thermally stratified into three different layers: the surface layer (0 – 2.5 m), hereafter called the epilimnion, the layer underneath (2.5 – 5 m), referred to as the hypolimnion, and the deepest layer (5 – 11 m). The temperature in the epilimnion was always adjusted to 20°C, while the temperature in the hypolimnion was adjusted to 10, 15, or 18 °C. This resulted in three different temperature gradients which are named according to the temperature difference between the epilimnion and the hypolimnion, i. e., 10 °, 5 ° and 2 °C temperature gradient. After the temperature stratification was established, the appropriate amount of algal suspension was injected into the hypolimnion to reach a final concentration of 0.5 mg C/L. The temperature in the deepest layer was always adjusted to 8 °C in order to keep the algae in suspension. I most intensively sampled the epilimnion and the hypolimnion, but also measured both, food and *Daphnia* abundance in the deepest layer in order to verify that at best very few individuals dwelled there. A day : night rhythm of 12 : 12 h was adjusted.

In order to determine the food concentration in the hypolimnion, I sampled hypolimnetic phytoplankton every morning (3, 3.5, 4, and 4.6 m depth) and measured the particle volume of these samples in a Cell Counter and Analyser System (CASY 1, model TCC, Schärfe System GmbH Reutlingen, Germany). By using a previously established calibration curve between particle volume and carbon concentration of *Scenedesmus* I determined the carbon concentration in the hypolimnion. In case the carbon concentration in the hypolimnion was lower than 0.5 mg C/L, the missing amount of *Scenedesmus* was injected through a hose with openings at 3 and 4 m depth. Algae in the hypolimnion were allowed to mix for four hours. Thereafter, phytoplankton was sampled in the entire water column at eleven different depths (0.1, 0.6, 1.2, 1.6, 2.1, 2.5, 3, 3.5, 4, 4.6, 5.5, and 6.5 m), and again these measurements were converted into mg C/L.

Zooplankton was sampled on the first, second and fourth day of each series. Every sampling day zooplankton was sampled during daytime and at night in eleven different depths (0.1, 1.2, 1.6, 2.1, 2.5, 3, 3.5, 4, 4.6, 5.5, and 6.5 m). In each depth 50 L were sampled and screened through glass traps retaining the zooplankton (Lampert & Loose 1992). All zooplankton samples were preserved with 4% sucrose formaldehyde. The samples were counted with the bench top version of an Optical Plankton Counter (OPC-1L, Focal Technologies Inc., Dartmouth, NS, Canada) which counts and sizes particles in a light beam (Kessler & Lampert 2003, i.e., Chapter I). Directly after the last sampling of one series, the temperature in the hypolimnion was altered to the temperature gradient of the next series. In order to allow *Daphnia* to acclimate to the new temperature gradient, no samples were taken for two days between successive series. The three successive samplings of a series were pooled and considered as one replicate. Although these replicates were in part obtained with the same population of *Daphnia* I considered them to be independent as the daphniids experienced several light cycles between the replicates, when they could redistribute. Moreover, there was turnover within the population, i. e., not all the individuals were identical.

Seven replicates were obtained from the 5 ° and 10 °C temperature gradients while the 2 °C temperature gradient was repeated five times. Unintended surface blooms developed in some of these replicates. Additionally, I deliberately created surface blooms in three further replicates with 5 °C temperature difference by adding 0.5 mg C/L *S. obliquus* to the epilimnion. All these replicates with algae in the epilimnion did not provide any trade-off and, thus, they were not considered in the statistical analysis.

Flow-through system: In parallel to the tower experiments, *Daphnia* were raised in flow-through systems under food and temperature conditions identical to the epilimnion and the hypolimnion in the plankton towers. Every day, three hours after the replenishment of the food concentration in the plankton towers, 2.5 L were withdrawn from 0.5 and from 1.6 m depth in the epilimnion and from 3 and 4 m depth in the hypolimnion. This water was pooled, filtered through a 150 µm gauze in order to eliminate *Daphnia* and then transferred into two reservoirs supplying two separate parts of a flow-through system. Each reservoir supplied three 120 mL flow-through vessels with each vessel containing 10 *D. hyalina x galeata*. The flow-through rate was adjusted to approximately 1.5 L d^{-1}. I determined the epilimnetic and hypolimnetic juvenile growth rates g_j because g_j is a good proxy for fitness (Lampert & Trubetskova 1996). The juvenile growth rate was calculated as $g_j = (\ln W_1 - \ln W_0)/(t_1 - t_0)$, where g_j is the juvenile growth rate, *W* is the dry weight at the beginning (0) and end (1) of the experiment, while t_1-t_0 is the elapsed time between the beginning and the end of the experiment. Synchronised offspring of the third and subsequent broods of *Daphnia* were inserted into the flow-through system at the age of two days. To measure the initial dry weight, two or three times ten randomly taken juveniles were transferred into pre-weighed aluminum containers, dried overnight at 60°C, cooled down in an desiccator and weighed on a Sartorius ultramicro balance (Sartorius GmbH Göttingen, Germany) to the nearest microgram. After four days, all *Daphnia* from one perspex tube were transferred into one pre-weighed aluminum container, dried and weighed to the nearest microgram.

Statistical analysis: The statistical package NCSS 2001 (Statistical Solutions, Cork, Ireland, Hines 2001) was used for all statistical analyses. For all vertical profiles the proportions of the total *Daphnia* population dwelling in the 11 different depths were calculated and subjected to a principal component analysis (PCA) which was based on a variance-covariance matrix (for details see Manly 1994, Lampert *et al.* 2003). PCA produces principal components (PC) which are linear combinations of the original dependent variables, i.e., the percentage of the population at the different depths. In order to establish linear combinations of the original dependent variables, each of these variables is multiplied with a coefficient called factor loading. Sign and value of each factor loading describe its relation to the PC, i. e., the contrasts in abundance at different depths. Inserting the measured percentages into the linear equation of a PC yields so called factor scores. These factor scores of the PCA were subjected to a 3-way ANOVA with the factors plankton tower (tower 1 and tower 2), temperature gradient (2, 5, and 10°C), and sampling time (day and night).

The juvenile growth rates were tested with 1-way ANOVA with the factors treatment (10° and additional food, 15° and additional food, 18° and additional food, and 20°C no additional food). Moreover the juvenile growth rates were subjected to Tukey's honest significant differences test as a post hoc comparison.

Additionally, I calculated a linear regression between the proportion of the population dwelling in the hypolimnion against the relative fitness in this layer. This relative hypolimnetic fitness Pg_{jH} was the fitness obtained in the hypolimnion in relation to the average fitness in the plankton towers, $Pg_{jH} = (g_{jH} \times 100)/[(g_{jE} + g_{jH})/2]$, where g_{jE} and g_{jH} were the juvenile growth rates in epilimnion and hypolimnion, respectively.

Results

Distribution of algae: The measurement of the food distribution showed that I was successful in establishing a stable hypolimnetic algal maximum. In the water layer between the surface and the thermocline the food concentration was on average 0.1 mg C/L. The food concentration in the hypolimnion ranged from 0.3 mg C/L just below the thermocline to 0.5 and 0.6 mg C/L in the other depths within this layer (not shown).

Vertical distribution of Daphnia: The temperature in the hypolimnion influenced the vertical distribution of the total *Daphnia* population (Figure II 1). The proportion of the population dwelling in the hypolimnion increased with an increasing temperature in this layer, i. e., with a decreasing temperature gradient between epilimnion and hypolimnion. Moreover, with a decreasing temperature gradient, fewer daphniids accumulated near the thermocline.

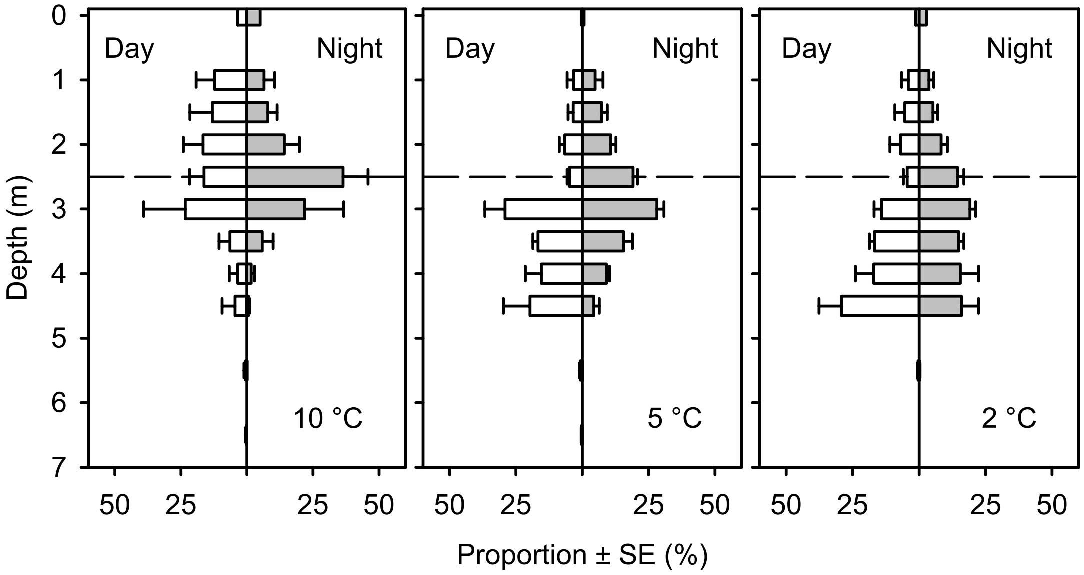


Figure II 1: *Vertical distribution of the total* Daphnia *population (± SE) at day (open bars) and night (shaded bars) in the three different temperature gradients. The temperature in the epilimnion was always 20 °C. The temperature differences between epilimnion and hypolimnion are given in the panels. The dashed line indicates the thermocline.*

Table II 1: *Factor loadings of the first three principal components (PC) of the principal components analysis. Major contrasts in bold. Additionally, the variation explained by the respective PC is given.*

Sampling Port	Depth (m)	PC1	PC2	PC3
1	0.1	0.101	- 0.105	- 0.015
3	1.2	0.164	- 0.203	- 0.363
4	1.6	0.179	- 0.230	**- 0.362**
5	2.1	0.257	- 0.194	- 0.253
6	2.5	**0.588**	0.107	**0.705**
7	3.0	- 0.101	**0.849**	- 0.210
8	3.5	- 0.261	0.148	0.021
9	4.0	- 0.359	- 0.062	0.190
10	4.6	**- 0.557**	- 0.314	**0.308**
12	5.5	- 0.011	0.008	- 0.010
14	6.5	- 0.001	- 0.004	- 0.010
Variance (%)		58.3	25.4	11.3

These observations are supported by the principal components analysis (PCA). This PCA resulted in three principal components (PC) explaining 95% of the total variation (Table II 1). The first PC (58.3% of total variation) contrasted the vertical distribution of *Daphnia* in the epilimnion, especially at port 6 (2.5 m) directly at the thermocline, with the distribution in the hypolimnion. Thus, the first PC indicated the shift of the population between these two layers. The second PC (25.4% of total variation) contrasted port 7, which is just below the thermocline, with all other depths, especially port 10, indicating the shift of the *Daphnia* population within the hypolimnion. The third PC (11.3% of total variation) contrasted port 6 with all other depths, especially the upper epilimnion and port 10 in the hypolimnion. Thus, the third PC represents the shift of the total population at the thermocline.

Table II 2: *Results of three way ANOVA on factor scores of the first three principal components. The factors tested were tower (left and right), temperature gradient (Temp., 2 °C, 5 °C, and 10 °C difference between epilimnion and hypolimnion), light (day and night) and the two-way interactions between these factors.*

	Factor	*d. f.*	*F*	*p*
PC 1	Tower	1, 14	0.01	0.920
	Temp.	2, 14	23.30	< *0.001*
	Light	1, 14	15.64	*0.001*
	Tower x Temp.	2, 14	0.51	0.612
	Tower x Light	1, 14	0.00	0.979
	Temp. x Light	2, 14	0.51	0.613
PC 2	Tower	1, 14	0.02	0.879
	Temp.	2, 14	1.75	0.209
	Light	1, 14	1.16	0.300
	Tower x Temp.	2, 14	0.20	0.820
	Tower x Light	1, 14	0.04	0.854
	Temp. x Light	2, 14	0.03	0.972
PC 3	Tower	1, 14	0.02	0.884
	Temp.	2, 14	0.72	0.502
	Light	1, 14	7.10	*0.019*
	Tower x Temp.	2, 14	0.40	0.679
	Tower x Light	1, 14	0.67	0.427
	Temp. x Light	2, 14	4.74	*0.027*

The ANOVA on the factor scores of the first PC showed that both the temperature in the hypolimnion and the light (day vs. night) had a significant influence on the vertical distribution of *Daphnia*, but there was no significant effect of the plankton tower (Table II 2). No significant effect of tower, temperature gradient or light for the factor scores of the second PC was detected. As the second PC indicated the shift of the population within the hypolimnion, it is not very important within the scope of this study. The factor scores of the third PC were significantly influenced by light and the interaction of temperature gradient and light (Table II 2), but this PC only accounted for 11.3 % of the total variation.

Fitness: The juvenile growth rate g_j was significantly influenced by the combination of temperature and food availability in the different layers and temperature gradients (1-way ANOVA, *d.f.* = 5, $F = 21.25$, $p < 0.001$, Figure II 2). Note that the epilimnetic g_j in all temperature gradients are similar while the hypolimnetic g_j increase with temperature (Figure II 2). In the 10 °C temperature gradient, the g_j in the hypolimnion was significantly lower than in the epilimnion (Tukey's HSD for unequal sample sizes, $p = 0.004$). In the 5 °C and 2 °C temperature gradient, g_j was significantly higher in the hypolimnion compared to the epilimnion (Tukey's HSD for unequal sample sizes, $p = 0.010$ and $p < 0.001$, respectively).

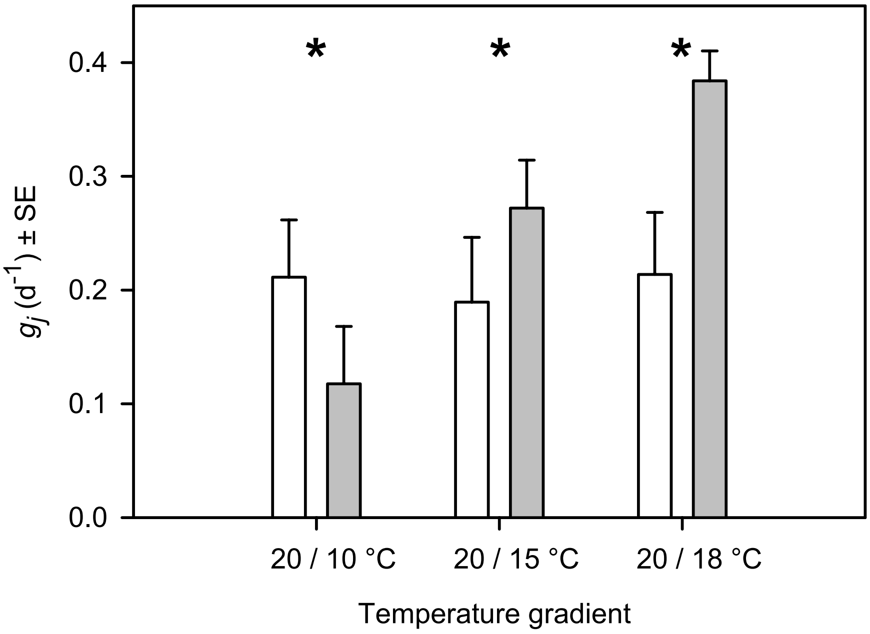


Figure II 2: *Juvenile growth rates (*g_j*) of* Daphnia *in epilimnetic water (open bars) at 20 °C and in hypolimnetic water (grey bars) at 10 °C, 15, and 18 °C. The epilimnetic and hypolimnetic* g_j *measured within the same temperature gradient are plotted next to each other. The asterisks indicate significant differences between the* g_j *in epilimnion and hypolimnion within one temperature gradient (Tukey's HSD post hoc test).*

Correlation between vertical distribution and fitness distribution: The proportion of *Daphnia* dwelling in the hypolimnion was positively correlated to the relative fitness (proportion of the hypolimnetic fitness of the average fitness in the total water column) the daphniids achieved in the hypolimnion ($r^2 = 0.65$; $p < 0.001$; Figure II 3). With an increasing relative fitness in the hypolimnion, the proportion of the population dwelling in the hypolimnion increased. Thus, the fitness distribution reflected the vertical distribution.

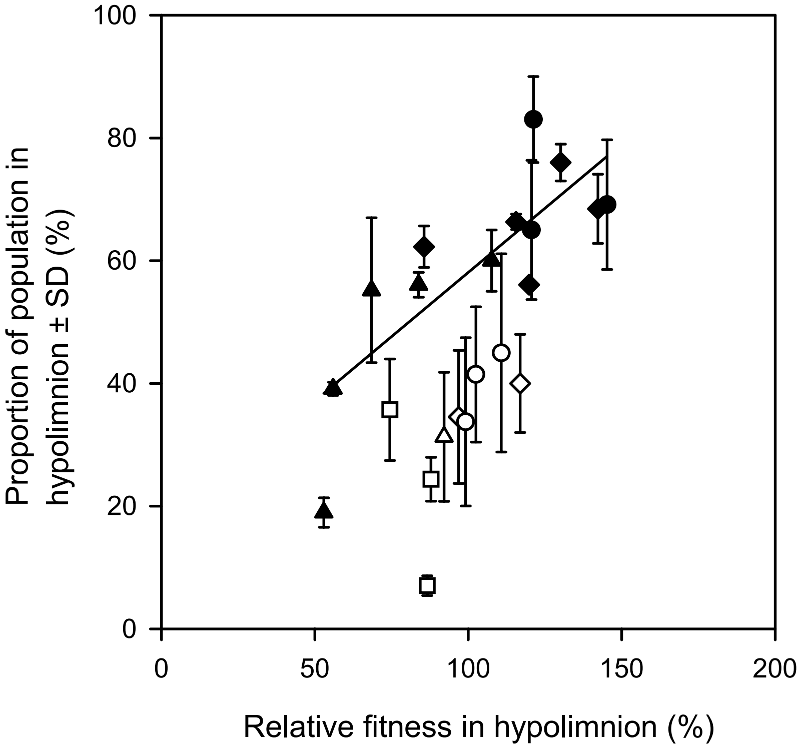


Figure II 3: *Regression of the proportion of the total* Daphnia *population in the hypolimnion measured in the towers, and the relative fitness in the hypolimnion measured in the flow-through system. Different symbols represent the temperature gradient in which the respective data point was measured: Circles represent a 2 °C temperature difference between epilimnion and hypolimnion, diamonds a 5 °C difference, and triangles a 10 °C temperature difference. Replicates measured in a water column with a deep water algal maximum are black. Only these replicates are included in the regression. Replicates with unintended blooms at the surface are depicted with open symbols with the shape of the corresponding temperature gradient. Open squares represent experiments at 5 °C temperature gradients with addition of 0.5 mg C/L* Scenedesmus *to the epilimnion.*

Discussion

In the trade-off between low food availability and high temperature on the one hand and high food availability and low temperature on the other hand, *Daphnia* distributed in accordance with the hypothesis: The vertical distribution of *Daphnia* reflected the fitness distribution.

The fitness measurements showed that there was indeed a trade-off between food and temperature. This trade-off was reflected by the juvenile growth rates g_j in the respective layers and in the different temperature gradients. In the epilimnion, the g_j was low due to the low food concentration. As no *Scenedesmus obliquus* were added to this layer, the only available food source were detritus particles (Lampert *et al.* 2003) which are of low food quality to *Daphnia* (Lampert 1987, Hessen *et al.* 2003). Thus, the fitness in the epilimnion was reduced by the low quantity and quality of the food. This effect might have been intensified by the higher temperature in the epilimnion which increases respiration (Giebelhausen & Lampert 2001, Cole *et al.* 2002). However, the juvenile growth rates were still positive, which means that despite the bad food conditions growth of the daphniids dwelling in the epilimnion was still possible.

In the hypolimnion, g_j was significantly reduced by the low temperatures although the good quality food *Scenedesmus obliquus* (Lampert 1987) was added to this layer. Furthermore, g_j decreased with decreasing temperature. Reduced growth of *Daphnia* at low temperatures has been reported in many studies (Kerfoot 1985, Dawidowicz & Loose 1992, Loose & Dawidowicz 1994).

Despite the low temperatures, the hypolimnetic g_j was significantly higher than the epilimnetic g_j in the two shallowest temperature gradients resulting in a deep-water fitness maximum. In other words, the high food concentration more than compensated the effects of low temperatures because high food concentrations increase growth even when the water temperatures are low (Orcutt & Porter 1984, Giebelhausen & Lampert 2001). Such a deep-water fitness maximum was found in several water columns with a deep-algal maximum, both in field studies (Williamson *et al.* 1996, Winder *et al.* 2003a) and in laboratory experiments (Lampert *et al.* 2003).

However, the fitness distribution was different in the steepest temperature gradient with 10 °C difference: There, the juvenile growth rate at 10 °C and high food was significantly lower compared to the g_j at 20 °C and low food, with both g_j being very low. Thus, the effect of additional food in the hypolimnion did not compensate for the low temperature.

The vertical distribution coincided very well with the fitness distribution. This observation is supported by the regression between the relative fitness and the proportion of the *Daphnia* population dwelling in the hypolimnion (Figure II 3): The warmer the temperature in the hypolimnion the lower the costs of dwelling there (Kerfoot 1985, Dawidowicz & Loose 1992, Loose & Dawidowicz 1994), and the larger the proportion of the population observed in this layer.

Also the gradients in food availability influenced both, fitness and vertical distribution. In some replicates, either *Scenedesmus obliquus* were deliberately added to the epilimnion or unwanted surface blooms developed (open symbols in Figure II 3), which increased the epilimnetic food concentration. In these situations, the *Daphnia* were not exposed to a trade-off anymore because the temperature and food optimum coincided in the epilimnion. *Daphnia* consequently avoided the cold hypolimnion. Such an avoidance of low hypolimnetic temperatures in water bodies with a surface algal maximum and without fish predation was also observed in other studies (Calaban & Makarewicz 1982, Haney 1993, Lampert *et al.* 2003). The intensity of this avoidance behaviour was dependent on the steepness of the temperature gradient (Lampert *et al.* 2003).

Assuming *Daphnia* optimise their individual fitness, the good match between the measured fitness distribution and the vertical distribution of the total population is rather surprising as one would expect all *Daphnia*, not only a proportion of the population, to dwell in the layer allowing for the highest g_j. Regardless of the absolute difference in fitness, if the fitness in the epilimnion is higher than in the hypolimnion *Daphnia* should all stay there and vice versa. However, the proportion of the *Daphnia* population dwelling in the more beneficial layer is always lower than 100 % (Figure II 3).

The fitness in the plankton towers might have been slightly different from the fitness measurements in the flow-through system. The food concentration in the flow-through system only represent snapshots for the fluctuating food concentrations in the plankton towers as water from the towers was only transferred once a day. Especially in very high population densities, competition for food in the DCM might have decreased the fitness of *Daphnia* in the hypolimnion to such an extent that it became similar to the epilimnetic fitness. In consequence, a proportion of the population would have migrated into the epilimnion with lower food concentration but also less competition. Thus, the daphniids would be distributed in the water column so that each individual achieved the same fitness. This is in accordance with the ideal free distribution (IFD) with costs model (Tyler & Gilliam 1995) which Lampert *et al.* (2003) discussed as a crude approximation to explain the close agreement between the vertical distribution and the fitness distribution of *Daphnia* in their study. According to Lampert *et al.* (2003) the prerequisite for such an agreement is a dynamic distribution of the daphniids.

A dynamic distribution of the *Daphnia* might have caused the discrepancy between the observed vertical distribution and the expectation that all daphniids should reside in the more profitable layer. Such a distribution implies that *Daphnia* allocated proportions of their time to the epilimnion and hypolimnion, respectively (Lampert *et al.* 2003) and frequently shifted between these two layers.

A dynamic distribution was also suggested by Haney (1988) to apply for zooplankton exposed to a trade-off between damage caused by high solar radiation and foraging in a food rich epilimnion. In such a lake, zooplankton might optimise their fitness with frequent shifts between the food rich epilimnion and the dark hypolimnion. The hypothesis of a dynamic distribution is further supported by a study of Lampert & Grey (2003) in which algae of a hypolimnetic algal maximum were labelled with ^{15}N. As epilimnetic *Daphnia* above and below the thermocline did not differ in ^{15}N enrichment over time they must all have been feeding in the hypolimnion and must thus have allocated their time to dwelling in the two different vertical habitats. Moreover, the observation of the migration of individual *Daphnia* in vertical perspex tubes with a deep algal maximum and similar temperature gradients showed that the daphniids distributed dynamically and that the time allocation depended on the temperature gradient (Chapter IV).

Time allocation might be a result of *Daphnia* searching the water column for the most profitable environmental conditions (Lampert *et al.* 2003). *Daphnia* quickly find food patches even over long distances in laboratory experiments (Haney 1993, Plath 1998) and in the field (Jensen *et al.* 2001). *Daphnia* reduce their swimming speed in patches with high food concentration (Cuddington & McCauley 1994, Larsson 1997). Thus, the time *Daphnia* dwell in a patch should increase with its profitability, i. e., with the fitness *Daphnia* can achieve there. After a certain period of time the daphniids may leave a profitable patch to search for another, more profitable patch. In other words, they would take advantage of profitable food patches, but also be able to find even better ones. In this case, always less than 100 % of the daphnids should reside in the most profitable vertical habitat similar to Figure II 3. Alternatively, some *Daphnia* might miss a profitable patch because of the time lag between sensing the food concentration and the behavioural response, especially when the food patch is small (Cuddington & McCauley 1994, Larsson & Kleiven 1996). However, this may not apply for the present study, as the hypolimnetic DCM represents a large food patch which should not have been missed because of its size.

An additional benefit of these migrations may be that *Daphnia* utilise the food in the DCM more efficiently as they can feed in the food rich hypolimnion and digest in the warm epilimnion, where high temperatures accelerate the assimilation of food (Lampert 1977). Such a strategy would reduce the trade-off between food and temperature and consequently increase the overall fitness of *Daphnia*.

The positive slope in the regression between the relative fitness and the proportion of the population in the hypolimnion (Figure II 3) may to some extent reflect how much time *Daphnia* spend in each layer. It is impossible to deduce the migration patterns of individuals from sampling a population (Pearré 1979, Lampert 2003). Nevertheless, the probability of catching *Daphnia* in a certain depth increases with the time they dwell there, and the time they reside in a certain patch should increase with its profitability. Therefore, the positive slope in the regression can indicate that *Daphnia* spent more time in the hypolimnion when the fitness in this layer was high. This was the case in the two shallow temperature gradients. There, the fitness in the hypolimnion was highest (Figure II 2 and II 3), and consequently the daphnids spent more time in this layer, as is reflected in the high proportion of *Daphnia* sampled in this layer. In the steepest temperature gradient, the fitness in the epilimnion and in the hypolimnion were equally low. Interestingly, in this gradient the daphnids aggregated at the thermocline, which might be a strategy to shift frequently between the two vertical habitats with short migration distances.

Migration in relation to time allocation must be well distinguished from a synchronised diel vertical migration of a *Daphnia* population. Possible metabolic gains should be independent of light and consequently not be synchronised within the population. Therefore, diel vertical migration triggered by changes in light intensity should not be a strategy to optimise the fitness gain in a trade-off between food and temperature. Nevertheless, I observed slight but significant differences in the vertical distribution of the *Daphnia* population between day and night, although not of the same order as diel vertical migration induced by the presence of fish. The slight upwards shift at night is probably a consequence of a genetically fixed residual response of egg-bearing females to light even in the absence of fish (Chapter III). In other studies performed in lakes with a deep algal maximum, diel vertical migration was suggested to be an adaptation of *Daphnia* to the trade-off between food and temperature (Williamson *et al.* 1996, Winder *et al.* 2003a). As these studies were performed in the field, the authors could not exclude the influence of predation (Williamson *et al.* 1996) on the vertical migration behaviour.

Time allocation of individual *Daphnia* is likely to be the reason for the good match between the measured fitness and the vertical distribution of the *Daphnia* population in the trade-off between food and temperature. There is still a lack of knowledge with regard to the exact mechanism and the influence of other additional trade-offs (e. g. oxygen distribution) on vertical distribution behaviour. Further studies will be needed to develop a theory of zooplankton habitat choice.

Chapter III

Depth distribution of *Daphnia* in response to a deep-water algal maximum: the effect of body size and temperature gradient.

Abstract

In the absence of fish predation, *Daphnia* exploiting a deep-water algal maximum are faced with a trade-off. They can either dwell in the epilimnion where development in the warm water is fast, but food shortage causes low egg production, or in the hypolimnion, where food availability is high but development is slow due to low temperatures.

We tested the hypotheses that (1) depth distributions of various ontogenetic stages (size classes and egg-bearing females) differ because daphnids react to light with size-specific diel vertical migration even in the absence of fish (residual predator avoidance hypothesis), and (2) differently sized daphnids select different depths because the relative importance of temperature and food varies for ontogenetic stages (physiological hypothesis). We used large indoor mesocosms (Plankton Towers) to test these hypotheses experimentally.

Temperature was the strongest factor governing the distribution, with larger proportions of the population dwelling in the food-rich hypolimnion if the temperature gradient was shallow. There were small but significant differences between ontogenetic stages during the day, but not at night. This suggested the existence of a “residual” effect of light on depth distribution in the absence of a fish cue.

Although large individuals exhibited a greater amplitude of diel vertical migration, the physiological hypothesis had to be rejected. A stage-specific physiological effect is unlikely to be directly triggered by light, hence vertical movement of the individuals should not be synchronised. Rather, being forced into deeper layers by the residual light response during the day, large and egg-bearing females experienced a lower average temperature during day than juveniles. They probably compensated for this by spending longer time periods in warm waters at night.

Introduction

The vertical distribution of zooplankton in a stratified lake results from a complex interaction between predator avoidance, food availability, and the vertical gradient of abiotic factors (e.g., temperature, oxygen). The dark hypolimnion is considered a refuge from visual predators (fish) and, thus, is a prerequisite for the advantage of diel vertical migration (DVM) as explained by the predator avoidance hypothesis (Lampert 1993). Zooplankton usually return to the warm, food-rich epilimnion at night when the risk of predation is low. Although this is the most common pattern, there are exceptional cases of zooplankton staying in deep waters all day (listed in Vos *et al.* 2002). Using a modelling approach, Vos *et al*. (2002) showed that under strong fish predation the risk for *Daphnia* of being eaten is still high at dawn and dusk, so that it may be profitable for them to stay in deep waters.

However, this is not the only reason why daphnids may stay in hypolimnetic waters. The assumption that food quality and quantity are better in the epilimnion is not always fulfilled. The food quality below the thermocline can be sufficient for growth (Boersma *et al.* 2001) and the availability of food in the hypolimnion can be even higher than in the epilimnion in lakes with a deep-water chlorophyll maximum (Winder *et al.* 2003a). Depending on the food quality of the algae present in the deep-water algal maximum, growth rates of *Daphnia* cultured in water from this layer are either lower (Cole *et al.* 2002) or higher (Williamson *et al.* 1996, Winder *et al.* 2003a) than in water taken from the epilimnion.

Lakes with a deep-water algal maximum are not uncommon (Fee 1976, Padisak *et al.* 1997, Barbiero & Tuchman 2001). Heavy grazing by zooplankton may contribute to the development of deep-water algal maxima (Pilati & Wurtsbaugh 2003), which can explain why they often occur in lakes with low fish predation.

Williamson *et al.* (1996) suggested that zooplankton may migrate into deeper waters to exploit hypolimnetic algal resources, but they still found DVM, which indicates that zooplankton must nevertheless profit from spending some time in warm surface waters even if the epilimnetic food is poor. The synchronisation of the migration indicates the presence of visual predators. In the absence of predation risk and when the quality of the algae in the deep-water algal maximum is sufficient, zooplankton in a lake with a deep-water algal maximum will be faced with a trade-off between high food availability but low temperature (slow development) in the hypolimnion and high temperature (fast development) but low egg-production in the sparse-food epilimnion.

Lampert *et al.* (2003) developed the idea that zooplankton allocate their time in the vertical profile in order to optimise their fitness, which would result in a dynamic vertical distribution. This distribution resembles an Ideal Free Distribution with Costs (Tyler & Gilliam 1995). Lampert *et al.* (2003) confirmed this hypothesis with experiments in large indoor mesocosms. As predicted, the percentage of the total *Daphnia* population residing below the thermocline was larger when the temperature gradient was shallower (i.e., temperature costs were lower). Although there is evidence for dynamic behaviour of individuals (Lampert & Grey 2003), the overall distribution was stable and there was no significant difference between day and night.

Lampert *et al.* (2003) looked only at the distribution of the total population, although the vertical distribution of *Daphnia* is influenced by the size structure of the population (Boronat & Miracle 1997). Large individuals, being more conspicuous than small ones, dwell deeper during the day (Dini & Carpenter 1988, Stirling *et al.* 1990, De Meester *et al.* 1995), and egg-bearing females migrate to greatest depths (Guisande *et al.* 1991). Hence, it is possible that the dynamic depth distribution of daphnids in a fishless environment varies for different ontogenetic stages due to at least two reasons.

(1) DVM is controlled by the change in light intensity and is modified by a chemical signal (kairomone) emitted by fish (Ringelberg 1991). Although DVM is strongly enhanced by the kairomone, there may still be a residual response to the light even in the absence of fish. This residual response may be size-dependent like the modified response, i.e., large *Daphnia* would migrate downwards some distance during the day while small ones would not. The lack of a significant day-night difference in the study of Lampert *et al.* (2003) may be a consequence of the size structure of the population, large individuals being less abundant than (non-migrating) small ones.

(2) The optimal depth distribution may not be identical for all ontogenetic stages. For example, for small individuals with higher food thresholds for growth (Gliwicz 1990) food may be of greater importance than temperature, compared to large individuals. On the other hand, egg-bearing females may spend more time in warm waters as high temperatures accelerate egg development and thus increase fitness.

Both reasons would result in differing depth distributions for ontogenetic stages. If the first one is relevant, we expect to find a greater proportion of the large than of the small size classes residing in the hypolimnion, but only during the day. In the second case, size classes should differ in their depth distribution, but no diel vertical migration should be found in the absence of predators. Using the same experimental approach as Lampert *et al.* (2003), we tested the hypotheses that (1) depth distributions of various ontogenetic stages (size classes and egg-bearing females) differ because daphnids react to light with size-specific diel vertical migration even in the absence of fish (residual predator avoidance hypothesis), and (2) differently sized daphnids select different depths because the relative importance of temperature and food varies for ontogenetic stages (physiological hypothesis).

Material and methods

Experimental system: The experimental design was adopted and slightly modified from Lampert *et al.* (2003). The experiment was performed in a large indoor mesocosm system, the Plön Plankton Towers. The two stainless steel columns, approximately 11.5 m high and one meter in diameter have been described by Lampert and Loose (1992). They were filled with 10-µm filtered water from a nearby mesotrophic lake (Schöhsee). The columns can be manipulated and sampled with a resolution of 50 cm. Vertical gradients of food and temperature were created by thermally stratifying the columns into four layers of approximately equal depth.

The surface layer, hereafter referred to as the epilimnion, was always set at 20 °C, while the temperature in the layer underneath, hereafter called the hypolimnion, was adjusted to either 18 °C (shallow gradient), 15 °C (intermediate gradient), or 10 °C (steep gradient). The temperature in the two lowest layers was always kept at 8 °C and 6 °C, respectively. Only the upper part of the towers (to 6.5 m depth) was considered in our experiments. The cool bottom layers served to keep the hypolimnetic algal maximum in place. Virtually no daphnids were found in the bottom layers below the second step in the temperature profile at 5.0 m. Consequently, we refer to the temperature transition from layer 1 to 2 as the metalimnion, and to the depth of greatest temperature change (at 2.5 m) as the_thermocline. *Scenedesmus obliquus* Meyen was always added to the second layer to produce a deep-water algal maximum. Daily adjustment of the algal density resulted in a distinct maximum despite algal sinking and grazing by daphnids. Mean vertical profiles of temperature and algal biomass for the three different treatments are depicted in Figure III 1. The vertical distribution of *Daphnia* in the temperature and food gradient was measured as the response variable. This distribution results from allocation of time by *Daphnia* to dwelling in the warm, nutritionally dilute epilimnion or the cooler, food-rich hypolimnion.

The *Daphnia hyalina x galeata* clone (originally isolated from Lake Constance, Germany, and kept in the laboratory for more than 10 years at approximately 20 °C and dim continuous illumination) is responsive to the presence of a chemical signal from fish (Loose 1993). Inoculum populations of *Daphnia* were raised in 100-liter containers and then transferred to the mesocosms. The green alga *Scenedesmus obliquus* (formerly known as *S. acutus* strain SAG 276-3a, algal collection Göttingen), which provides good growth of *Daphnia* in our laboratory was used as unialgal food. Algae were cultured in 10-liter jars in dilute (1:4) Z4 medium (Zehnder & Gorham 1960) under continuous light. Although the plankton towers cannot be kept sterile, *Scenedesmus* was almost the only food source for *Daphnia*. However, in some replicates blooms of other algae developed at the surface, which reduced the intensity or even abrogated the trade-off. Replicates with more than 0.2 mg C/L in the epilimnion were not included in the analysis.

Preparation and sampling of the mesocosms: Detailed information on the set-up of an experiment and sampling is given in Lampert *et al.* (2003). Basically, the towers were filled with 10-µm filtered lake water and stratified. The temperature control in the hypolimnion was always set to different temperatures in the two parallel towers. A concentrated suspension of *Scenedesmus* was added to the hypolimnion when the temperature profile was stable. The amount of algal suspension needed to enrich the layer (volume approximately 1.7 m^3) with an algal carbon biomass of 1 mg C L^{-1} was estimated by measuring the light absorption (800 nm) of the algal suspension in a photometer and using a pre-established calibration curve (extinction vs. particulate organic carbon). Daphniids added to the surface layer were then allowed to build up a dense population, which took about one week to ten days, depending on the inoculum density. Algal biomass was monitored and replenished, when the hypolimnetic food concentration was lower than 1.0 mg C/L.

Vertical profiles of algal biomass were estimated from small samples taken at the sampling ports. The particle density (volume) was measured by a CASY (Schärfe GmbH) particle counter and was converted into POC using a calibration curve. *Daphnia* were sampled in the epilimnion, hypolimnion and in the remaining water column at 9 or 11 depths from 0.1 to 6.5 m by simultaneously pumping a volume of 48 litres from each depth through glass traps (Lampert & Loose 1992). They were preserved in sucrose formaldehyde (Haney & Hall 1973).

Table III 1: *Experimental design*: *The experiment was conducted in two different series. Each series consisted of two blocks, with one block being the data obtained during one filling of the plankton towers.*

Experimental series	Treatment: Temperature gradient (°C)	Block	Replicates in block	Profiles per replicate
1 (1998)	10	2	3	1
	5	1	1	1
	5	2	2	1
	2	2	3	1
2 (2001)	10	1	2	3
	10	2	3	3
	5	1	5	3
	2	1	1	3
	2	2	2	3
Total	10		8	
	5		8	
	2		6	

Experimental procedure: The data in this study originated from two experimental series, one performed in 1998 and one in 2001 (Table III 1). The series differed with respect to the sampling strategy but were identical in basic design and routine program.

A daily routine program was carried out in both series. The light period was set from 4 a.m. to 6 p.m.. A profile of algal biomass was measured every morning in order to calculate the amount of algae necessary to replenish an algal concentration of 1 mg C L^{-1} in layer 2. Depending on the density of the *Daphnia* population, the algal concentration was reduced during the day. However, a second measurement of the algal profile four to five hours after the replenishment of food showed the existence of a distinct algal maximum in the hypolimnion with average values around 0.6 mg C L^{-1} (Figure III 1). This food concentration is above the incipient limiting concentration for *Daphnia* (Lampert 1987).

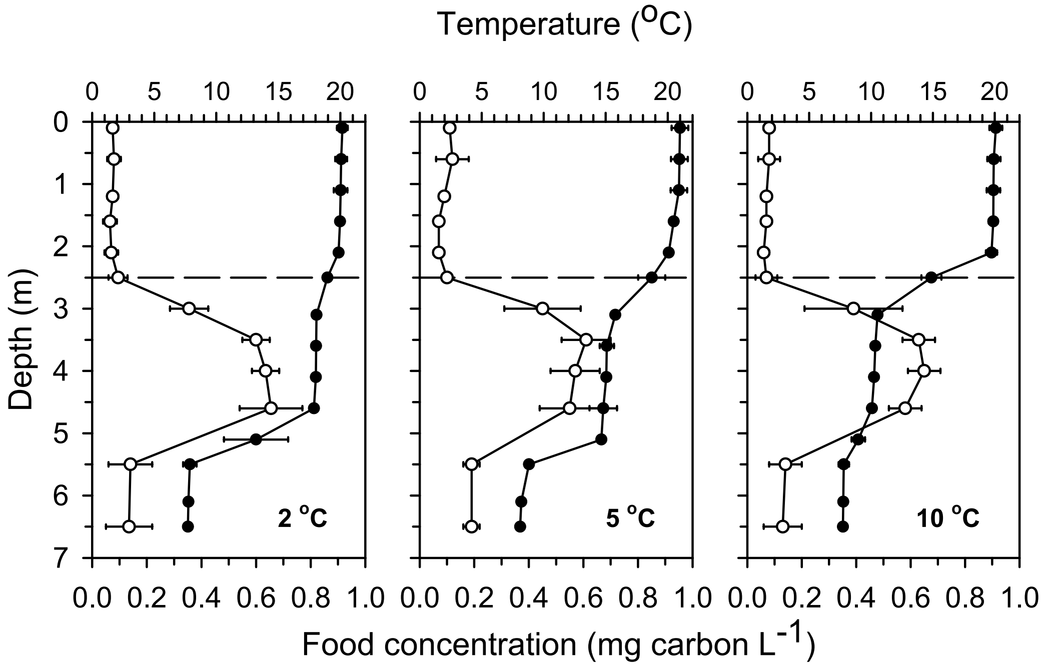


Figure III 1: *Vertical profiles of temperature (solid circles) and algal concentration (open circles) in the three experimental treatments. Bold numbers denote the temperature difference between epilimnion and hypolimnion (steepness of the gradient). Values represent means (± 1 SE) of seven replicates. The dashed line indicates the thermocline.*

The program of measuring the depth distribution of *Daphnia* started immediately after the daphniids had established populations sufficiently dense to be sampled with good precision (1-15 ind. L^{-1}). Two profiles were taken on each experimental day, one in the afternoon (day) and a second one two hours after the lights had been switched off (night). Due to the growth of the population and differences in light/dark sampling efficiencies, the absolute numbers of *Daphnia* caught in a vertical profile varied between sampling dates and between day and night. The sample collected at each sampling port was therefore expressed as the percentage of the total. The relative depth distributions of the population were used for statistical analysis. In the first series, *Daphnia* were sampled at nine different depths (0.1, 1.1, 1.6, 2.1, 2.5, 3.0, 3.5, 4.6, and 6.0 m), while in the second series, two more depths were sampled (4.1 and 5.1 m). The missing data in the first series were interpolated as the mean between the two neighbouring ports in order to combine the two series.

The first experimental series comprises size-structured data from the experiments described in Lampert *et al.* (2003). In this series, the temperature settings were switched in the towers immediately after each night sampling and the sampling routine was repeated two days later. Although we sampled the same *Daphnia* population repeatedly, each sampling was considered a replicate. This was justified as due to reproduction and senescence there was a continuous turnover of the population within the two days and as the daphniids experienced two full light cycles to adjust to the new conditions. The series was divided into two blocks, each block starting with new filling of the towers.

The data in the second series originate from two blocks (Table III 1). In this series, temperature settings were changed every six days and daphniids were sampled on days 3, 4 and 6. This resulted in three day and night profiles for each temperature setting, which could not be considered independent. Consequently, the three samplings were combined to form one replicate.

Our clone of *Daphnia* has a neonate body size (from the top of the head to the base of the tail spine) of about 0.6 mm and a size at maturation of about 1.7 mm. We divided the total population into four size classes (< 1.0 mm, 1.01-1.40 mm, 1.41-1.70 mm, >1.70 mm), the last size class including the majority of egg-bearing females. In the first series, *Daphnia* were counted under a dissecting microscope, 100 individuals of each sample were sized, and the total number of egg-bearing females determined. Three (of 5 -7) replicates for each treatment were sized and could be used for this study. In the second series, all samples were counted and sized with a bench top model of the Optical Plankton Counter (OPC, Focal Technologies Inc.). They were size-corrected using a species-specific calibration factor (Kessler & Lampert 2003, i.e., Chapter I). Data on egg-bearing daphniids were not available in this case. The second series contributed 3 to 5 replicates for each treatment. Combining the two experimental series resulted in 6 to 8 replicates with four size classes for each treatment (3 temperature gradients, day and night) and 3 replicates for egg-bearing females.

Statistics: The method of characterising the distribution patterns with different treatments has been described in detail in Lampert *et al.* (2003). Percentages of the total population at each of the 11 ports were subjected to a principal component analysis (PCA). PCA produces linear combinations of the original variables that represent different aspects of the distribution of daphniids over the 11 ports in a tower in terms of contrasts between the percentages at different ports. Depending on the amount of the total variation explained by them, the first few principal components can be used to describe the main aspects of the distribution of daphniids, i.e., they provide measures for the shape of the distributions.

The factor scores of the PCs explaining most of the variance (about 95% of the total) were analysed further by ANOVA to relate them to the experimental factors. A general linear model was fitted for each of the PCs, with estimation of the main effects (temperature gradient, time of the day, size class) and the two-way interactions between them. The ANOVA showed which of the experimental factors were significantly related to different aspects of the vertical distribution of daphniids.

The proportion of daphniids residing in the food-rich layer (i. e., the hypolimnion) between 3.0 and 4.6 m (cf. Figure III 1) was calculated for each size class and for the egg-bearing females in order to quantify responses to the environmental factors. These data were analysed by two-way ANOVA or Kruskal-Wallis test. All statistical analyses were carried out using the NCSS statistical package (Hines 2000).

Results

Depth distributions: Vertical distribution patterns of *Daphnia* in relation to the thermocline varied depending on the temperature gradient (Figure III 2). The shallower the temperature gradient, the deeper was the population maximum located. At 10 °C difference, daphniids tended to accumulate in the thermocline, at 5 °C difference they preferred the lower thermocline depths, while the majority resided in the area of highest food density when the difference was only 2 °C. This trend was similar during day and night although the distributions of the individual size classes showed more similarity in their distribution at night. The spreading of the distributions during the day was caused by small differences between the size classes. In deep waters (4 – 5 m), the percentages of large *Daphnia* were always higher during the day than at night. In contrast, the population in the epilimnion was always dominated by small *Daphnia*. Evidently, part of the large daphniids migrated deeper during the day.

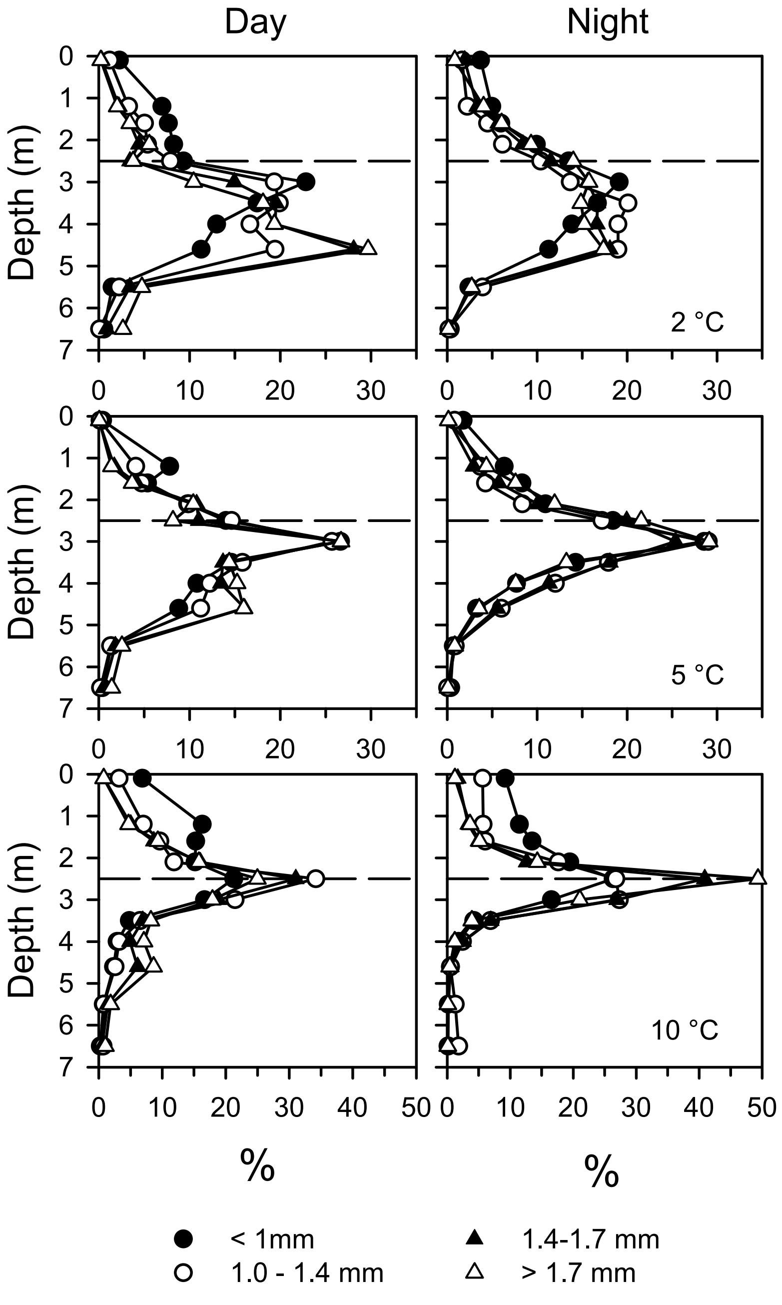


Figure III 2: *Relative vertical distributions (means of 6-8 replicates) of different size classes of* Daphnia *in the varying temperature gradients during day and night. Temperature difference between epilimnion and hypolimnion indicated in the right panel (cf. Figure III 1). Error bars omitted for clarity. The dashed line indicates the thermocline.*

Table III 2: *Eigenvectors for principal components 1-3 (PC1 - PC3) accounting for more than 90 % of the variation. Bold numbers depict major contrasts.*

Sampling Port	Depth (m)	PC1	PC2	PC3
3	1.2	- 0.066	0.123	0.359
4	1.6	- 0.082	0.147	0.385
5	2.1	- 0.180	0.151	**0.464**
6	2.5	**- 0.759**	0.057	**- 0.547**
7	3.0	0.047	**- 0.898**	0.028
8	3.5	0.289	- 0.128	- 0.159
9	4.0	0.322	0.092	- 0.241
10	4.6	**0.436**	0.317	- 0.352
12	5.5	0.030	0.068	- 0.031
14	6.5	- 0.001	0.029	0.015
Variance (%)		48.0	24.7	17.6

These qualitative impressions are corroborated by the principal component analysis (PCA) that compares the shapes of the distributions (Table III 2). More than 90 % of the variation was explained by the first three principal components. PC1 explained nearly half of the variation. It contrasted port 6 (thermocline) and port 10 (near food maximum) and thus reflected the movement through the thermocline into deeper waters. Another 25 % of the variation was explained by PC2. It pointed to a contrast between port 7 (just below the thermocline) and the deeper waters, indicating a shift within the hypolimnion. Finally, and least important (17.6 %), PC3 showed a contrast between port 6 and ports 5 and above, which indicated exchange of daphniids between the thermocline and the epilimnion. In summary, the most important process affecting the depth distribution was the exchange between thermocline and food maximum, represented by PC1.

The ANOVA on PC1 (Table III 3) shows significant effects of time (day vs. night) and temperature gradient, but not of size class. There is, however, a significant time x size interaction, indicating that different size classes distribute differently during the day but not at night. Temperature was the only significant factor affecting PC2 ($F_{2,153}$ = 12.77, $p <$ 0.001). PC3 was significantly affected by temperature gradient ($F_{2,153}$ = 3.42, p = 0.035) and size class ($F_{1,153}$ = 6.40, p < 0.001). Hence, size influenced the distribution above the thermocline (i.e. the tendency of small individuals to stay closer to the surface), but this was of minor importance to the overall distribution.

Table III 3: *Results of the general linear model ANOVA on principal component PC1 factor scores with estimation of the main effects of the factors time of day (time), temperature gradient (Temp), size class (Size), and the two-way interactions between these.*

Factor	*d.f.*	MS	*F*	*p*
Time	1,153	9.99	25.25	< *0.001*
Temp	2,153	47.07	118.93	< *0.001*
Size	3,153	0.28	0.70	0.554
Time x Temp	2,153	0.03	0.07	0.930
Time x Size	3,153	2.13	5.39	*0.001*
Temp x Size	6,153	0.52	1.31	0.255

Egg-bearing daphniids were not included as a factor in the PCA as they represent only a subset of the experiment. They showed distributions (Figure III 3) rather similar to the largest size class in the total population, which could be expected as they all belong to the largest size class. Due to the lower numbers of egg-bearing animals in the vertical profile, percentages were much more variable than for the total population. Nevertheless, the effect of the temperature gradient was clearly visible as well as the higher proportion of daphniids at greater depths during the day.

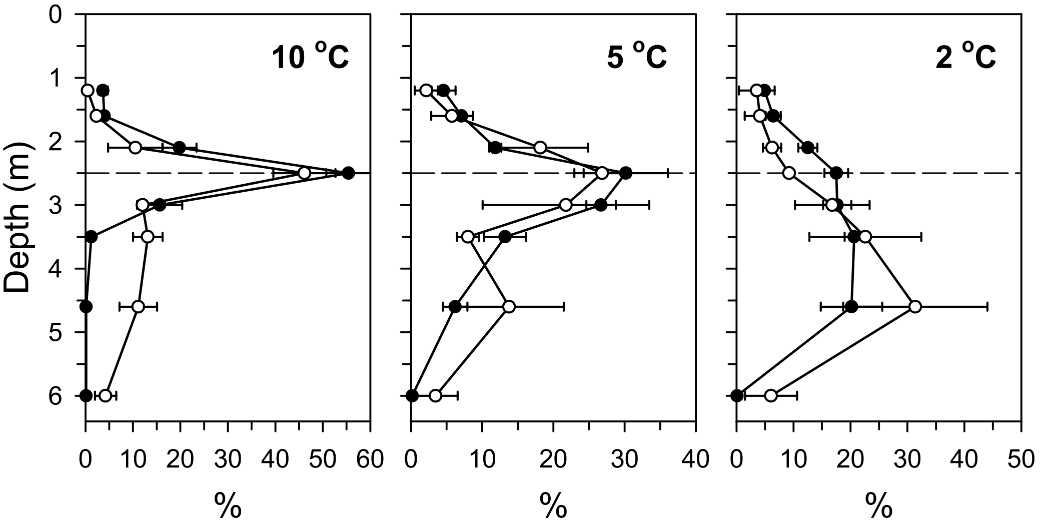


Figure III 3: *Relative vertical distributions of egg-bearing* Daphnia *in the three experimental treatments during day (open circles) and night (closed circles). Values represent means (± 1 SE) of 3 replicates. Temperature differences between epilimnion and hypolimnion indicated in bold. The dashed line indicates the thermocline.*

Proportions in deep layers: The depth distribution curves (Figure III 2) and the ANOVA on PC1 (Table III 3) suggest that the proportion of both the total population and the individual size classes dwelling in the food rich hypolimnetic area varied between temperature gradients and between day and night. We quantified this impression by calculating the proportion of the total population of each size class present between 3 and 4.6 m depth (Figure III 4). This showed a strong effect of the temperature gradient, which was confirmed by a Kruskal Wallis test ($H = 92.01$, d.f. = 2, $p < 0.001$). All size classes showed the smallest proportions in the hypolimnion when the temperature difference was large (10 °C), and the largest proportions with a 2 °C difference. During day, there was some tendency that the proportion of *Daphnia* dwelling in deep water increased with size. At night, size groups 2 and 3 seemed to dwell deepest at all temperatures (Figure III 4). However, neither daytime nor size had a significant effect on the proportions dwelling at the algal maximum (Kruskal Wallis test, daytime, $H = 3.68$, d.f. = 1, $p = 0.055$; size, $H = 5.0$, d.f. = 3, $p = 0.172$).

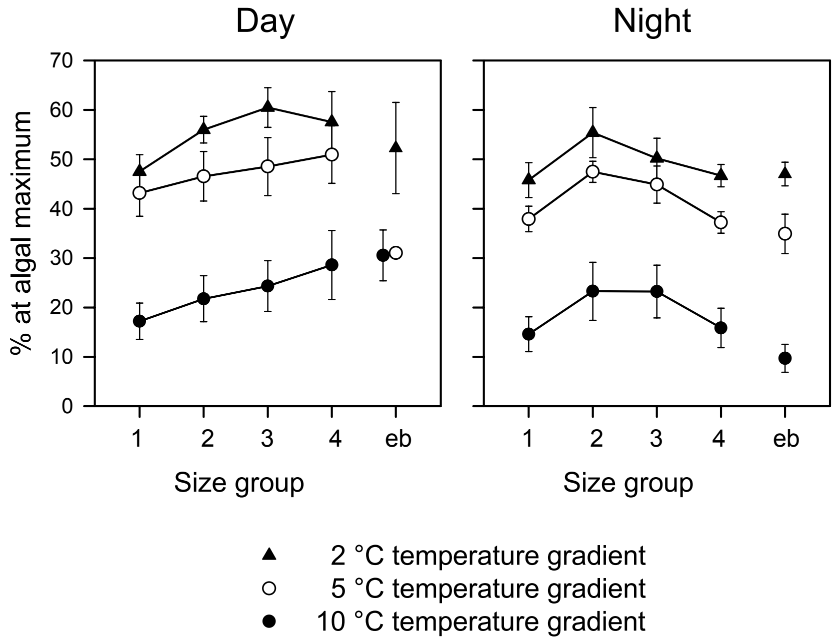


Figure III 4: *Proportions (means ± 1 SE) of the total populations of different size classes and egg-bearing females (eb, n = 3) found in the zone of maximum food (3.0-4.6 m depth) during day and night in experiments with varying temperature gradients. Size classes (n = 6-8): 1 = <1.0 mm, 2 = 1.01-1.4 mm, 3 = 1.41-1.7 mm, 4 = > 1.7 mm.*

Deep-water proportions of egg-bearing daphniids (cf. Figure III 3) were analysed separately by Kruskal-Wallis test. Again, only temperature was significant (H = 12.12, d.f. = 2, p = 0.002) , indicating that a larger proportion of the egg-bearing population resided in deeper waters when the temperature gradient was shallow. These proportions were similar as for the largest size class, only in the 5 °C temperature gradient the proportion in the algal maximum was unusually low. Separate analyses of the temperature data subsets (Wilcoxon rank-sum test) showed that the deep-water proportion was significantly larger during the day only with a 10 °C temperature difference (Z = 1.96, p = 0.025).

Discussion

Overall, the vertical distributions found in this study resemble earlier results of Lampert *et al.* (2003). Also, the temperature gradient being the main factor that determined the proportions of daphniids feeding in the food-rich layer is consistent with the earlier study. Contrary to Lampert *et al.* (2003), we found a significant effect of the time of day, probably due to the larger number of replicates.

However, we have additional information as Lampert *et al.* (2003) did not separate size classes. The differences between size classes only during the day, though small, supported the “residual predator avoidance” hypothesis. DVM in response to light was strongly reduced in the absence of fish, but did not cease completely. The presence of fish greatly enhances DVM in the tower system with a deep algal maximum (P. Larsson and W. Lampert, personal observation). The “residual” response in the absence of fish causes the differences between size classes as large *Daphnia* respond stronger than small ones. This cannot be a “memory effect” as found by Ringelberg and van Gool (1995) as the daphniids used for this experiment came from stock cultures that had never experienced fish kairomones. Also, it is unlikely that the lake water in the towers still contained kairomones originating from the lake. The measurements never began less than a week after the towers had been filled. This time should be sufficient for decay of the fish kairomone (Loose 1993). Thus, DVM has a genetic basis as found for phototactic behaviour (De Meester 1989), although its extent is largely controlled by the phenotypic response to fish.

In the absence of visual predators, there is no reason for the synchronous response to light except in the unlikely case that light had a direct deleterious effect. If different ontogenetic stages occupied different depths for physiological reasons, they would do this continuously. The separation of size classes, however, did not persist at night. Based on these results, the ”physiological” hypothesis must be rejected.

Since the light response was absent, night distributions could be expected to reflect the true trade-off between temperature and food, in which case potential differences in the vertical distribution of ontogenetic stages should be visible in the dark. The lower the proportion in the hypolimnion, the more important temperature should be relative to food. The deep-water proportions at night (Figure III 4), besides the strong temperature effect, showed a curvilinear trend for the size groups, and the egg-bearing females matched that pattern, although the size effect was not significant. Temperature seemed to be most important for the smallest size group. This group included neonates that may still live on egg-yolk reserves and are known to survive starvation for several hours (Tessier & Consolatti 1989). Hence, high temperatures may be more profitable for them than feeding in the cold hypolimnion. Although not significant, there seems to be a trend for decreasing proportions in the hypolimnion at night for larger individuals. However, this may have been an indirect effect of the light response. The longer daphniids stayed in the cold water during day, the lower the average temperature the experience. In order to compensate for the low temperature sum they must stay longer in the sparse-food warm water at night. Egg-bearing females are most conspicuous and most threatened by fish predation (Tucker & Woolpy 1984). Therefore, they may experience the greatest conflict between the “residual” light response driving them into deep waters and the temperature sum necessary for the development of the eggs, in particular in the steep gradient. Consequently, they have to compensate the largest temperature effect and their proportion in the deep waters at night is the lowest of all ontogenetic stages.

Vertical distributions of zooplankton are the result of a complex interplay of mortality risk, food availability and abiotic factors. Differences between ontogenetic stages and species in the sensitivity to these factors can lead to habitat partitioning (Leibold & Tessier 1997). Fish predation seems to be most important in a hierarchy of factors causing DVM (Dini & Carpenter 1992), but it varies considerably between lakes and seasons (e.g., Ringelberg *et al.* 1991). If predation is less severe, food availability becomes important and day and night differences in vertical distribution become less pronounced (Leibold 1990, 1991). Abiotic factors, in particular gradients of temperature (e.g., Kerfoot 1985) and oxygen, cause costs that *Daphnia* need to compensate for. Careful analyses of vertical gradients of zooplankton distributions and related factors (e.g., resource availability and quality, Tessier *et al.* 2001), particularly in deep-water algal maxima (Williamson *et al.* 1996), in combination with modelling exercises (Vos *et al.* 2002) and experimental studies as presented here, will help us to understand the importance of these factors and to develop a theory of zooplankton distribution based on the concept of individual fitness.

Chapter VI

Distribution of *Daphnia* in a trade-off between food and temperature: individual habitat choice and time allocation

Abstract

In a thermally stratified water column with a deep-water algal maximum, *Daphnia* face a trade-off between food (high fecundity) and temperature (fast development). Recent studies showed that *Daphnia* populations move up and down the entire water column to take advantage of both, high food availability and high temperatures, but the exact proportions of time *Daphnia* allocate to the epilimnion, metalimnion, and hypolimnion with their specific food and temperature conditions are not yet known.

In a system of 1 m high, vertical perspex tubes, I adjusted three different temperature gradients with 2 °C, 5 °C, and 10 °C difference between the surface (epilimnion) and the bottom layer (hypolimnion). Algae were added to the hypolimnion to simulate a deep-water algal maximum. The migration behaviour of individual neonate and egg-bearing *Daphnia hyalina x galeata* was monitored in order to measure the proportions of time the individuals allocated to the different vertical habitats and to assess the frequency of their shifting between epilimnion and hypolimnion.

Neonates continuously stayed at the surface, taking advantage of the higher temperature. Most likely feeding was not yet necessary for them due to egg yolk reserves. The egg-bearing females spent more time feeding in the hypolimnion when the temperature was high (lower costs), but also migrated into the epilimnion to take advantage of the higher temperature. In the steepest temperature gradient, the egg-bearing females either shifted between epilimnion and hypolimnion, or they constantly dwelled in the metalimnion with intermediate conditions.

Introduction

In their natural environment, *Daphnia* are exposed to a variety of trade-offs. For example, in lakes with a surface algal maximum *Daphnia* face the trade-offs between avoidance of predation (e.g., Gliwicz & Pijanowska 1988, Ringelberg & Van Gool 2003), or high UV-radiation on the one hand (Leech & Williamson 2001, Rhode *et al.* 2001), and foraging on the other. *Daphnia* experience another trade-off in thermally stratified water bodies with a deep-water algal maximum (DCM) (Williamson *et al.* 1996, Lampert *et al.* 2003). In this situation, the surface layer is warm, which accelerates development, but it is food deficient, which reduces growth and fecundity. In the DCM, the water is cool but food availability is high.

When exposed to vertical trade-offs, *Daphnia* populations may perform diel vertical migration (DVM) (e.g., Gliwicz & Pijanowska 1988, Ringelberg & Van Gool 2003), which is a synchronised migration of a high proportion of the population (Guisande *et al.* 1991, Duncan *et al.* 1993). DVM is known to be an induced defence to either predation risk caused by visually hunting planktivorous fish (Stich & Lampert 1981, De Meester *et al.* 1995, Ringelberg &Van Gool 2003), or to high UV-radiation (Leech & Williamson 2001, Rhode *et al.* 2001). By performing DVM, *Daphnia* escape into the dark hypolimnion during the day but ascend into the food rich epilimnion at night (Ringelberg *et al.* 1991, Loose *et al.* 1993). DVM has also been discussed as an adaptation to the trade-off between food availability and temperature in thermally stratified lakes with a DCM (Williamson *et al.* 1996, Winder *et al.* 2003a), but the influence of predation and other factors on the vertical distribution of *Daphnia* has never been excluded (Williamson *et al.* 1996).

Vertical migration of zooplankton may also occur on a shorter time scale (Haney 1988). For example, Haney (1988) suggested, that zooplankton hide in the dark hypolimnion but undertake short forays into the food rich epilimnion when they are exposed to a trade-off between damage caused by high solar radiation and foraging in a food rich epilimnion. Similarly, Lampert *et al.* (2003) hypothesised that *Daphnia* distribute dynamically in a thermally stratified water column with a deep-water algal maximum, i.e., the *Daphnia* move up and down the water column to take advantage of the high temperatures in the epilimnion and the high food availability in the hypolimnion. Thus, they allocate certain proportions of their time to dwelling in the different vertical habitats with particular food and temperature conditions. In contrast to DVM, this migration is not synchronised within the population. Lampert and Grey (2003) demonstrated this dynamic distribution with an experiment performed in large indoor mesocosms, the Plön plankton towers (Lampert & Loose 1992), where they established a thermally stratified water column and a DCM with ^{15}N labelled algae. As *Daphnia* above and below the thermocline did not differ in ^{15}N enrichment over time, they must all have been feeding in the hypolimnion and thus, have been migrating between the two layers.

The intensity of the trade-offs experienced by *Daphnia* changes with their age and size. Neonates contain egg-yolk reserves, which may reduce the necessity for them to feed at least during the first hours of their life (Tessier & Consolatti 1989). With these energy reserves, they may not experience the trade-off as opposed to the adults without egg-yolk reserves, and therefore, neonates and egg-bearing females would be expected to migrate differently.

The behavioural adaptations of *Daphnia* to the trade-off between food and temperature have only been studied on the population level (Williamson *et al.* 1996, Cole *et al.* 2002, Lampert & Grey 2003, Lampert *et al.* 2003, Winder *et al.* 2003a, Winder *et al.* 2003b). However, from samplings of a population it is not possible to deduce the migration pathways of individuals, which means neither the time *Daphnia* individuals spent in the different vertical habitats, nor the frequency of the shifts between them can be determined (Pearré 1979, Lampert 2003).

In this study, I monitored the migration behaviour of individual neonate and egg-bearing *Daphnia hyalina x galeata* in a temperature gradient and a hypolimnetic algal maximum. I hypothesised that neonates and egg-bearing females migrate differently. Further, I hypothesised that adult *Daphnia* shift between the different vertical habitats and alter their time allocation behaviour and migration frequency depending on the temperature gradient.

Material and Methods

Daphnia hyalina x galeata were synchronised over three to four generations in order to exclude maternal effects, and to obtain experimental females which were born within 12 h of each other. *Daphnia* were raised at 20 °C and continuous dim light. They were kept singly in 100 mL jars containing a suspension of 1 mg C/L *Scenedesmus obliquus* in 0.45 µm filtered water from Lake Schöhsee, a mesotrophic lake in Northern Germany. *Daphnia* were fed daily with 1 mg C/L *Scenedesmus obliquus* which were taken directly from the outlet of a chemostat containing Chu-12 medium (Müller 1972), and transferred into fresh medium every other day. The third and fourth generation of *Daphnia* served as experimental egg-bearing females and neonates.

The experiment was performed in two “plankton organs”, which are systems of 1 m long and 2 cm wide perspex tubes (Dawidowicz & Loose 1992), filled with 0.45 µm filtered lake water, and inserted into a water bath. Both systems were positioned in the same climate room and both were illuminated by dim light.

The first system contained six perspex tubes. The water temperature was adjusted to 20 °C, and no food was added. Three neonate and adult individuals were placed in these columns which served as unstratified controls.

In contrast, the tubes in the second system were thermally stratified. The water column was divided into a warm surface layer (epilimnion), an intermediate layer (metalimnion), and a cold layer underneath (hypolimnion). The epilimnion was always kept at 20 °C. The temperature in the hypolimnion was adjusted to 10 °C, 15 °C, or 18 °C, which corresponded to metalimnetic temperature gradients of 10 °C, 5 °C , and 2 °C. 1 mg C/L of *Scenedesmus obliquus* was added to the hypolimnion. The food concentration in each of the layers within the tubes was measured at the end of the experiment to assure that grazing by *Daphnia* did not severely reduce the hypolimnetic food concentration. The food concentration was measured as particle volume per mL with a Cell Counter and Analyser System (CASY 1, model TCC, Schärfe System GmbH Reutlingen, Germany), which in turn were converted into mg C/L, using a calibration curve. The second system contained 20 perspex tubes, ten each for neonates and egg-bearing females.

Prior to the experiment, 13 neonates and 13 egg-bearing females were distributed randomly over the two plankton organs. Each individual was released singly in 20 cm depth. They were allowed to acclimatise over 2 hours prior to the first observation.

Swimming behaviour of the daphniids was characterised by recording the depth where the individuals dwelled at fixed intervals. The individuals were observed one after another in a fixed order with 49 s intervals between the observations of two succeeding *Daphnia*. Each individual was observed 20 times with 26 min intervals between these observations.

Three different temperature gradients were run one after another. The 10 °C gradient was run twice, the two remaining temperature gradients only once. New individuals were used in each experimental block. In order to test whether the observations of all individuals in the different temperature gradients were comparable, the unstratified controls of all temperature gradients were tested for significant differences. The number of control *Daphnia* was restricted to three. To compensate for the low number, I ran an additional experiment with 13 neonates and 13 egg-bearing females in an unstratified water column without food. A three-way repeated measurements ANOVA on all 20 observations of all *Daphnia* showed no significant difference between unstratified controls run at different times (Table IV 1). Therefore, the experimental data from all three temperature gradients were compared in one statistical analysis. Individuals that were dead or moribund were omitted from the analysis.

Table IV 1: *Repeated measurement ANOVA on all observations of the individuals in unstratified columns without food.*

Factor	*d. f.*	*F*	*p*
Group	3	2.14	0.110
Age	1	26.95	< 0.001
Time	19	1.26	0.206
Group x Age	3	4.93	0.005
Group x Time	57	1.34	0.053
Age x Time	19	1.98	0.008
All	57	1.07	0.345

The migration behaviour was analysed with respect to two different parameters, the mean average depth of the individuals, and the time the *Daphnia* spent in the epilimnion, metalimnion, or hypolimnion, respectively.

Depth measurements of the individuals were log-transformed and subjected to a 2-way ANOVA to test for the influence of age (neonate and egg-bearing female) and temperature gradient (2 °C, 5 °C, and 10 °C). In addition, the influence of the temperature gradient on the two age groups was compared using Tukey's HSD post hoc test for unequal sample sizes.

The time allocation of the neonates and the egg-bearing females was analysed separately. To test the influence of temperature gradient (2° C, 5° C, and 10 °C) and habitat preference on the proportions of time *Daphnia* spend in the epilimnion, metalimnion, or hypolimnion, respectively, the ranks of the percentages of the observations were subjected to a 2-way Kruskal Wallis test with a Scheirer Ray Hare extension. The factors tested were temperature gradient (2° C, 5° C, and 10 °C) and stratum (epilimnion, metalimnion, and hypolimnion).

Results

This study subjected individual neonate and egg-bearing *Daphnia hyalina x galeata* to a trade-off between food availability and temperature. The behavioural response of the individuals to the trade-off was assessed in terms of their average depth (i. e., vertical habitat choice) and the time *Daphnia* spent in the different vertical habitats.

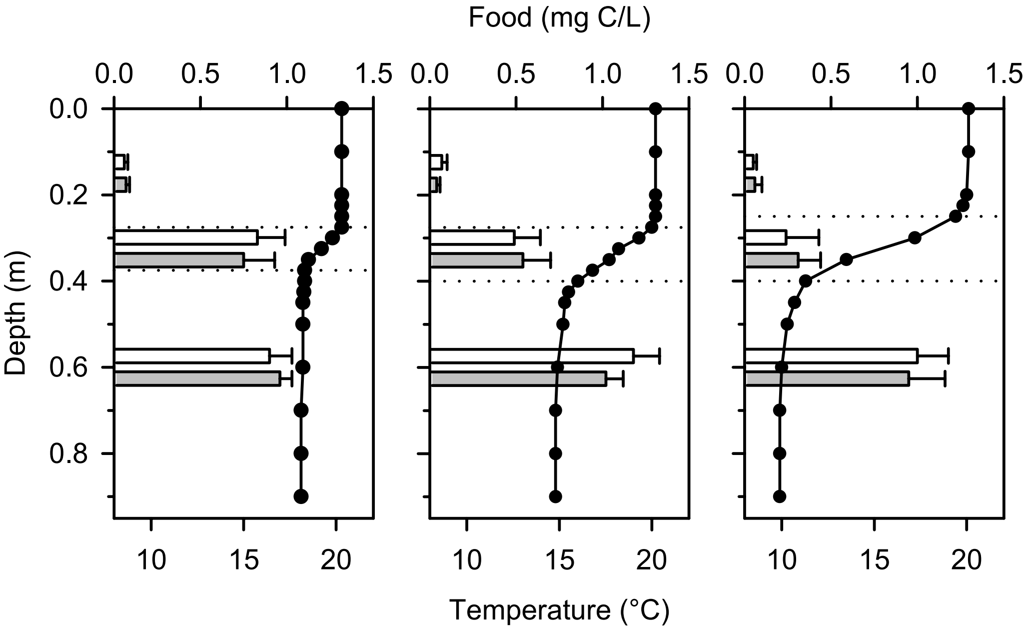


Figure IV 1: *Temperature profiles (black circles) and average final food concentration (± SD) in the epilimnion, metalimnion and hypolimnion in tubes with neonates (open bars) and in tubes with egg-bearing females (shaded bars). The dotted lines indicate the boundaries between the metalimnion and the two other layers.*

Food and temperature gradients: In all three temperature gradients, a clear deep-water algal maximum could be established (Figure IV 1). The *Scenedesmus* concentration was highest in the hypolimnion (1 mg C/L) and lowest in the epilimnion (approximately 0.05 mg C/L). The algal concentration in the metalimnion was intermediate. Thus, the water column was divided into three different vertical habitats, each containing its particular food and temperature conditions.

Comparison of migration behaviour between neonates and egg-bearing females: Neonates and egg-bearing females differed in their migration behaviour (Figure IV 2), which is reflected in their average depth, their habitat selection, and the frequency of shifts between the epilimnion and the hypolimnion. Except for two individuals, all neonates stayed in the epilimnion almost constantly regardless of the temperature gradient. In contrast, the egg-bearing females moved in the entire water column (c.f., Figure IV 3), but their migration was different in the three temperature gradients (Figure IV 3).

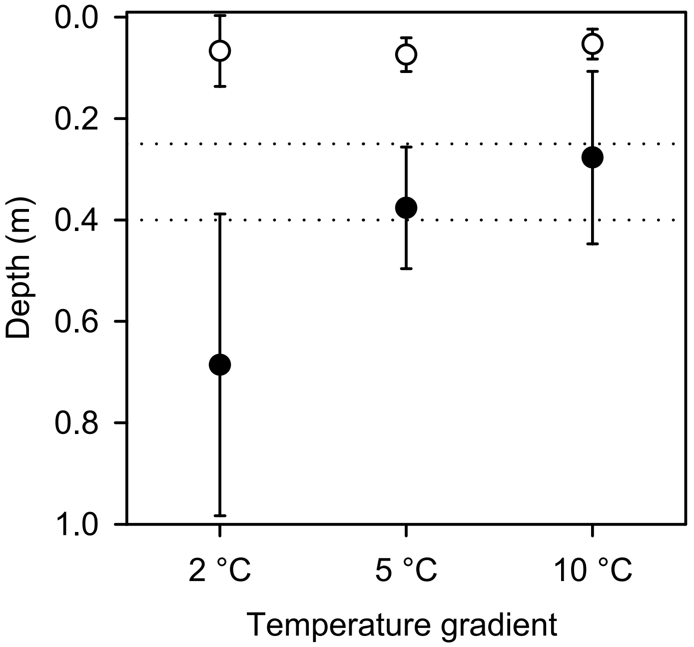


Figure IV 2: *Average depth of neonates (open dots) and egg-bearing females (black dots) ± SD in the three temperature gradients. The temperature in the epilimnion was always 20 °C. The labels of the x-axis denote the temperature difference between epilimnion and hypolimnion. The dotted lines indicate the boundaries between the metalimnion and the two other layers.*

Average depth: The two age groups dwelled in significantly different average depths (2-way ANOVA, effect of age group, $F_{1, 66} = 137.6$, $p < 0.001$), and the average depth in turn depended on the temperature gradient (2-way ANOVA, effect of temperature, $F_{2, 66}$, = 7.6, $p = 0.001$). However, the temperature gradient only effected the average depth of egg-bearing females, but not of neonates (2-way ANOVA, interaction temperature gradient x age group, $F_{2, 66}$, = 6.6, $p = 0.002$). Neonates stayed near the surface almost constantly regardless of the temperature gradient (Tukey's HSD post hoc test on the interaction of temperature gradient x age group, all $p > 0.980$; Figure IV 2 and IV 3). Egg-bearing females dwelled deeper when the temperature in the hypolimnion was higher. However, the difference in the mean depth was only significant between the *Daphnia* in the 2 °C and the 10 °C temperature gradient (Tukey's HSD post hoc test on the interaction between temperature and age, $p < 0.001$). In the 2 °C temperature gradient, egg-bearing individuals dwelled in the hypolimnion (0.69 ± 0.30 m depth; Figure IV 2), while they dwelled at the boundary layer between epilimnion and metalimnion (0.27 ± 0.17 m depth) in the 10 °C gradient. In the intermediate temperature gradient (5 °C) *Daphnia* generally stayed in the metalimnion (0.38 ± 0.12 m).

Table IV 2: *Kruskal Wallis test with Scheirer Ray Hare extension on the ranks of the mean observations in the epilimnion, metalimnion, and hypolimnion (stratum) within each temperature gradient (temp).*

Age group	Factor	*d. f.*	*H*	*p*
Neonate	Temp.	2	0.0	1.000
	Stratum	2	102.7	< *0.001*
	Temp x Stratum	4	0.1	0.999
Egg-bearing female	Temp.	2	3.3	0.192
	Stratum	2	1.6	0.458
	Temp x Stratum	4	32.4	< *0.001*

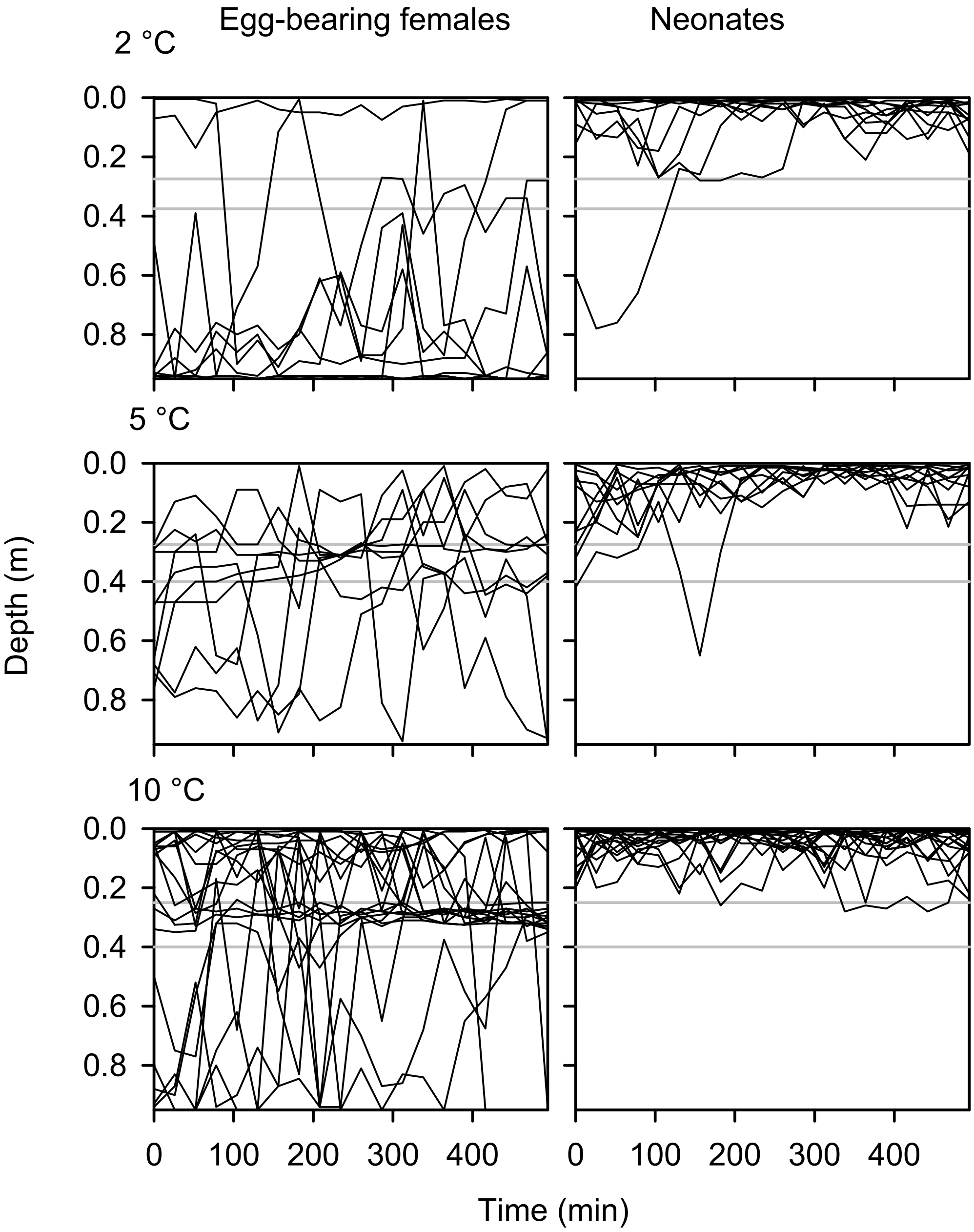


Figure IV 3: *Migration pathways of individual egg-bearing females (left panels) and neonates (right panels) in water columns with 2 °C, 5 °C, and 10 °C temperature difference between the epilimnion and the hypolimnion. The grey lines indicate the boundaries between the metalimnion and the other two layers.*

Time allocation: The neonates greatly preferred the epilimnion in all three temperature gradients, i.e., they spent almost all of their time in the epilimnion, resulting in significantly different proportions of time spent in epilimnion, metalimnion, and hypolimnion (Kruskal Wallis test with Scheirer Ray Hare extension, *p* < 0.001, Table IV 2). Their habitat choice was the same in all temperature gradients (Kruskal Wallis test with Scheirer Ray Hare extension, *p* = 1.000, Table IV 2). In contrast, egg-bearing females allocated their time differently between the three vertical habitats in the three temperature gradients (Figure IV 3 and IV 4). In only two of them did the females clearly prefer one of the vertical habitats (significant interaction temperature gradient x stratum, Kruskal Wallis test with Scheirer Ray Hare extension, *p* < 0.001, Table IV 2, Figure IV 4): In the 2 °C temperature gradient they spent most of their time in the hypolimnion, while in the 10 °C temperature gradient they spent most of their time in the epilimnion and metalimnion. The mothers in the 5 °C temperature gradient stayed almost equally long in all three vertical habitats (Figure IV 4).

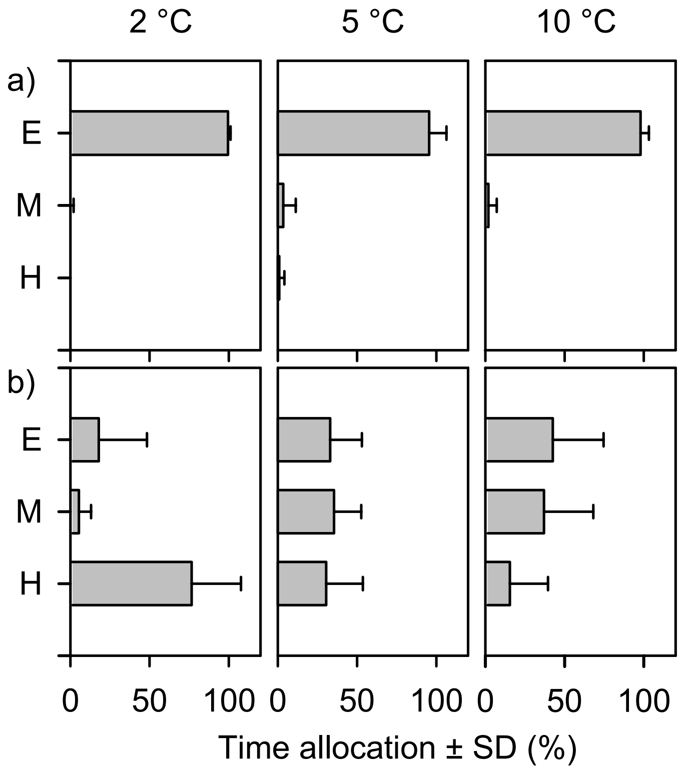


Figure IV 4: *Average proportions of time ± SD neonates (upper panels, a) and egg-bearing females (lower panels, b) allocate to dwelling in the epilimnion (E), metalimnion (M), and hypolimnion (H) in the 2 °C, 5 °C, and 10 °C temperature gradient.*

Table IV 3: *Average time (h) between the shifts from the epilimnion to the hypolimnion and vice versa of neonates and egg-bearing females (egg-bearing f.).*

Temperature gradient	Age group	Time between shifts ± SD (h)
2 °C	Neonate	7.8 ± 1.3
5 °C	Neonate	7.7 ± 1.7
10 °C	Neonate	no shifts
2 °C	Egg-bearing f.	5.9 ± 2.9
5 °C	Egg-bearing f.	4.5 ± 2.3
10 °C	Egg-bearing f.	5.5 ± 2.5

Frequency of shifts: Only one neonate in the 5 °C and in the 2 °C temperature gradient, respectively, undertook a short foray into the hypolimnion (Figure IV 3, Table IV 3), while the other neonates did not shift at all. The egg-bearing females in the 5 °C temperature gradient migrated between the epilimnion and hypolimnion in the shortest time intervals. The females in the two other gradients clearly preferred one of the vertical habitats, and the time intervals between the shifts were longer (Table IV 3).

Discussion

In a thermally stratified water column with a deep-water algal maximum, *Daphnia* are exposed to a trade-off between food and temperature. While the epilimnion is warm but food deficient, the hypolimnion is colder but food rich. The experiment was carried out to test the hypothesis that, firstly, neonates and egg-bearing females migrate differently, and, secondly, that *Daphnia* move up and down the water column and alter their time allocation behaviour and migration frequency depending on the temperature gradient.

The first hypothesis was confirmed. Neonates and egg-bearing females differed in their migration behaviour. Egg-bearing females distributed dynamically, whereas the neonates remained near the surface. The second hypothesis was confirmed only for the egg-bearing females, as only the these altered their time allocation and migration frequency depending on the temperature gradient.

Neonates and egg-bearing females migrated differently, because they experienced the food and temperature trade-off differently. Almost all neonates remained near the surface, only one neonate in the 5 °C and one in the 2 °C temperature gradient undertook a short foray into the hypolimnion. As they were at the most 12 h old at the beginning of the observations, they may still have contained egg yolk. Neonate *Daphnia parvula*, a species of approximately the same size as *D. hyalina x galeata*, are known to survive for at least 40 h on their egg-yolk reserves at 20 °C without food (Tessier & Consolatti 1989). Most likely, the neonates in the present study did not yet need to feed and remained in the epilimnion to take advantage of the higher temperature (Venkataraman & Job 1980, Vijverberg 1980, Hanazato & Yasuno 1985, Giebelhausen & Lampert 2001). Consequently, they did not yet experience the trade-off between high temperatures and food availability and dwelled near the surface in all temperature gradients, resulting in a shallow average depth (Figure IV 2) and a very low frequency of shifts between the epilimnion and hypolimnion (Table IV 3).

This finding seems to be inconsistent with the findings of Kessler and Lampert (Chapter III), who monitored the vertical distribution of different size classes of a *Daphnia* population in a thermally stratified water column with a hypolimnetic DCM. In their study, small *Daphnia* (total length up to 1 mm) were sampled in the hypolimnion. However, this size class did not only include neonates, but also older *Daphnia*, that lacked egg-yolk reserves and consequently moved down into the DCM to feed.

In contrast to neonates, egg-bearing females experienced the trade-off between food and temperature: The adults needed food to produce eggs (Lampert 1978, Orcutt & Porter 1984), but foraging in low temperatures is costly (Vijverberg 1980, Kerfoot 1985, Dawidowicz & Loose 1992). However, even at 10 °C *Daphnia* can be expected to achieve positive growth rates due to the high food concentration (Orcutt & Porter 1984, Giebelhausen & Lampert 2001, Lampert *et al.* 2003). The food concentration in the epilimnion was either slightly above or below the threshold food concentration for *Daphnia hyalina* and *Daphnia galeata* (Gliwicz 1990), i.e., *Daphnia* would either have very low or even negative growth rates in epilimnetic water (Gliwicz 1990, Giebelhausen & Lampert 2001). However, high temperatures accelerate metabolic processes and the development of eggs (Kerfoot 1985, Dawidowicz & Loose 1992), which increases their fitness.

In this food and temperature trade-off, the egg-bearing females migrated between the different layers and spent proportions of their time in all vertical habitats, resulting in deeper average depths compared to the neonates (Figure IV 2). The time adults spent in the DCM decreased with a decreasing hypolimnetic temperature, because low temperatures are costly (Vijverberg 1980, Kerfoot 1985, Dawidowicz & Loose 1992) and cause higher costs associated with foraging. Especially in the 10 °C temperature gradient, adult *Daphnia* spent most of their time in the epilimnion and the metalimnion and avoided the hypolimnion. Such an avoidance of cold temperatures by *Daphnia* has been shown in several other studies (Calaban & Makarewicz 1982, Haney 1993, Lampert *et al.* 2003).

In all temperature gradients, they left the food rich hypolimnion for periods of time and dwelled in the epilimnion. By moving between the epilimnion and the hypolimnion, they took advantage of both, food availability and high temperatures. Such dynamic distribution behaviour under reversed conditions was also suggested by Haney (1988) for zooplankton in a water column with an epilimnetic algal maximum and high UV radiation, where the *Daphnia* would stay in the dark hypolimnion to avoid the damage caused by light, but also undertook short forays into the epilimnion to feed.

The frequency with which the egg-bearing females shifted between the epilimnion and the hypolimnion differed between the three temperature gradients (Table IV 3). The frequency of shifts was lowest in the 2 °C temperature gradient, where the individuals spent most of their time in the hypolimnion with relatively high temperatures and thus low costs associated with foraging. The egg-bearing females in the 10 °C applied two different “strategies”: Either they frequently migrated between the epilimnion and the hypolimnion, or they remained in the metalimnion and took advantage of intermediate food and temperature conditions. As the average frequency of these shifts was calculated from both, the “resident” and the migrating individuals, the time between the shifts was relatively long.

While the migration into the hypolimnion can obviously be ascribed to foraging, there are several explanations for the migrations of adult *Daphnia* back into the epilimnion: *Daphnia* may have been searching for better food patches. *Daphnia* distribute evenly over a food deficient water column, but after the insertion of food patches, *Daphnia* are able to quickly locate and aggregate in these food patches (Haney 1993) even over long distances (Plath 1998). Jensen *et al.* (2001) demonstrated that horizontal long-distance migration into a food patch also occurred in the field. The ability of *Daphnia* to find food patches even over long distances was attributed to food search migrations when they were hungry (Plath 1998, Jensen *et al.* 2001). In the present study, *Daphnia* may have probed the water column for even better food patches. However, the migrations into the epilimnion cannot have been food search behaviour alone, because the food concentration in the hypolimnion was high, which is known to attract *Daphnia* (Jakobsen & Johnsen 1987, Neary *et al.* 1994, Larsson & Kleiven 1996). Even if *Daphnia* searched for food, the time *Daphnia* spent in the epilimnion was too long to be food search behaviour alone. *Daphnia* are able to find food patches very quickly. In a 2.5 m high water column they found a newly added food patch within 30 min (Haney 1993), and in a 7 m high water column within 2.5 h (Plath 1998), respectively. If *Daphnia* had searched in vain for food at the surface of the 1 m high water columns used in the present study, they would have returned to the hypolimnion earlier than every 4.5 to 5.9 h.

Alternatively, the forays into the epilimnion may increase or accelerate somatic growth (Venkataraman & Job 1980, Vijverberg 1980, Korpelainen 1986) and the development of eggs (Venkataraman & Job 1980), which increased their fitness. Especially in the hypolimnion at 10 °C, growth and egg-development was very slow (Orcutt & Porter 1984, Kerfoot 1985, Lampert *et al.* 2003), and these females can be expected to have a high need to compensate for the overall heat loss by dwelling in the epilimnion. This is in agreement with the observation in the present study, as these females allocated more time to dwelling in the epilimnion than did *Daphnia* in the other temperature gradients (Figure IV 4). However, the effect of temperature interacts with the effect of the available food concentration (Orcutt & Porter 1984, Giebelhausen & Lampert 2001), which means, the warmer temperature in the epilimnion only leads to increased growth when enough food is available (Giebelhausen & Lampert 2001). By migrating between the warm epilimnion and the food rich hypolimnion, they could both accelerate growth and egg-development and also obtain enough food for growth and egg-production.

Although a dynamic distribution would be expected to increase the fitness of *Daphnia* in a food-temperature trade-off, it is very difficult to measure the benefit of this strategy. Further experiments and modelling are needed to assess the fitness benefit of such a behaviour.

Conclusion

Conclusion

Several studies investigated the distribution of *Daphnia* populations in thermally stratified lakes with a deep-water algal maximum, where *Daphnia* are exposed to a vertical trade-off between food and temperature. Most of these studies suggested diel vertical migration to be an adaptation to this vertical trade-off, but the influence of fish predation could never be excluded. In the present thesis, I found evidence that the weak vertical displacement of large *Daphnia* in a fishless environment is a "residual" response against the potential threat posed by visually hunting fish rather than an adaptation to the food-temperature trade-off. Instead of migrating in diel cycles, *Daphnia* actively chose the best vertical habitat and performed frequent, asynchronous migrations between the different layers, resulting in a match between the vertical distribution of the population and the corresponding fitness distribution. *Daphnia* changed their vertical distribution when the temperature gradient, i.e., the intensity of the trade-off, was slightly altered. Thus, *Daphnia* are able to correctly assess the profitability of the different vertical habitats and adjust their behaviour accordingly.

In the field, the fitness distribution and the vertical distribution of *Daphnia* populations will be influenced by additional vertical gradients, such as gradients in predation risk, UV-radiation, anoxia, or competition. Most likely *Daphnia* respond to these additional vertical trade-offs with similar behavioural mechanisms as in the food and temperature trade-off alone, but this needs to be verified in future studies.

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Lebenslauf

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Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertation, abgesehen von der Beratung durch meine akademischen Lehrer, selbstständig angefertigt habe und keine anderen als die angegebenen Quellen und Hilfsmittel von mir eingesetzt worden sind. Des weiteren versichere ich, dass die vorliegende Dissertation weder ganz noch zum Teil bei einer anderen Stelle im Rahmen eines Prüfungsverfahrens im Fach Limnologie vorgelegen hat.

Das erste Kapitel dieser Arbeit ist bereits veröffentlicht: (Kessler K. & Lampert W. (2003) Note - Counting and sizing preserved *Daphnia* with the Optical Plankton Counter. Archiv für Hydrobiologie, 156, 485-493). Kapitel 3 mit Winfried Lampert als Koautor befindet sich bei Freshwater Biology in Revision. Kapitel 2 wurde bei Oecologia und Kapitel 4 bei Freshwater Biology eingereicht.

Kiel, den 18.12.2003