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Influence of food quality on depth selection of *Daphnia pulicaria*

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We studied the habitat choice of juvenile and adult Daphnia pulicaria in thermally stratified water columns (plankton towers) with a deep water algal maximum (DCM). The DCM consisted of either filamentous cyanobacteria (Planktothrix agardhii), non-filamentous Chlorophyceae (Scenedesmus obliquus) or a mixture of both. Adult D. pulicaria spent more time at colder temperatures in the presence of P. agardhii than in the presence of S. obliquus, either as the sole food source or when mixed with P. agardhii. Juvenile D. pulicaria did not show a different habitat choice in the three food treatments. In a fourth treatment, we also determined Daphnia distribution in the absence of food. Comparing the habitat choice of juveniles and adults in each of the four treatments, the latter spent more time at colder temperatures when food was absent or when in the sole presence of P. agardhii. Additional grazing and stable isotopic marker experiments showed that D. pulicaria ingested and assimilated Planktothrix filaments. The results suggest that the differences in habitat choice between adult D. pulicaria in the presence of different food types were influenced by food quality effects: adult Daphnia which move to colder waters in the presence of low quality P. agardhii decrease their metabolic rate and might thus be able to invest more resources into reproduction when environmental conditions improve.

INTRODUCTION

In most ecosystems, including lakes, food is distributed in a patchy manner so that animals have to actively search for food. The observable behaviour of animals resulting from this can have important cascading effects on the functioning of ecosystems.

Temperature varies consistently in stratified lakes of temperate regions. This may have strong consequences for the habitat choice of aquatic organisms such as *Daphnia*. For instance, if a stratified lake contains food for daphnids only in the metalimnion (deep water algal maximum, DCM) (Abbott *et al.*, 1984; Barbiero and McNair, 1996; Barbiero and Tuchman, 2004), daphnids are faced with a trade-off between staying in the epilimnion at warm temperatures (high growth rates) but with little food (small energy intake) and staying in the metalimnion at lower temperatures but in the presence of abundant food. As *Daphnia* represent an important link between primary producers and fish, their behavioural response in such a situation will strongly affect trophic transfer in the food web of the lake.

Additionally, as temperature declines steadily within the thermocline, this layer represents an area with numerous different combinations of food and temperature for daphnids. If such an environment is predator-free, *Daphnia* can experience distinct combinations of food and temperature and they should spend most of their time in the combination that optimizes their fitness (Lampert *et al.*, 2003; Kessler and Lampert, 2004; Lampert, 2005). The optimal habitats are often different between *Daphnia* species and between size classes within species. There are studies which show that for young, small daphnids that still feed on egg yolk

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reserves during their first few days, warm temperature is more important than it is for large daphnids (Tessier and Consolatti, 1989; Kessler, 2004) (Kessler, personal communication, Plön).

Daphnia are generally regarded as unselective filter feeders which can ingest particles between 2 and ~50 μm, depending on the species, size and age (Geller and Müller, 1981; Brendelberger, 1985; Brendelberger, 1991). Filamentous cyanobacteria are often associated with algal blooms which in turn usually only support low biomass of daphnids (Moss *et al.*, 1991; Ghadouani *et al.*, 2006). The reasons for this phenomenon seem to be mechanical interference of filaments with the filtering process of daphnids, toxicity and the absence of essential biochemical compounds such as sterols in cyanobacteria. Numerous studies have investigated these three mechanisms over the last few decades. However, as results are controversial, this topic continues to attract major attention and is still being studied intensively (Wilson *et al.*, 2006).

For a long time, filamentous cyanobacteria were considered as inedible for daphnids due to their filamentous nature, and studies indicated that mechanical interference in the filtering process was the reason for inferior assimilation of filamentous cyanobacteria (Burns, 1968; Hawkins and Lampert, 1989). However, experimental indication of mechanical interference is contradictory (DeBernardi and Giussani, 1990) and there is evidence for interference (Burns *et al.*, 1989; DeMott *et al.*, 2001), for non-interference (Müller-Navarra *et al.*, 2000; von Elert *et al.*, 2003) and for ingestibility (Knisely and Geller, 1986; Gilbert and Durand, 1990; Hartmann and Kunkel, 1991; Kurmayer, 2001). Generally, interference is due to clogging of the filtering structures by filaments. The subsequent cleaning process with the abdominal claw can lead to rejection of the total content of the food groove (Burns, 1968). Consequently, this decreases the total filtration rate, resulting in decreased *Daphnia* growth in the presence of filamentous, non-toxic cyanobacteria (Gliwicz, 1990; Gliwicz and Lampert, 1990; DeMott *et al.*, 2001). However, there is also evidence that long filaments can be broken into pieces by being handled by daphnids (Dawidowicz, 1990), which can lead to improved grazing.

These conflicting results suggest that the process of mechanical interference of filaments depends on grazer species, cyanobacterial species and filament concentration. However, interference of cyanobacterial filaments with filtering structures is also size-dependent in daphnids: in the presence of filaments, the filtration rate of large daphnids is decreased more than the filtration rate of small ones (Hawkins and Lampert, 1989; Gliwicz and Lampert, 1990; DeMott *et al.*, 2001). Two reasons were suggested to be responsible for the different ability of small and large bodied *Daphnia* to cope with the filaments: first, the carapace gap is usually narrower in smaller individuals, which prevents filaments from entering the filtration chamber inside the carapace (Gliwicz and Siedlar, 1980), second, it was suggested that the flow around the filtering appendages of small and large individuals is different (Abrusán, 2004). In smaller individuals, due to the lower Reynolds number of the flow, the filtering structures might work more like paddles than like sieves, which decreases the possibility of the filaments clogging the filters. In this context, Abrusán (Abrusán, 2004) showed that growth of large *Daphnia* was higher in water with higher viscosity compared to water with a lower viscosity in the presence of filaments (*Cylindrospermopsis raciborskii*). This shows that viscosity is an important factor when considering the process of interference of *Daphnia* with filamentous food. Together with the results of Geller and Müller (Geller and Müller, 1981), who have demonstrated that the mesh sizes of the filtering structures of some *Daphnia* clones change with age, these results of Abrusán (Abrusán, 2004) infer that interference with food and food requirements might also change with age and therefore size. This altogether indicates that large and small size classes of a *Daphnia* species should have different optimal habitats in the same environment.

However, to date, this habitat selection behaviour of *Daphnia* in the presence of filamentous non-toxic cyanobacteria has largely been ignored. Bednarska and Dawidowicz (Bednarska and Dawidowicz, in press) studied the variability of the combined behavioural and phenotypic adaptations of nine *D. longispina* clones in the presence of *C. raciborskii*. In this study, the behaviour of *Daphnia* depended largely on the mesh size of the filtering appendages: animals with larger mesh size descended into colder parts of the water column of plankton organs (glass tubes: 60 cm long, 1 cm diameter) (Dawidowicz and Loose, 1992) when exposed to filamentous cyanobacteria than animals with smaller mesh size.

In addition to filament interference, poor nutritional quality is the other main reason for the low growth rate of daphnids in the presence of cyanobacteria. Prokaryotic cyanobacteria are generally known to be inadequate food of a low quality for *Daphnia* as most of them lack sterols (Ahlgren *et al.*, 1990; DeBernardi and Giussani, 1990; Volkman, 2003). Sterols play an important role in eukaryotic organisms as precursors of steroid hormones and in lipid biostructures (Goad, 1981). As sterols cannot be synthesized by arthropods (e.g. *Daphnia*) *de novo* (Goad, 1981), they must be obtained from their food. Von Elert *et al.* (von Elert *et al.*, 2003)

provided evidence that when supplemented with sterols, the filamentous cyanobacteria *Anabaena variabilis* supports growth rates of *Daphnia* that are almost as high as when fed *Scenedesmus*. Subsequent studies further confirmed that sterols are essential dietary compounds for daphnids which significantly affect their growth and reproduction rates (Martin-Creuzburg and von Elert, 2004; Martin-Creuzburg *et al.*, 2005). Recent estimates indicate that the lack of sterols constrain the quality of natural seston considerably, if >80% (von Elert *et al.*, 2003) or >50% (Martin-Creuzburg *et al.*, 2005) of the total biomass is prokaryotic.

In our experiment, we studied the habitat choice of different size classes of *D. pulicaria* by investigating their vertical distribution in the presence of different food patches over a temperature gradient (plankton tower experiment). We used either filamentous cyanobacteria, non-filamentous green algae or a mixture of both as food in a DCM to investigate whether (i) different size classes of *D. pulicaria* had different optimal habitats and (ii) whether food quality can influence habitat choice. We also studied habitat choice of different size classes in the same temperature gradient without food. Additionally, we investigated whether *D. pulicaria* is able to ingest and assimilate *P. agardhii* filaments.

METHOD

Cultures

All experiments were carried out with *D. pulicaria* which has been cultivated at the Max Planck Institute for Limnology for several years. Daphnids used in the habitat choice experiments were cultured in 200 L containers filled with 10-μm filtered lake water from Lake Schöhsee, a mesotrophic lake near to the institute. *S. obliquus* served as food. Daphnids used in the grazing experiment were cultured in 2 L jars in 0.45 μm filtered lake water from Lake Schöhsee and *S. obliquus* served as food.

As food for the daphnids in the experiments we chose the Chlorophyceae *S. obliquus* (SAG 276–36) and the filamentous, non-toxic cyanobacterium *Planktothrix agardhii* (NIVA cya 116). Both food types were cultured in batch systems under continuous light conditions. We used Z/4 medium (Zehnder and Gorham, 1960) for *S. obliquus* and WC medium for *P. agardhii* (Modified Woods Hole MBL after Guillard and Lorenzen, 1972). To obtain high growth rates and dense cultures, *S. obliquus* cultures were continuously aerated with a mixture of CO_2 and air, *P. agardhii* cultures were aerated with air only.

Tower experiment

This experiment was performed in two large indoor mesocosms, the plankton towers in Plön (Germany), which were randomly assigned to the different treatments. The plankton towers are 11.5 m high and have an inner diameter of 0.85 m. Sample ports for withdrawing phyto- and zooplankton samples are located at every 0.5 m. The towers are described in detail by Lampert and Loose (Lampert and Loose, 1992). The photoperiod during the experiment was 12:12 h D:L. During the day, the towers were lit from the top.

For each replicate, the towers were filled with 10-μm filtered water from Lake Schöhsee, and a temperature profile was established with 20°C in the epilimnion (0–3.0 m), 13°C in the hypolimnion (7.0–11.5 m) and a broad, 4 m thermal discontinuity layer in-between (in the following termed thermocline). A typical temperature profile is shown in Fig. 1. After thermal stratification of the towers, we put ~10 000 daphnids into each tower and added a moderate concentration of *S. obliquus* as food into the epilimnion (~0.2 mg C L^{-1}). For the next 5–6 days, we allowed the *Daphnia* population to grow undisturbed. The purpose of this pre-experimental period was to obtain large and rapidly growing *Daphnia* populations in the towers. The experiment started as soon as *S. obliquus* in the water column was almost entirely grazed down (algae POC: not detectable to 0.02 mg C L^{-1}).

Altogether 15 runs were conducted in the towers. Of these, 10 randomly chosen runs started with the measurement of the vertical distribution of *Daphnia* in

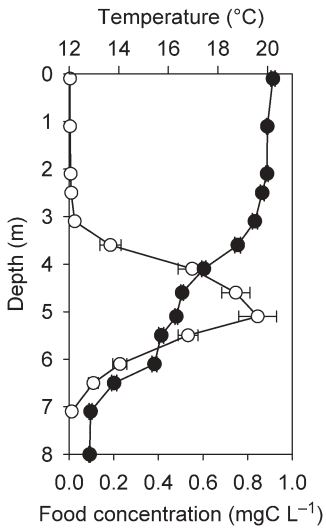

Fig. 1. Mean temperature (closed symbols) and mean food concentration (open symbols) profiles averaged over all replicates. Error bars represent 1 SE, $N = 15$.

the first night after the pre-experimental population growth period (thus in the absence of food). We used this as the no-food treatment. *Daphnia* distribution was always sampled 2 h after the beginning of the dark period to obtain a light-independent distribution. Sampling was done simultaneously at 12 different depths using glass traps and pumps which filtered the zooplankton from ~30 L of water ("*Daphnia* traps") (Lampert and Loose, 1992). Daphnids were immediately fixed in sucrose formaldehyde (4%) (Haney and Hall, 1973).

On the next morning, all 15 runs received either *P. agardhii* (0.65 mg C L^{-1}), *S. obliquus* (0.65 mg C L^{-1}) or a mixture of both species (*P. agardhii*: 0.65 mg C L^{-1}, *S. obliquus*: 0.2 mg C L^{-1}) as food for the daphnids in the thermocline. We thus performed three food treatments, which we replicated five times each. The food was situated in the thermocline over a temperature gradient as we wanted to give the daphnids the opportunity to choose their optimal temperature × food combination. After adding the food, we allowed *Daphnia* to acclimate to their food source for 2 days and one night before sampling them during the second and third night. We averaged the distributions of these two consecutive night samples and considered this mean as one replicate. Although the *Daphnia* distribution in the absence of food (10 replicates) and the consecutive distributions in the presence of food were done with partly the same *Daphnia* population, we considered these treatments as independent because the conditions in the towers were different and *Daphnia* had enough time to redistribute according to the new conditions. Furthermore, there is generally a constant turnover of the populations due to births and deaths so that the populations are never completely identical.

During the experiment, food carbon concentration in the water column of each tower was measured every morning and afternoon by taking water samples at 13 depths (0.1–7.1 m) and by measuring fluorescence with a FluoroProbe (bbe Moldaenke, Kiel, Germany). The FluoroProbe is able to distinguish between the pigments of green algae (*S. obliquus*) and cyanobacteria (*P. agardhii*). The fluorescence of each food type was converted into particulate carbon using previously established calibration curves. Because of grazing, the carbon concentration within the algal maximum was always lower than 0.65 mg C L^{-1} (or 0.2 mg C L^{-1} for *S. obliquus* in the Mix treatment) in the morning and the missing amount was then added. This daily addition of fresh *Planktothrix* and/or *Scenedesmus* also helped to counteract the possible qualitative decrease of the available food source which might have occurred over time. After this replenishment procedure in the morning, we allowed algae to mix within the respective water layer, and the food concentration was sampled again ~0.5 h before the onset of darkness. The average of all afternoon food profiles is given in Fig. 1.

Sampled daphnids were counted and divided into two size classes (neonates and juveniles: 0.6–1.5 mm; adults: 1.5–3.5 mm) with an optical plankton counter (OPC-1l, Focal technologies, Dartmouth, Nova Scotia, Canada) (Kessler and Lampert, 2003).

Although concentration of living algae/cyanobacteria in the epilimnion (three food treatments) or the whole water column (no-food treatment) never exceeded 0.02 mg C L^{-1} when measured with the FluoroProbe, measurements of total POC in the epilimnion during two replicates (Mix treatment) via filtration and combustion indicated 0.1–0.15 mg C L^{-1} [method: filtration of water on precombusted GF/C filters and subsequent combustion (CN Analyser Fisons instruments)]. This shows that the epilimnion was probably never free of food but that this food was likely to be of a very low quality as it mainly consisted of "non-living" POC.

We also determined the length distribution of *P. agardhii* filaments in four of the five *Planktothrix* replicates from two depths within the food patch (4 and 5 m) to see whether it differed between depths, due to either *Daphnia* grazing, temperature or sedimentation effects. This was done by fixation of seston samples with acid Lugol's iodine during the afternoon sampling procedure. Filament length was measured by using an inverted microscope connected to a computer-measuring program (analySIS 2.11). We measured at least 100 randomly chosen filaments per sample (×100 magnification).

Labelling of *P. agardhii* with ^{15}N and isotope measurement

To test whether small and large *D. pulicaria* were able to ingest *P. agardhii* filaments, we used ^{15}N-labelled *P. agardhii* in one *P. agardhii* replicate and all the five Mix replicates. *Planktothrix agardhii* for labelling was grown in a 5-L culture in WC medium with 15% of the inorganic nitrogen exchanged with ^{15}N (NH_4–$^{15}NO_3$, Chemotrade, Leipzig) for 3–4 days. Before adding *P. agardhii* to the towers, we allowed the filaments to sediment for 12 h in the batch culture, the supernatant was then removed and the filaments were diluted again with unlabelled WC solution. We mixed labelled and unlabelled *P. agardhii* in such a way that the label in the tower was between $\delta^{15}N = 345–955‰$ in the different replicates. The isotopic signatures ($\delta^{15}N$) of *Daphnia* and seston in the towers were measured on day 0 (start), day 1, 2 and 3 of each replicate's run. To measure $\delta^{15}N$ of

seston, water samples were collected through 2–3 ports from the metalimnion. Volumes of 0.5–1.5 L were filtered onto precombusted and preweighed GF/C glass-fiber filters (Whatman). Daphnids were sampled from two depths per tower by "*Daphnia* traps" (Lampert and Loose, 1992). Daphnids were sorted by the two size classes into preweighed tin cups and dried at 60°C for 24 h.

We calculated the incorporation of ^{15}N (%) of small and large daphnids after 1, 2 and 3 days as $(\delta^{15}N_D start - \delta^{15}N_D end) * 100/(\delta^{15}N_D start - \delta^{15}N_{food})$, where $\delta^{15}N_D start$ is the $\delta^{15}N$ of the daphnids at day 0, $\delta^{15}N_D end$ the $\delta^{15}N$ of the daphnids at day 1, 2 or 3, respectively, and $\delta^{15}N_{food}$ the mean $\delta^{15}N$ of the algae during the experiment. We calculated linear regressions for the five Mix treatments in which we used labelled *P. agardhii*. $^{15}N/^{14}N$ isotopic ratios were measured by a Eurovector EuroEA 3000 Series elemental analyzer coupled to a Micromass Isoprime mass spectrometer.

Grazing experiment

We calculated the clearance rate of *D. pulicaria* on *P. agardhii* at 12°C and 20°C in a laboratory experiment by measuring changes in *Planktothrix* biomass. Ten adult *D. pulicaria* were allowed to graze for 24 h either in the presence of *P. agardhii* alone (0.65 mg C L^{-1}) or in the presence of a mixture of *S. obliquus* and *P. agardhii* (0.2 and 0.65 mg C L^{-1}, respectively). Each treatment was replicated five times. All experimental animals were born within 24 h and were put into the experiment at an age of 9 days. We used 1 L glass vessels filled with 0.45-μm filtered lake water for each replicate. All vessels were kept in a climate chamber at a constant temperature of 20°C, the vessels of the 12°C treatments were additionally cooled in water baths. The experiment was performed in darkness, although we switched on dim light for ~5 min every 2 h to stir the water in the vessels to avoid sedimentation of *P. agardhii* and *S. obliquus*. Controls without *Daphnia* were replicated three times. Additionally, one treatment (five replicates) with only *S. obliquus* as food (0.65 mg C L^{-1}) was performed to evaluate whether the lack of temperature adaptation of the animals prior to the experiment lead to an exceedingly strong decrease in their grazing rate in the 12°C treatment. At the beginning and the end of the experiment, seston was sampled and fixed with acid Lugol's iodine. The density of *P. agardhii* filaments or *S. obliquus* cells was determined with sedimentation chambers (Utermöhl, 1958) and an inverted microscope (×100 magnification). We counted at least 400 filaments (*P. agardhii*) or cells (*S. obliquus*) per sample to keep the counting error <10% (Lund *et al.*, 1958). Again we also determined the relative filament length distribution (see tower experiment). We then calculated the total filament length (in mm mL^{-1}) for each replicate by multiplying the density of *Planktothrix* filaments by the mean filament length. The mean clearance rate (mL h^{-1}) per *Daphnia* for each treatment was then calculated according to Lampert and Sommer (Lampert and Sommer, 1999).

Statistics

The median depths of each size class of *Daphnia* in each replicate were calculated based on the total amount of sampled individuals in a profile. Animals from all sampling ports were included. The median depths were then compared either between size classes within each treatment (Mann–Whitney *U*-test) or within each size class between food treatments (Kruskal–Wallis *H*-test).

Filament length distributions at 4 and 5 m in the tower experiment, and in all treatments of the grazing experiment, were analysed with Mann–Whitney *U*-test. Differences between clearance rates in warm and cold vessels of the *Planktothrix*, Mix and *Scenedesmus* treatments of the grazing experiment were calculated separately by Mann–Whitney *U*-tests. To test, if clearance rates were different from zero, we used one sample *t*-test. To compare slopes of regressions showing the ^{15}N uptake of small and large daphnids in the tower experiment, we used ANCOVA (Sokal and Rohlf, 1981). All analyses were calculated with Statistica 6.1 (StatSoft, Inc).

RESULTS

Tower experiment

The two size classes of *D. pulicaria* reacted differently to the various food treatments. In the presence of *S. obliquus* in the thermocline, the distributions of small and large daphnids were almost identical (Fig. 2). Both size classes stayed mainly at the upper zone of the food patch, i.e. at the interface between the warm layer and the food rich layer. The median depth was not significantly different between size classes (Mann–Whitney *U*-test, $U = 10.5$, $P = 0.65$) (Fig. 2).

In the presence of the mixed food source (*S. obliquus* and *P. agardhii*), the median depths of small and large daphnids were also not significantly different (Mann–Whitney *U*-test, $U = 10.0$, $P = 0.55$) (Fig. 2).

In the presence of *P. agardhii*, small and large daphnids showed different vertical distributions (Fig. 2). The majorities of both size classes, the small and the large *Daphnia* were still present in the upper, relatively warm

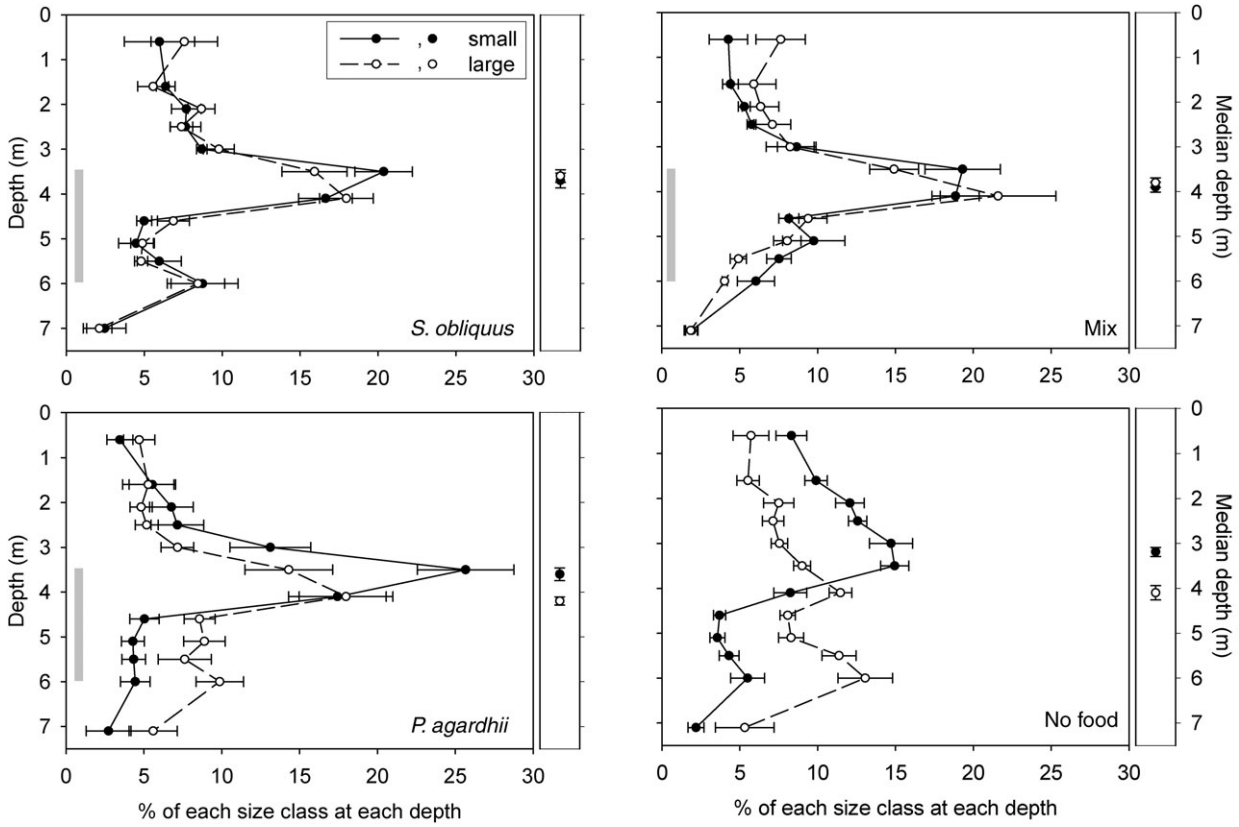

Fig. 2. Vertical distribution and median depth of small and large *D. pulicaria* in the four treatments (*P. agardhii*, *S. obliquus*, Mix- and no-food). $N = 10$ for the no-food treatment; $N = 5$ for all other treatments. Error bars represent 1 SE. The grey box indicates the position where food concentration was higher than 0.2 mg C L^{-1}.

part of the food patch. However, there was a higher percentage of large daphnids within the colder part of the food patch (4.1–6.0 m) compared with the small daphnids. This difference in the distributions between the two size classes is reflected by the different median depths of the large and small *Daphnia* populations (Mann–Whitney *U*-test, $U = 2.0$, $P = 0.02$) (Fig. 2).

In the no-food treatment, the distributions of small and large *Daphnia* were radically different, showing that the two size classes had different optimal habitats under these circumstances. The main part of the population of small daphnids remained within the epilimnion, whereas the distribution of the large ones was biased towards the hypolimnion (5.5–6.0 m) (median depths: Mann–Whitney *U*-test, $U = 9.0$, $P = 0.001$) (Fig. 2).

When the median depths of large *Daphnia* were compared across the three food treatments, they differed significantly (Kruskal–Wallis H test, $H_{2,15} = 7.1$, $P = 0.029$). There was a higher percentage of the population in the colder parts of the food patch in the *P. agardhii* treatment compared with the Mix and the *S. obliquus* treatment. Thus, large daphnids dwelled on average deeper in the *P. agardhii* treatment than in the other two treatments.

The median depths of small *D. pulicaria* between the three food treatments were not significantly different (Kruskal–Wallis *H*-test, $H_{2,15} = 1.9$, $P = 0.392$).

Filament length of *P. agardhii* in the tower experiment was between 12 and 713 μm. There was no significant difference between the length distributions of *P. agardhii* filaments at different depths of the metalimnion (Mann–Whitney *U*-test, $U = 7.0$, $P = 0.77$). Thus, the *Daphnia* distributions in the Mix and *Planktothrix* treatment did not result from different filament lengths at different depths.

The use of *P. agardhii* as a food source

There was a significant increase in the $\delta^{15}N$ of the daphnids after the addition of the labelled filaments in the towers (Fig. 3). Thus, both size classes ingested and assimilated *P. agardhii*. There was no difference between slopes of regressions (ANCOVA, $F_{(1,26)} = 0.000$, $P = 0.990$) (Fig. 3).

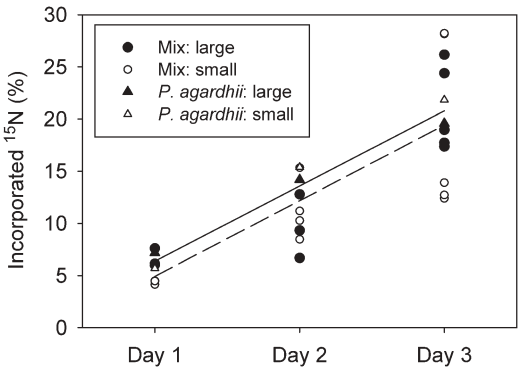

Fig. 3. Incorporated ^{15}N (%) of small and large *D. pulicaria* in the Mix and the *Planktothrix* treatment over time. Regressions are calculated for the Mix treatment only and for each size class separately (large: $y = 7.2x - 0.9$, $r^2 = 0.68$, $P < 0.001$; small: $y = 7.2x - 2.3$, $r^2 = 0.53$, $P < 0.001$). Solid line is the regression for large daphnids, broken line for small daphnids.

Grazing experiment

The grazing experiment clearly showed that *D. pulicaria* was able to process *P. agardhii* filaments. Generally, *Daphnia* treatments contained a higher relative frequency of small filaments (<50 μm) than the respective control treatments (Fig. 4), which probably resulted from handling and breaking of filaments by daphnids. The median filament length in the controls and *Daphnia* treatments were significantly different in both *Planktothrix* experiments (12°C and 20°C) and in one Mix treatment (20°C) (Mann–Whitney *U*-test, $U = 0.0$, all $P < 0.025$) (Table I). The difference between control and *Daphnia* treatment in the Mix treatment at 12°C experiment was only marginally significant (Mann–Whitney *U*-test, $U = 1.0$, $P = 0.052$) (Table I). The clearance rate was different from zero only in the 20°C Mix treatment (one sample *t*-test, $t_{(4)} = 5.75$, $P = 0.0046$) (Fig. 5).

Clearance rates in the treatment with *S. obliquus* as sole food source were 5.0 (± 0.3) and 3.0 (± 0.3) mL h^{-1} individual^{-1} in the 20°C and 12°C treatment, respectively (Mann–Whitney *U*-test, $U = 0.0$, $P = 0.009$). Both clearance rates were different from zero (one sample t-test; 20°C: $t_{(4)} = 18.99$, $P < 0.0001$ and 12°C: $t_{(4)} = 8.51$, $P = 0.001$).

DISCUSSION

Earlier studies in the plankton towers of Plön have shown that daphnids spend more time in optimal habitats compared with suboptimal habitats (Lampert *et al.*, 2003; Kessler and Lampert, 2004; Lampert, 2005). Thus, the vertical distribution of *Daphnia* in the plankton towers reflects the fitness distribution (Lampert *et al.*,

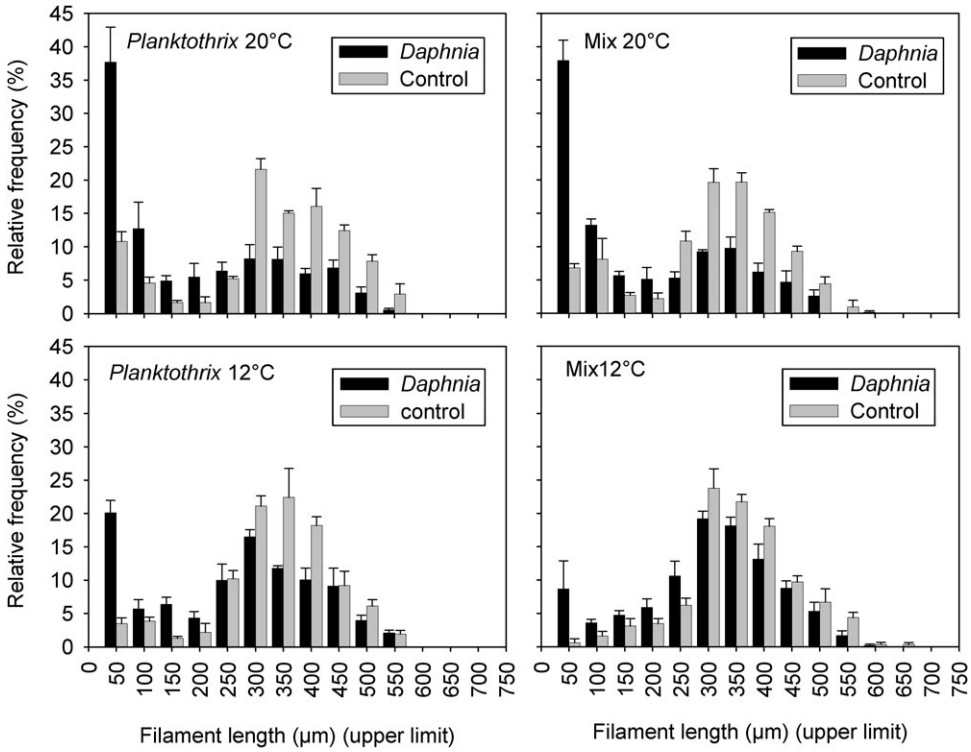

Fig. 4. Mean filament length distribution of *Planktothrix agardhii* in the four treatments of the grazing experiments. Each treatment was either performed with 10 adult *D. pulicaria* per liter (*Daphnia*) or without *D. pulicaria* (control). Error bars represent 1 SE, $N = 5$.

Table I: Median filament length (μm) (± SE) of Planktothrix agardhii in the controls and the Daphnia treatments of the grazing experiment

		Control ($N = 3$)			*Daphnia* ($N = 5$)	
		Median	SE		Median	SE
P. agardhii	20°C	316.2	7.7	**	125.2	40.0
P. agardhii	12°C	315.1	4.2	**	261.8	6.7
Mix	20°C	298.6	6.5	**	94.3	22.2
Mix	12°C	327.1	4.1	*	292.8	10.6

All treatments contained either *P. agardhii* or a mixture of *P. agardhii* and *S. obliquus*. Asterisks indicate differences between control and *Daphnia* treatment calculated with Mann–Whitney *U*-test (** $P < 0.05$ and * $P < 0.1$).

2003). The optimum in those studies was estimated by determining the growth rates of individual *Daphnia* in seston collected from the different layers of the towers. Additionally, Lampert and Grey (Lampert and Grey, 2003) have shown that the distribution of the daphnids in the plankton towers is a dynamic one, which means that each individual continuously searches for its optimal habitat within the water column but spends proportionally more time in its optimal habitat. Thus, in general, the depth at which the highest proportion of daphnids is seen in the towers can be interpreted as the depth of their optimal habitat under these circumstances.

Taking these earlier experiments and results into consideration, our tower experiment indicates that the optimal habitat of *D. pulicaria* depends on the food type and we could show that different size classes of *D. pulicaria* have different optimal habitats in the presence of filamentous cyanobacteria and non-filamentous green algae.

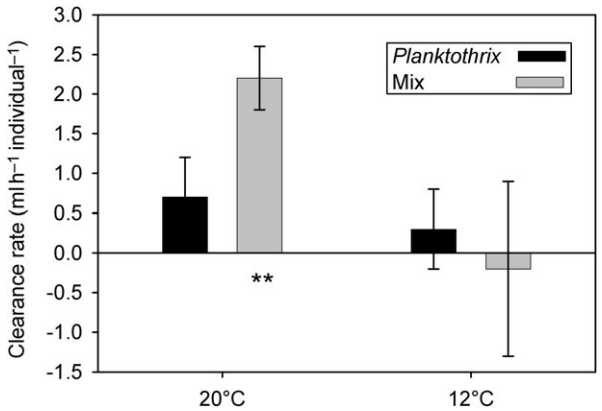

Fig. 5. Clearance rate (mL h^{-1} individual^{-1}) on *Planktothrix agardhii* filaments by large *D. pulicaria* in the four treatments of the grazing experiment. Error bars represent 1 SE, $N = 5$. Asterisk indicates clearance rate being significantly different from zero ($P = 0.005$). Food concentration was 0.65 mg C L^{-1} in the *Planktothrix* treatment and 0.2 mg C L^{-1} *S. obliquus* and 0.65 mg C L^{-1} *P. agardhii* in the Mix treatment.

In the presence of *S. obliquus*, large and small daphnids behave similarly and spend most of their time at the interface between the warm surface layer and the food rich, colder layer. Earlier experiments in the plankton towers with slightly different temperature and food profiles gave comparable results (Lampert and Grey, 2003; Kessler and Lampert, 2004). This behaviour can be explained as an adaptation to the trade-off between temperature and food where both size classes make short excursions from the warm layer, in which they spend most of their time, to the food rich colder layer for feeding.

The habitat choice of daphnids in the presence of a mixed food source (*Planktothrix* and *Scenedesmus*) is essentially similar to the behaviour in pure *Scenedesmus*. However, when *Planktothrix* is the only available food source, small and large *D. pulicaria* have different vertical distributions. Although both size classes have their optimal habitat in the interface between the warm and the food-rich layer, large daphnids spend significantly more time in the colder part of the food patch than small *Daphnia*. This is also supported by the comparison of the distribution of only large daphnids between the treatments: in the presence of *P. agardhii* as the only food, large daphnids always spend more time in the colder part of the food-rich layer compared with a situation with only *S. obliquus* or a mixture of *S. obliquus* and *P. agardhii*.

As large daphnids spend more time in colder parts of the tower in the presence of *P. agardhii*, they experience on average a lower temperature compared with the other food treatments. A lower temperature entails physiological disadvantages for daphnids, such as slower growth rates (Orcutt and Porter, 1983; Orcutt and Porter, 1984; Sakwinska, 1998; Mitchell and Lampert, 2000; Giebelhausen and Lampert, 2001) and higher ages at first reproduction (Orcutt and Porter, 1983; Orcutt and Porter, 1984; Doksaeter and Vijverberg, 2001). On the other hand, a lower temperature also involves a lower metabolism and therefore decreases metabolic costs. Thus, at a lower temperature, animals can prolong their lifespan (MacArthur and Baillie, 1929) by saving energy.

There are two possible reasons why large *D. pulicaria* spend more time in the colder part of the food patch: first, large daphnids can utilize filamentous food better at lower temperatures with higher water viscosity, and therefore should spend more time in the colder parts of the food patch (Abrusán, 2004). However, this explanation is not consistent with the results of our grazing experiment (Fig. 5), where the clearance rate at 20°C

was higher. The second possible explanation may be deduced from the *Daphnia* distribution in the no-food treatment. Here, large daphnids are more or less evenly distributed but with a higher percentage in the colder strata of the water column (5.5–6.0 m), i.e. the optimal habitat in the absence of food seems to be at greater depths. Large daphnids here possibly try to keep their metabolic costs as low as possible in the absence of food to aid survival until the environmental conditions improve. This strategy may also apply in the filamentous food situation: this food source might be of such a low quality that daphnids try to keep costs as low as possible. It is unlikely that the inedibility of filaments is the cause for the low quality because our labelling and grazing experiments showed that *D. pulicaria* is able to feed on filaments of *P. agardhii*. Therefore, the poor nutritional quality of the filaments might be due to the biochemical composition. Being a prokaryote, *P. agardhii* lacks sterols that are essential for *Daphnia* reproduction (Goad, 1981). Thus, even though *D. pulicaria* can utilize *P. agardhii*, they would not be able to reproduce when this cyanobacteria is the only food available (von Elert *et al.*, 2003; Martin-Creuzburg *et al.*, 2005). We infer from our results that large *D. pulicaria* spend more time in colder parts of the food patch to save energy by decreasing metabolic costs. Thus, they are able to survive until sterol-containing food is available again. This is supported by the fact that large daphnids do not migrate down into the colder strata in the Mix treatment, i.e. when *S. obliquus* is present as an additional sterol-rich food source.

It is important to note that the distribution of large *Daphnia* in the *Planktothrix* treatment was not influenced by filament concentration. This can be seen by the fact that the deeper distribution resulted both in exposure to higher concentration (4.5–5.5 m) and in exposure to lower concentration of filaments (5.5–6.5 m).

In contrast to the large size classes (adults), juvenile *D. pulicaria* were not influenced by food type in our experiments. It was shown in earlier experiments that juveniles can survive and grow on a sterol free, pure cyanobacterial diet (Martin-Creuzburg *et al.*, 2005). Martin-Creuzburg *et al.* (Martin-Creuzburg *et al.*, 2005) showed that juveniles from mothers grown on 100% *S. obliquus* had a low but significant positive growth over 6 days even when fed 100% cyanobacterial food (*Synechococcus elongatus*), which could explain the lack of any significant effect of the food type on the behaviour of juveniles in our experiments. Additionally, DeMott and Müller-Navarra (DeMott and Müller-Navarra, 1997) showed that juvenile growth of *D. pulicaria* was high during the first 4 days when fed on 100% *Synechococcus elongatus* but weight declined slightly during the next 3 days. Identical experiments with *D. magna* and *D. galeata* present a continuous weight gain over 7 days (DeMott and Muller-Navarra, 1997). Daphnids in our experiment have all started with some reserves on sterols as the mothers were fed with *S. obliquus* and juveniles that were born during the experiment probably had enough reserves to be independent from high food quality for the duration of our experiment. Thus, they were able to stay in the warmer part of the food patch due to their ability to use their reserves and therefore grow on pure cyanobacterial food. Contrary to that, adults which primarily invest in reproduction would not be able to reproduce on a pure diet of cyanobacterial food (Martin-Creuzburg *et al.*, 2005), such as *P. agardhii*. Thus, food quality seems to be a plausible explanation for the different behavioural patterns of small and large daphnids in our tower experiments.

It is unclear how daphnids recognize the quality of their food. However, such behaviour is likely to be adaptive: during non-toxic cyanobacterial blooms, individuals who outlive the bad conditions by spending more time in the cold hypolimnion could allocate more resources into reproduction once the conditions improve.

It is also difficult to evaluate how important this behavioural adaptation of *Daphnia* is for explaining *Daphnia* distribution in natural situations, as there are other factors like small amounts of alternative food sources in cyanobacterial blooms or predators that will influence the distribution. Still, the behaviour seen here provides an additional factor that shapes the distribution of *Daphnia* in natural systems.

Both the stable isotope analysis (SIA) and the grazing experiment support our food quality explanation. They confirm that *D. pulicaria* is able to process and ingest *P. agardhii* filaments. SIA seems to be the more suitable method here, probably because it is capable of measuring small changes on a finer scale. The fact that both juvenile and adult *Daphnia* ingest and assimilate *Planktothrix* filaments indicates that the different behaviour of the two size classes is not caused by their different ability to mechanically process the filaments but rather due to their different demands on food quality. The grazing experiment additionally shows that the clearance rate depends on the temperature and on the presence of an alternative (qualitatively better) food source. In all treatments, there was a clear increase in small filaments (<50 μm) in the *Daphnia* treatments compared with the controls and a strong decrease in long filaments (300–400 μm). This was probably due to longer filaments being handled by daphnids and broken into pieces by them, which is consistent with the results from earlier experiments (Dawidowicz, 1990). This effect was greater in the 20°C

than in the 12°C treatment. However, statistical analysis showed that the actual clearance rate of filaments was only significantly different from zero in the warm Mix treatment (20°C). This suggests that grazing of filaments is highest in the presence of an additional food source at warm temperatures and that grazing on filaments is generally very low with pure filamentous food especially at low temperatures. These results corroborate the tower experiment: in the Mix treatment, most large daphnids stay in the warm part of the food patch where they have a higher clearance rate.

Daphnia in our grazing experiment were not pre-adapted to the 12°C treatment. This could lead to a strongly decreased clearance rate. However, as animals in the 12°C treatments with *S. obliquus* as the sole food source showed a high, positive clearance rate, there is no reason to assume that the lack of temperature pre-adaptation lead to a lack of grazing on *P. agardhii* filaments in the 12°C treatment.

In conclusion, we showed that small (juvenile) and large (adult) *D. pulicaria* behave differently in the presence of pure filamentous cyanobacteria (*P. agardhi*): the latter spend on average more time in colder water. We suggest that due to their behaviour, *D. pulicaria* might avoid unnecessary metabolic costs during a period in which they are unable to reproduce due to the low nutritional quality of *P. agardhii*, and that daphnids are able to actively respond to different food types by choosing between suitable habitats.

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