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Competitive ability of *Daphnia* under dominance of non-toxic filamentous cyanobacteria

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Abstract

It is generally assumed that *Daphnia* is more susceptible to the inhibitory effects of filamentous cyanobacteria than small cladocerans since daphnids have a larger gape size and filtrate the filaments, whereas small cladocerans do not. This study addresses the question whether food limitation has the potential to modify this scenario of cladoceran response to dominance of non-toxic filamentous cyanobacteria. *Daphnia galeata* was grown under limited (0.1 mg C l^{-1}) and unlimited concentrations (1.0 mg C l^{-1}) of high-quality food algae both in the absence/presence of non-toxic filamentous *Aphanizomenon flexuosum*. As the effects of these cyanobacteria on *D. galeata* were positive under food limiting conditions and negative at the high food density, it was concluded that *D. galeata* was mainly affected by nutritional quality due to its ability to ingest the filaments, while mechanical interference with food collection was not important. In competition experiments between *D. galeata* and *Bosmina longirostris*, *D. galeata* was the dominant species at regular additions of food (1.0 mg C l^{-1}) in the absence of *Aphanizomenon*. In the presence of these cyanobacteria, *D. galeata* was inhibited during the first days of the experimental period. However, the negative effect at the initially high food density was outweighed by nutrition at food limiting conditions and the outcome in competitive dominance was not changed. The results demonstrate that the ability of *D. galeata* to ingest large-sized non-toxic cyanobacteria can be considered as advantageous under food limiting conditions.

Introduction

In general, even non-toxic filamentous cyanobacteria are considered as poor-quality food for *Daphnia*, due to the interference of the filaments with the collection of available food sources, size- and shape-related constraints on ingestion and a low nutritional quality (reviewed by Hanazato, 1996). Several studies have concentrated on the deleterious effects of mechanical interference with the collection of available food sources in *Daphnia* (Webster & Peters, 1978; Porter & McDonough, 1984; Hawkins & Lampert, 1989) showing that respiration rates are increased and feeding and assimilation rates are reduced, resulting in a decrease in body growth and reproduction in the laboratory (Gliwicz & Lampert, 1990) and in the field (Edmondson & Litt, 1982). In addition, a number of studies demonstrated that cyanobacterial filaments not only interfere with *Daphnia* feeding, but also can be ingested (Lampert, 1981; Peterson Holm et al.,1983; Gilbert & Durand, 1990; Kirk & Gilbert, 1992). Consequently, filamentous cyanobacteria cannot be considered solely a source of mechanical interference for *Daphnia* but affect *Daphnia* also via their food value. This is considered to be low (Lampert, 1981; examples in Gulati & DeMott, 1997).

The widely accepted hypothesis suggests that *Daphnia* is more susceptible to the inhibiting effects than smaller cladocerans, copepods and rotifers (Sommer et al., 1986; DeMott, 1989). This is because *Daphnia* spp. have the largest gape size and the broadest food spectrum and are, therefore, more likely to filtrate the filamentous particles (Burns, 1968; Kirk & Gilbert, 1992). Thus, a high abundance of filament-

ous cyanobacteria could be expected to selectively suppress growth and survival of *Daphnia* spp. while leaving smaller cladocerans unaffected and thus shift zooplankton community structure to smaller sizes.

In contrast to this hypothesis, it has been observed that *Daphnia* cannot only ingest but even grow on a sole diet of cyanobacteria (DeBernardi et al., 1981; Brett, 1993; Repka, 1998). Even if cyanobacteria lack some essential compounds, they are sources of energy, and they can build up large detritus pools in the seston of eutrophic lakes (Gons et al., 1992). Bacteria attached to larger sestonic particles such as cyanobacteria are supplementary food items for cladocerans (Schoenberg & Maccubbin, 1985). Gilbert & Durand (1990) speculated that *Daphnia's* ability to ingest the cyanobacterial filaments, even if they were of poor nutritional quality, would be advantageous when food is limiting, e.g. under a competitive situation. This hypothesis is also invoked to explain the dominance of *Daphnia* over smaller cladocerans in fishless environments under natural conditions (Hall et al., 1976).

Given this conflicting pool of evidence, it is difficult to predict the outcome of competition between cladocerans under dominance of filamentous cyanobacteria. In fact, a large body of literature on field observations reports both exclusion (Gliwicz, 1977; Edmondson & Litt, 1982; Vaga et al., 1985; Threlkeld, 1986; Zankai & Ponyi, 1986; Burns et al., 1989) and coexistence (Hrbáček, 1964; Knisely & Geller, 1986; Gulati et al., 1992; Christoffersen, 1993; Matveev et al., 1994; Schaffner et al., 1994; Epp, 1996) of *Daphnia* with filamentous cyanobacteria. The role of toxins might account for one part of the discrepancies in the literature, however, the question arises, to what extent the other mechanisms by which cyanobacteria affect food uptake and nutrition are important. For this purpose, differentiation between the mechanical effects of interference of filamentous cyanobacteria and the effects of ingestion and nutrition would be useful. This can be achieved by comparing *Daphnia* growth when fed with cyanobacterial filaments at limited and unlimited concentrations of high quality food. Susceptibility to mechanical interference with food collection has been suggested to increase at limited food concentrations (Gliwicz & Lampert, 1990; Fulton & Jones, 1991; Kurmayer, 2001). As a consequence of a high filament–food ratio, the encountered filaments disturb the process of food collection more severely when food is low. Every rejection movement reduces the time available to collect food particles, which is most constrained under food limiting conditions. On the other hand, if mechanical interference is not important and a grazer ingests the cyanobacterial filaments together with high quality food, then inhibition by cyanobacteria cannot increase when food is low as compared to high food availability. Indeed, non-toxic filamentous cyanobacteria do not necessarily interfere with feeding but enhance survival and even reproduction under conditions of low food availability (Gilbert & Durand, 1990; Kurmayer, 2001).

It was the aim of the study to investigate which of the two possible effects of non-toxic filamentous cyanobacteria – mechanical interference with food collection or utilisation as supplementary food – on *Daphnia* is important. It is hypothesized that in spite of inhibition in the presence of high food densities, *Daphnia* may actually benefit from non-toxic cyanobacterial filaments in a competitive situation due to its ability to ingest the large particles. *Daphnia* was grown under limited and unlimited food conditions mixed with filamentous cyanobacteria for 15 days. To include maternal effects in nutrition, the effects of cyanobacteria on *Daphnia* were compared over four generations at the high food ratio. Then competition experiments were performed at regular additions of high food and filament densities for 4–5 weeks. The species used were *Daphnia galeata* and *Bosmina longirostris* which can be found in eutrophic to hypertrophic water bodies often dominated by blooms of filamentous cyanobacteria (Gliwicz, 1985; Gulati et al., 1992). Though small-bodied daphnids may coexist with filamentous cyanobacteria in nature (e.g. Gulati et al., 1992), inhibition in growth and reproduction has been observed in the laboratory (Gliwicz & Lampert, 1990) and in the field (Gliwicz, 1977; Gliwicz, 1985; Zankai & Ponyi, 1986).

Materials and methods

Experimental organisms

The filamentous cyanobacterium *Aphanizomenon flexuosum* (Komárek & Kovácik, 1989) was isolated from Lake Alte Donau in Vienna (Austria). This species generally occurs in small and shallow water bodies of Eurasia (J. Komarék, pers. com.). For cladoceran growth experiments, *Aphanizomenon* was cultured in a 1:4 mixture of a nutrient medium according to Zehnder & Gorham (1960) and Millipore water. Bottles (2–6 l) were filled with autoclaved medium, inoculated and bubbled with sterile air at 20

°C and a light intensity of 10–30 μmol m^{-2} s^{-1} at a 16:8 h light and dark cycle. The algae were harvested via filtering on a 25 μm mesh screen and then re-suspended in membrane filtered (0.45 μm) lake water. During the exponential growth phase, *Aphanizomenon* formed single filaments of 517±67 (95% C.L.) μm in length (n=127) and 2.4 μm in width. Only 3% and 7% were smaller than 50 μm and 100 μm, respectively, and 51% did not exceed 400 μm. The largest filaments were 2.5 mm in length and 2250 filaments ml^{-1} corresponded to 1 μg C (measured by a CN-analyser). Microscopical measurements revealed that the cyanobacterial filaments maintained their length at least for 1 day after re-suspension in lake water. For the competition experiments, a high biomass of *Aphanizomenon* was needed. Consequently, the cyanobacteria were cultured in the cyano medium (Jüttner et al., 1983) which is six times more concentrated than the Zehnder & Gorham (1960) medium, but very similar in its chemical composition. For both media, pH was 9.0 prior to autoclaving.

The high-quality food algae used were *Scenedesmus acutus* (obtained from the Max-Planck-Institute in Plön, Germany). *Scenedesmus* was grown in 250 ml Erlenmeyer flasks filled with autoclaved Zehnder & Gorham medium (1:4 diluted with water) at the same light and temperature conditions as for the cyanobacteria and harvested after three weeks. The cells were 14.2±0.4 (C.L.) μm in length and 4.7μm± 0.2 μm in width. One cell contained 35 pg C (carbon measured by a CN-analyser).

For competition experiments, *Cryptomonas ovata* (SAG 979.3) was grown in Erlenmeyer flasks filled with autoclaved cyano medium (pH 7.0) and shaken continuously (80 r.p.m.). The cells were 18.4±0.5 μm in length and 11±0.5 μm in width. For the experiments, *Scenedesmus* was added directly to the membrane filtered lake water while the *Cryptomonas* species were centrifuged at 4000 g for 10 min and then re-suspended in membrane filtered lake water. Sterile glassware was used, however the cultures were not axenic. All cell densities were adjusted by measuring absorbance at 550 nm and estimating cell concentrations from previously established calibration curves.

Daphnia galeata (0.54–1.8 mm) and *Bosmina longirostris* (0.2–0.45 mm) were isolated from the same lake as *Aphanizomenon*. Stock cultures of the species were grown in beakers (1.0 l) at 20 °C filled with membrane filtered (0.45 μm) lake water and a food level adjusted to 1.0 mg C l^{-1} of *Scenedesmus* every day. Cladocerans were thinned out and transferred to fresh medium every week. Neonates (< 24 h) were used to start all experiments.

Toxicity of Aphanizomenon flexuosum

To test for neurotoxins present in this strain of *Aphanizomenon*, doses of up to 1250 mg of freeze-dried material per kilogram were injected into mice and the mice observed up to 36 h for any signs of poisoning by V. Vasconselos. No toxicity was detected and in addition no microcystins and other polypeptides frequently observed in *Microcystis* and *Planktothrix* were found by matrix assisted laser desorption - time of flight mass spectrometry (MALDI-TOF) performed by J. Fastner according to Fastner et al. (1999). Since the toxic effects of cyanobacteria on crustaceans were not always caused by the well-known polypeptides (Jungmann, 1992; Reinikainen et al., 1995), these tests do not mean that toxic effects on *Daphnia* were completely absent.

Growth experiments

Preliminary experiments revealed increased survival for *Daphnia* when grown on *Aphanizomenon* alone relative to controls without food, however, none of the animals reached maturity. Growth experiments were performed from October to December 1998: (1) *Daphnia* growth was measured at unlimited (1.0 mg C l^{-1}) and limited (0.1 mg C l^{-1}) concentrations of high-quality food (*Scenedesmus*) both in the absence and presence of 10 000 filaments ml^{-1} of *Aphanizomenon*. Since neonates originated from the same mothers and were incubated at the same time, this experiment provided an explicit test for the role of nutritive vs. interfering effects of *Aphanizomenon* filaments on *D. galeata*. (2) The effects of *Aphanizomenon* on *D. galeata* over four generations were compared at the highest food concentration.

To start an experiment, neonates of the second brood from mothers from stock cultures were pipetted into bottles (1 individual per 100 ml) and placed on a plankton wheel (0.5 rpm) at 20 °C under dim light. A timer ran the plankton wheel for 15 min every 2 h. In the generation experiment, neonates of controls were generally from the second brood number. Neonates in the cyanobacteria treatment originated from the second brood number until the second generation, however, stemmed from mixed brood numbers of very few surviving females in the third and fourth generation.

The experimental animals were transferred to fresh food suspensions every day for 15 days and examined for survival, increase in length and egg production. Offspring were counted and removed. Each treatment consisted of 12 parallels. The length was measured from the top of the head to the base of the tail spine.

Competition experiments

Competition experiments between *D. galeata* and *B. longirostris* were carried out from July to September 1998 and started at unlimited concentrations of high-quality food (*Scenedesmus* and *Cryptomonas*) in the presence or in the absence of filamentous cyanobacteria (10 000 filaments ml^{-1}). In addition, each species was grown alone in each algal treatment in parallel (controls). *Scenedesmus* (1.0 mg C l^{-1}) was mixed with *Aphanizomenon* in experiment 1. It is further known that the maximum level of population growth is obtained with cryptomonads (examples in Gulati & DeMott, 1997). To test whether the presence of food algae of highest quality can affect the outcome in competition between the two cladocerans, 20% of *Cryptomonas* (in terms of fresh weight) were added in experiment 2.

To start an experiment, neonates released from egg-bearing females from stock cultures during 24 h were pipetted into bottles (720 ml) and placed on a plankton wheel. The initial inoculum consisted of 10 neonates of *Bosmina* and 10 (experiment 1) or 5 (experiment 2) neonates of *Daphnia*. The animals were transferred with a 100 μm mesh screen to new food suspensions every other day and counted once per week. This was continued for 4–5 weeks with three parallel bottles per treatment. By transferring the animals with a 100 μm mesh screen, the long filaments of the cyanobacteria were partly transferred into the new food suspension. No data on bacterial densities are available; however, microscopic counts showed an increase of *Aphanizomenon* biomass over time and probably bacteria and detritus particles increased as well. Cyanobacterial filaments generally become a better food source to filter feeding cladocerans when decaying and overgrown by bacteria (Gliwicz, 1990; Repka, 1998).

The animals were counted by sub-sampling and placed back into the bottles. Cetyl alcohol was used to keep the animals out of the surface film. The eggs were counted for *Daphnia* while pipetting the animals into new food media but not for *Bosmina* because of their small size. During the first 3 weeks of a competition experiment, population growth (r) was determined using the following equation:

$$r = (\ln N_t - \ln N_0)/t,$$

where N_0 is the initial population size and N_t is the population size at time t.

Phytoplankton was preserved with acid Lugol's solution and enumerated using a compound microscope at 400× in the course of experiment 2 for one parallel per treatment. For each treatment, the mean filament size of *Aphanizomenon* was determined by measuring 50 randomly selected filaments.

Statistical methods

Since observations from the same bottles in competition experiments over time were not independent, two-way repeated measures analysis of variance was applied to test the differences between treatments. The assumptions of normality and homoscedasticity were violated. However, because of the robustness of ANOVA to violations of normality and homoscedasticity if the sample sizes are equal (Sokal & Rohlf, 1995: chapter 13), retention of all variables was deemed permissible. The differences between population growth rates were analysed by a two-factor ANOVA. An Spss statistical package (release 6.0 for Windows) was used for all statistical analyses.

Results

Daphnia *growth experiments*

While pipetting the animals from one food suspension to another, some specimens were regularly found with yellowish coloured guts, which were clearly distinct from the green guts of the control animals. Since *Aphanizomenon* contains higher amounts of xanthophylls than *Scenedesmus* (pers. obs.), the yellow coloured guts may indicate ingestion of the cyanobacteria. Even the smallest daphnids contained at least one or two filaments in the food groove and rejected these filaments by movements with the abdominal claw during the measurements under the microscope. Later stages were often observed with high numbers of aggregated filaments in the food groove indicating that the filaments were not rejected immediately, but the animals were able to continue their filtering movements and retain the filaments at least for some time.

Aphanizomenon significantly enhanced survival of *Daphnia* at 0.1 mg C l^{-1} of *Scenedesmus* and did not

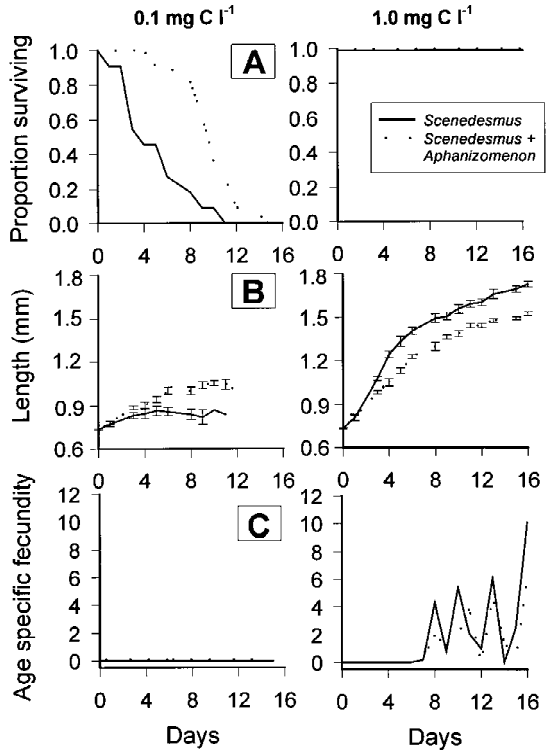

Figure 1. Life history of *Daphnia galeata* at limited (0.1 mg C l^{-1}) and unlimited (1.0 mg C l^{-1}) food concentrations of *Scenedesmus* each in the presence (dotted line) or absence (solid line) of *Aphanizomenon*: (A) Age specific survival, (B) mean length (± SE) and (C) age specific fecundity.

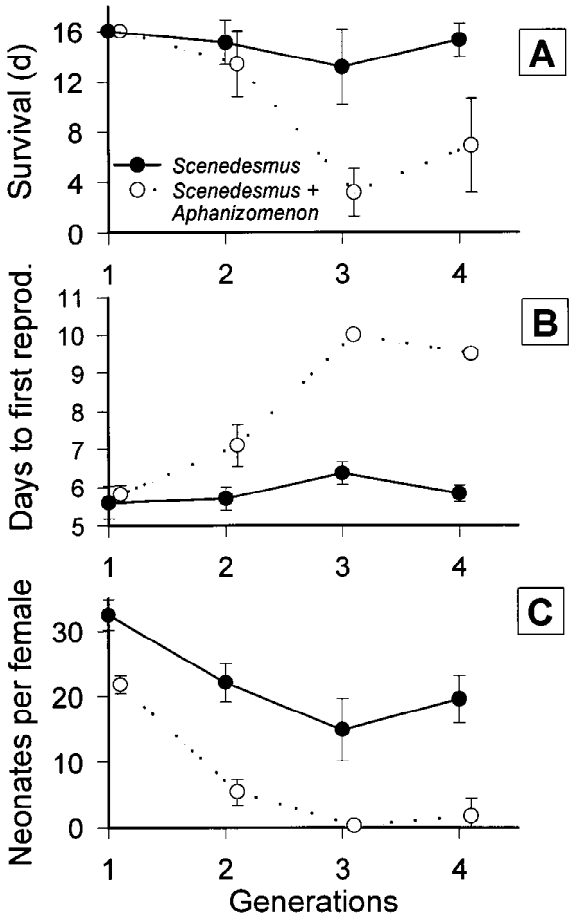

Figure 2. Life history of *Daphnia galeata* during four generations at unlimited (1.0 mg C l^{-1}) food concentrations of *Scenedesmus* in the presence (dotted line, white circles) or absence (solid line, black circles) of *Aphanizomenon*: (A) Mean (± C.L.) (95%) survival′ (B) mean (± C.L.) time to first reproduction and (C) mean (± C.L.) production of viable neonates. The error bars are missing for the two last generations in graph (B).

affect survival at unlimited food conditions (Fig. 1a). The somatic growth of *Daphnia* was enhanced at 0.1 mg C l^{-1} in the presence of cyanobacteria, however reduced at the same time for the same individuals under non-limiting food conditions (Fig. 1b). Since the animals were not adapted to the food limiting conditions, no eggs were produced at 0.1 mg C l^{-1}, while *Aphanizomenon* significantly decreased egg production at 1.0 mg C l^{-1} (Fig. 1c). The result is consistent with the hypothesis that *Daphnia's* ability to ingest the filamentous cyanobacteria (even if they are of low nutritional quality) is an advantage if food density is generally low but reduces growth if the filaments are ingested at the expense of abundant high quality food particles.

The generation experiment revealed no mortality in the first generation, but significantly increased mortality in the presence of *Aphanizomenon* during subsequent generations (Fig. 2a). Most of the offspring (60%) died within 4 days after two generations in the presence of *Aphanizomenon*. However, a few individuals survived until the end of the experiment and even longer (until the fifth generation, which is not shown here). In contrast, survival remained high on *Scenedesmus* alone. Correspondingly, the time to first reproduction in the presence of *Aphanizomenon* increased during subsequent generations (Fig. 2b). The size at maturity decreased from 1.17 mm in the absence of *Aphanizomenon* downto 1.05 mm in the presence of *Aphanizomenon* from the second generation onwards. The production of viable neonates approximated zero after three generations in the presence of *Aphanizomenon* (Fig. 2c). Most of the daughters died without reproducing. This decline in growth over time indic-

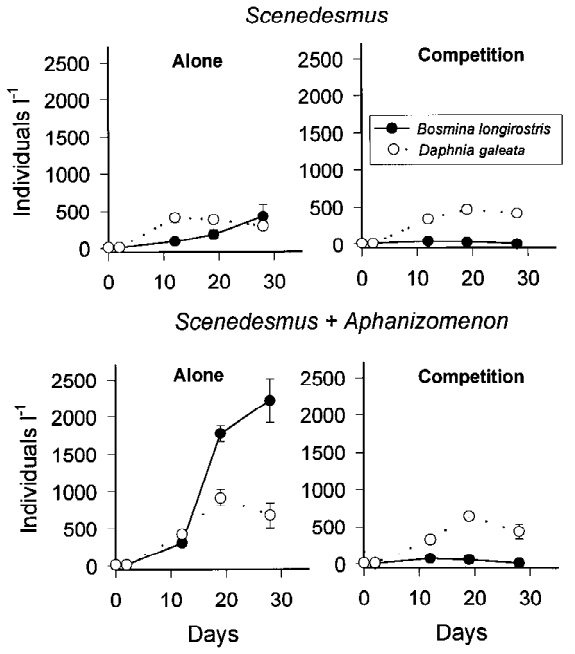

Figure 3. Mean (± SE) densities of *Daphnia galeata* (broken line) and *Bosmina longirostris* (solid line) in the absence (upper part) or presence (lower part) of cyanobacteria added to high-quality algae. Each species was grown alone and in competition on *Scenedesmus* (1.0 mg C l^{-1}) and *Aphanizomenon* (4.0 mg C l^{-1}). If the error bars are not visible they are hidden behind the symbols.

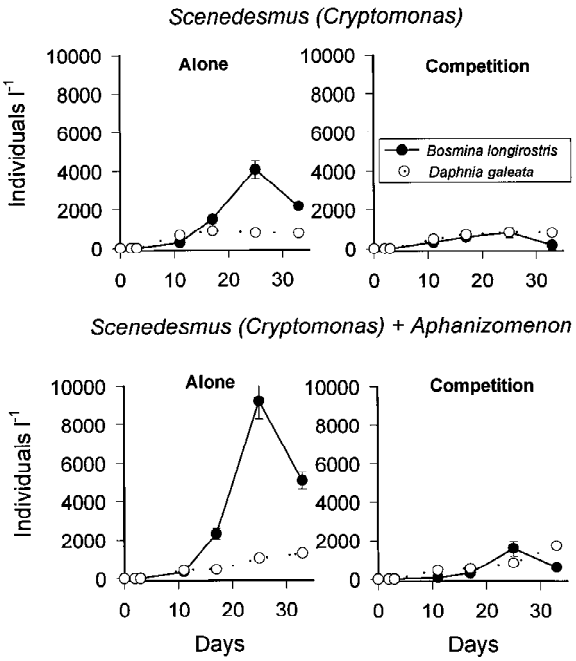

Figure 4. As Figure 3 but the diet of *Scenedesmus* was supplemented with *Cryptomonas* for each treatment.

ates maternal effects caused by a gradual depletion in the reserves of an essential nutrient.

Competition experiments

Averaged over the entire experimental period, the effects of the filamentous cyanobacteria on both cladoceran species grown individually were positive rather than negative (Figs 3 and 4). Their positive effect on mean population density and biomass of *Daphnia* was significant in experiment 1 (Table 1) and comparing the last two sampling dates also for experiment 2. For *Bosmina*, the effect was significant for both experiments (Table 2) and peak densities of 9000 ind. l^{-1} were observed. These results indicate that the filamentous cyanobacteria and/or the resulting detritus were a useful food source for both species.

Averaged over the entire period, *Daphnia* had significantly higher egg numbers in the presence of *Aphanizomenon* than in its absence (Table 1). As in growth experiments, a high mortality in neonates and juveniles was observed, indicating that the nutritional quality of *Aphanizomenon* for *Daphnia* was low. Yet the inhibiting effects did not become apparent due to the general food limiting situation and the very low production of newborn in controls without cyanobacteria (Table 1). Consequently, the inhibition was rather small and only measurable during the first weeks when comparing the population growth rate (r) of *Daphnia*. Since *Daphnia* had significantly lower growth rates on *Scenedesmus* alone than on the *Scenedesmus*/*Cryptomonas* mixture, the effects of *Aphanizomenon* were already positive during the first 19 days of experiment 1, but negative in experiment 2. Hence, I conclude that the negative effects of *Aphanizomenon* on *Daphnia* only became apparent due to the better food situation in the controls of experiment 2.

In both experiments, population growth of *Daphnia* was unaffected by *Bosmina* when compared with controls ($p > 0.1$, Table 1). The addition of *Aphanizomenon* did not change the outcome of competition between the two species. The strong effects of *Daphnia* in the competition experiments overrode the highest densities of *Bosmina* in the absence of *Daphnia*. Competitive exclusion did not occur within 35 days. However, in both experiments, the competition effect of *Daphnia* on *Bosmina* was highly significant ($p < 0.001$, Table 2). This reduction was more severe in the presence of *Aphanizomenon* than in its absence, e.g. *Bosmina* achieved the highest growth rates on a

Table 1. Means ± SE of variables for *Daphnia galeata* in triplicates of both competition experiments. The variables were analysed for the effects of competition (C), cyanobacteria (Cy) and their interaction (C*Cy) by two-factors (repeated measures) ANOVA. r = population growth rate from day 0 to day 19 (experiment 1) and day 0 to day 17 (experiment 2). n = number of samples. P = significance level calculated by ANOVA

	n	Control alone	Competition alone	Cyanobacteria	Competition	P (C)	P (Cy)	P (C*Cy)
Experiment 1 (*Scenedesmus*)								
density (ind l^{-1})	15	222±49	248±53	406±105	286±70	0.159	0.006	0.043
eggs ($female^{-1}$)	9	1.0±0.5	1.1±0.5	2.6±0.7	1.7±0.7	0.34	0.031	0.277
r (d^{-1})	3	0.17±0.003	0.19±0.003	0.22±0.006	0.2±0.003	0.487	<0.001	0.022
Experiment 2 (*Scenedesmus* and *Cryptomonas*)								
density (ind l^{-1})	21	444±90	409±84	463±112	515±133	0.824	0.108	0.248
eggs ($female^{-1}$)	12	1.2±0.4	1.1±0.3	1.9±0.3	1.7±0.2	0.318	0.001	0.619
r (d^{-1})	3	0.28±0.01	0.27±0.006	0.25±0.007	0.26±0.008	0.992	0.022	0.302

Table 2. Means ± SE of variables for *Bosmina longirostris* in triplicates of both competition experiments. The variables were analysed for the effects of competition (C), cyanobacteria (Cy) and their interaction (C*Cy) by two-factors (repeated measures) ANOVA. r = population growth rate from day 0 to day 19 (experiment 1) and day 0 to day 17 (experiment 2). n = number of samples. P = significance level calculated by ANOVA

	n	Control Alone	Competition	Cyanobacteria Alone	Competition	P (C)	P (Cy)	P (C*Cy)
Experiment 1 (*Scenedesmus*)								
density (ind l^{-1})	15	155±55	20±6	865±260	33±12	<0.001	<0.001	<0.001
r (d^{-1})	3	0.13±0.02	0.04±0.01	0.26±0.003	0.01±0.06	0.003	0.205	0.088
Experiment 2 (*Scenedesmus* and *Cryptomonas*)								
density (ind l^{-1})	21	1146±326	278±81	2422±745	375±136	<0.001	<0.001	<0.001
r (d^{-1})	3	0.27±0.008	0.21±0.02	0.30±0.007	0.17±0.03	0.002	0.667	0.13

cyanobacteria dominated diet in the absence of *Daphnia* (0.26±0.003), but the growth rates approximated zero (0.01±0.06) in the presence of the competitor. It is interesting to note that the population growth rates of *Bosmina* were higher than the growth rates of *Daphnia* when *Bosmina* was grown alone and under a dominance of cyanobacteria.

Averaged over the experimental period, the fresh weight of *Aphanizomenon* was 31±1 (SE) mm^3 l^{-1} (corresponding to 198±31 μg l^{-1} of chlorophyll*a*). In most cases, the biomass of *Aphanizomenon* increased during 2 days after re-suspension of the cells in membrane filtered lake water (Fig. 5a). Averaged over the entire period, the mean filament size of the freshly re-suspended filaments was 280±34 μm. With the exception of the last sampling date, the mean filament length remained constant during 2 days or differed only marginally from the mean size of the freshly re-suspended filaments (Fig. 5b). The mean filament size in the single species treatment with *Bosmina* was higher than in both treatments with *Daphnia* (Fig. 5c). Compared with controls without animals, the reduction of *Aphanizomenon* biomass in the treatments with *Daphnia* was more pronounced than in the treatment with *Bosmina* alone (Fig. 6). Not even the high *Bosmina* density of 9 individuals ml^{-1} showed a measurable reduction of *Aphanizomenon* biomass. Taking into account that the production of biomass by *Bosmina* in single species treatments was roughly the same as for *Daphnia*, it is concluded that *Bosmina*

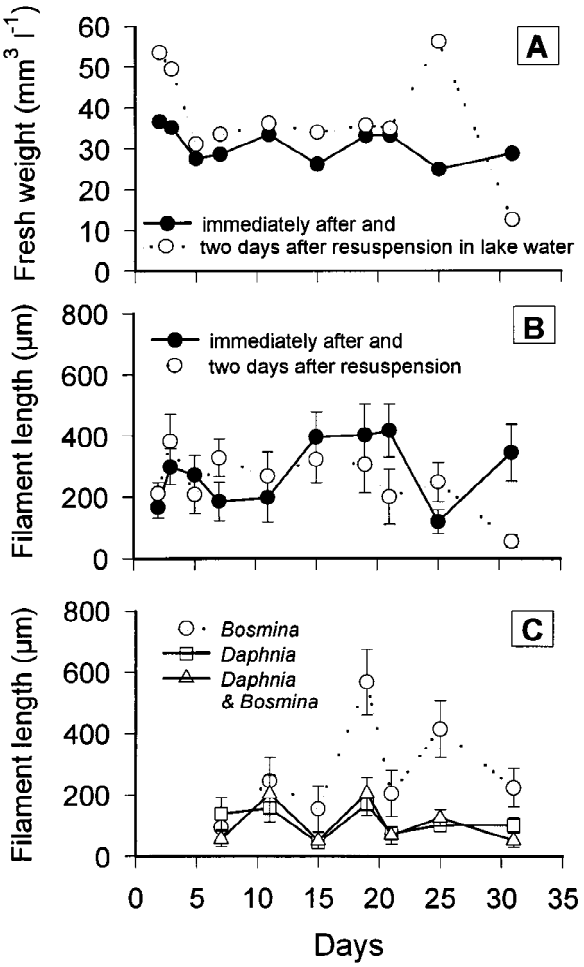

Figure 5. (A) Biomass of *Aphanizomenon* in the course of competition experiment 2 immediately after resuspension of the filaments in filtered lake water (black circles) and 2 days after (white circles) in controls without animals. (B) As above but mean (± C.L.) length of *Aphanizomenon* filaments. (C) Mean (± C.L.) length of *Aphanizomenon* filaments in single species treatments with *Bosmina* (circles) and *Daphnia* (squares) and competition treatments (triangles) of experiment 2.

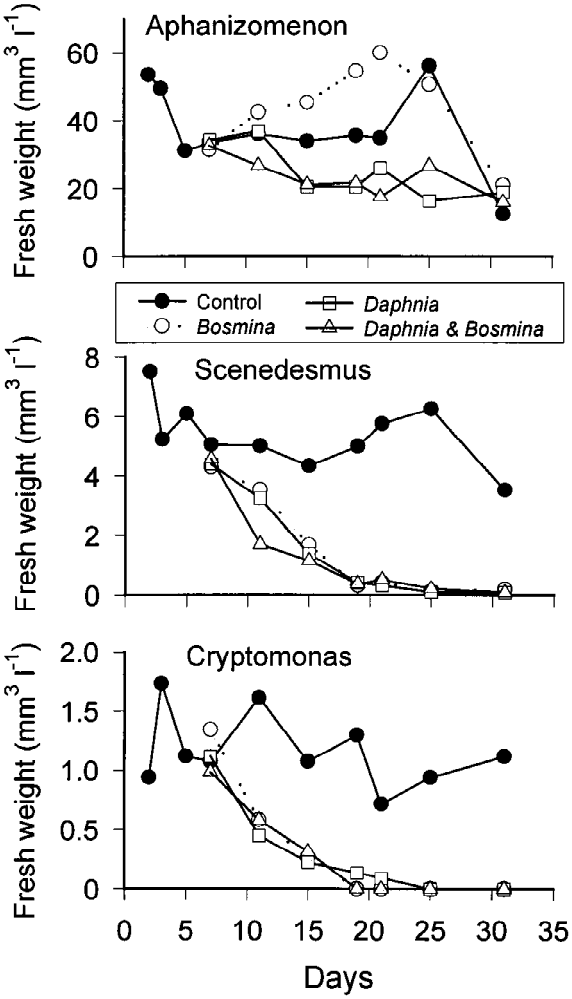

Figure 6. Algal biomass in the course of competition experiment 2 in controls without animals (black circles), in single species treatments with *Bosmina* (white circles) and *Daphnia* (white squares) and competition treatments (white triangles).

did not feed on *Aphanizomenon*, but rather on detritus and bacteria resulting from cyanobacteria. Both cladoceran species reduced *Scenedesmus* and *Cryptomonas* down to levels below detect ability.

Discussion

Growth experiments

This study attempted to differentiate between the effects of nutritional quality, mechanical interference and toxicity of filamentous cyanobacteria on *Daphnia*. The effects of *Aphanizomenon* observed in this study were positive at the high filament–food ratio but negative when the ratio was low. It is concluded that *Daphnia* was mainly affected by the ingestion of the filaments while mechanical interference with food collection was not important. The observation of no interference of the filaments with feeding in *Daphnia* can be explained by the soft and flexible morphology of the filaments. DeMott (1995) demonstrated that hardness of particles strongly influences *Daphnia's* selectivity for large food items and *Daphnia* can ingest large particles with softer cell walls but not polystyrene beads of the same size. As suggested by

one referee, an alternate hypothesis of *Daphnia* inhibition could be that the costs of food rejection become measurable only when *Daphnia* has a surfeit of food (at 1.0 mg C l^{-1}) and probably an elevated rate of rejection. However, the results of the gradual inhibition over generations do not fit to this hypothesis.

The possibility cannot be ruled out that an unknown toxic compound present in *Aphanizomenon* may affect *Daphnia*. However, experiments comparing the growth response of *Daphnia* on toxic cyanobacteria at different food levels consistently revealed much more pronounced negative effects when food is low (Reinikainen et al., 1994; Hietala et al., 1997; Kurmayer, 1999). Consequently, it is concluded that nutritional quality of *Aphanizomenon* is more important than the production of toxic compounds.

When mixed with high amounts of *Scenedesmus*, *Aphanizomenon* gradually reduced *Daphnia* survival, growth and reproduction from one generation to another. The daphnids should be able to ingest enough high-quality food in the presence of *Aphanizomenon* since 1.0 mg C l^{-1} of *Scenedesmus* is far above the incipient limiting level of 0.3 mg C l^{-1} (Kurmayer, 1999). It is speculated that *Daphnia* has difficulty to attain its nutrient requirements when the total carbon concentration is high (5 mg C l^{-1}) and the mixture consists of large amounts of low quality cyanobacteria. Indeed, earlier studies support the idea that the assimilation efficiency decreases at high food concentrations (Porter et al., 1982) because the gut retention time of the food becomes shorter. As stated by DeMott & Müller-Navarra (1997), a decline in growth over time might indicate a gradual depletion in the reserves of an essential nutrient. Initial growth is possible due to reserves, i.e. polyunsaturated fatty acids present at the onset of the generation experiments. In contrast to the study of DeMott & Müller-Navarra (1997), *Aphanizomenon* contains the important linolenic and linoleic acid (G. Ahlgren, pers. com.). Compared with *Scenedesmus*, linoleic acid is slightly lower, but it is not yet known whether this difference can cause the low nutritional quality of *Aphanizomenon* in general.

Competition experiments

Competitive dominance of *Daphnia* over *Bosmina* is known from other studies (e.g. review by DeMott, 1989). My results support the size efficiency hypothesis predicting that larger cladocerans have a competitive advantage over smaller cladocerans in exploiting limited food resources (Hall et al., 1976). The maximum population growth rates (r) of *D. galeata* were always higher (0.5 d^{-1}) than the maximum growth rates of *B. longirostris* (0.4 d^{-1}). In addition, neonates of *D. galeata* survived significantly longer than neonates of *B. longirostris* in the absence of food (2.7 ± 0.4 (C.L.) vs. 1.7 ± 0.4 days, $p < 0.001$, log-rank test). Hence, the results support the model of Romanovsky & Feniova (1985), which predicts that the larger species is able to build up a cohort of adults when initially resources are high. The big adults are best able to withstand starvation and to maintain food at low levels, thus preventing reproduction of both species. If the food level rises again, the surviving adults of the larger cladoceran are able to depress the average food concentration very quickly while the smaller species is driven to extinction.

In both experiments, *Bosmina* exhibited the highest growth rates in the control treatments without *Daphnia* and in the presence of *Aphanizomenon*. Though the biomass of *Bosmina* was quite comparable to the biomass of *Daphnia*, *Bosmina* did not reduce the cyanobacterial filaments to a measurable extent. Therefore, I conclude that *Bosmina* fed selectively on bacteria and detritus resulting from the cyanobacteria in addition to high quality food algae. G.-Tóth & Kato (1997) found that *Bosmina longirostris* is able to graze efficiently on large sized bacteria. Although the population growth rates of *Bosmina* where higher when compared with *Daphnia* during the first half of both experiments, they were not good predictors of *Bosminas* competitive ability under dominance of filamentous cyanobacteria. The advantage of detritus was not able to outweigh the general reduction of high-quality algae by the competing daphnids and the interaction between limitation of high-quality food (competition) and the cyanobacteria was highly significant. The average individual numbers of *Bosmina* were roughly the same in both competition experiments whether cyanobacteria were present or not. Consequently, this observation does not necessarily mean that *Bosmina* was more sensitive to competition when reared with cyanobacteria, but rather that it was doing extremely well when fed cyanobacteria in the absence of the other species. On the other side, preliminary experiments revealed inhibition of *Bosmina* by filamentous cyanobacteria especially when food availability is low (Kurmayer, 1999) and in this study *Bosmina* density approximated zero on day 12 of the competition experiments when the difference in population density between single species treatments was rather low. Therefore, one could speculate that the filaments in-

terfere with the food searching behaviour of *Bosmina*. For example, Balseiro et al. (1991) observed mechanical interference of *Asterionella formosa* with feeding of *Bosmina longirostris* in the field.

Conclusions

In conclusion, measures of population growth rates (r) alone were not sufficient to predict competitive ability under dominance of filamentous cyanobacteria. This was mainly due to the interaction effects between limitation of high-quality food sources and cyanobacteria. In general, the interaction effects influenced *Daphnia* less than *Bosmina* and with regard to population size, the positive interacting effects between food availability and cyanobacteria on *Daphnia* were overriding. Though population growth rates of *Daphnia* were reduced in the presence of cyanobacteria during the first days, *Daphnia's* population size was less affected by the competitive effects of *Bosmina* than the population growth of *Bosmina* by *Daphnia*. The latter can only be explained by the overall food limitation caused by the population increase of *Daphnia*, while *Daphnia's* ability to monopolize the use of large-sized particles in the diet can be considered as advantageous under these food-limiting conditions.

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