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## *Daphnia* food limitation in three hypereutrophic Dutch lakes: Evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria

**Abstract**—The Loosdrecht Lakes comprise three shallow, hypereutrophic lakes in The Netherlands. Research conducted over the past two decades suggests that absence of large-bodied *Daphnia* from these lakes can be explained by size-specific effects of both poor food quality and predatory mortality. The phytoplankton is dominated by filamentous cyanobacteria, which interfere with the feeding of large-bodied *Daphnia*. Moreover, dense populations of planktivorous fish are expected to feed selectively on larger prey. More recent research, however, suggests that the growth of the small native *Daphnia* species, *Daphnia cucullata*, is limited by low dietary phosphorus (P). In this study, we compared the growth and feeding rates of four *Daphnia* taxa representing a gradient in body size. In each of 10 growth experiments, native *D. cucullata*

feeding in natural seston exhibited improved growth in response to additions of phosphate and the P-rich cyanobacterium *Synechococcus*. The two largest species, *Daphnia magna* and *Daphnia galeata*, however, failed to grow in natural seston, even when the seston was supplemented with phosphate, *Synechococcus*, or *Scenedesmus*. A *D. galeata* × *cucullata* hybrid exhibited weak growth in natural seston and no response to the supplements. Feeding experiments with polystyrene beads show that feeding inhibition in natural seston increased markedly with increased *Daphnia* size. Our results verify that *Daphnia* differing in size face different food quality constraints. The growth of native *D. cucullata* (adult size 0.6–0.8 mm) is reduced by P deficiency, whereas larger *Daphnia* appear to be excluded by interfering filaments of cyanobacteria.

The dominance of small-bodied zooplankton in eutrophic lakes is often attributed to size-selective predation by planktivorous fish. Fish predation can be especially intense in shallow lakes where zooplankton lack a deep-water refuge (Tessier and Welser 1991). Food limitation is also important in eutrophic lakes. Despite high food abundance, food quality is often poor, especially in lakes dominated by cyanobacteria. Laboratory experiments show that cyanobacteria can be poor quality foods for zooplankton for a variety of reasons, including toxicity, digestion resistance, difficulties in handling and ingesting filaments and colonies, and nutritional inadequacy (see reviews by Lampert 1987a; de Bernardi and Giussani 1990; Brett and Müller-Navarra 1997). However, despite evidence from laboratory experiments, the roles of these various food quality mechanisms in nature are poorly understood (reviewed by Haney 1987). Filamentous cyanobacteria can inhibit the feeding of daphnids, thereby reducing the collection and ingestion of more nutritious resources. Since feeding inhibition increases with increasing *Daphnia* body size, both feeding inhibition and fish predation favor small-bodied daphnids in eutrophic lakes (reviewed by Gliwicz 1990a).

Both poor food quality and fish predation are considered to have strong impacts on the zooplankton communities of our three shallow (mean depths = 1.45–1.85 m), interconnected study lakes (reviewed by DeMott and Gulati 1999). The phytoplankton of all three lakes is dominated by cyanobacteria year-round, mainly *Oscillatoria limnetica* and the prochlorophyte *Prochlorothrix hollandica*. Together, these two filamentous forms comprise, on average, about 90% of the algal mass in suspension and often exceed  $10^5$  filaments  $\text{ml}^{-1}$  (Van Tongeren et al. 1992). Fish are very abundant (about 300 kg  $\text{ha}^{-1}$ ) and are dominated by stunted, planktivorous bream (*Abramis brama*; Lammens et al. 1992). The zooplankton in all three lakes is dominated by small species, including *Bosmina* spp. (*longirostris* and *coregoni*), *Chydorus sphaericus*, cyclopoid copepods, and rotifers (Gulati et al. 1992). The genus *Daphnia* is represented almost exclusively by the smallest Eurasian species, *Daphnia cucullata*. *Daphnia galeata*, which is common in other shallow hypereutrophic Dutch lakes, is conspicuous by its absence.

Laboratory and field experiments support the notion that the combined effects of interfering filaments and fish predation are responsible for the absence of larger *Daphnia* species in the Loosdrecht Lakes. A series of laboratory experiments showed that *Daphnia magna* was able to grow and reproduce in seston from Lake Loosdrecht only after the filament concentration was reduced by dilution (Davidowicz et al. 1988). The lack of success of a biomanipulation project in Lake Breukeleveen was attributed to high densities of filamentous cyanobacteria (Van Donk et al. 1990). Further experiments showed that *D. galeata* could flourish in mesocosms in Lake Breukeleveen when the fish abundance was reduced to about 15% of the lake density (Van Donk et al. 1994). However, in addition to the fish reduction, the concentration of cyanobacteria filaments declined in the mesocosms, presumably due to reduced turbulence. Thus, a reduction in both fish and filaments may have been necessary for the successful establishment of *D. galeata*.

More recently, however, DeMott and Gulati (1999) found

a strong inverse relationship between the abundance of *D. cucullata* and the phosphorus (P) content of seston, using a 9-yr data set from the Loosdrecht Lakes. This finding suggests an additional explanation for the poor food quality of lake seston. Thus, it becomes important to consider how the effects of interfering filaments might interact with P deficiency. High concentrations of filaments reduce feeding rates on alternative food resources, leading to energy limitation (Gliwicz 1990a). Theory predicts an interaction between energy limitation and P limitation (Sterner 1997). When available energy is very low, near the threshold food concentration for individual growth, most assimilated carbon is respired, and P-sufficient and P-deficient resources support equivalent, very low growth rates (Sterner and Robinson 1994). Thus, strong energy limitation due to feeding inhibition should prevent P limitation.

Here, we compare the growth and feeding rates of the small native *D. cucullata* and three larger *Daphnia* taxa feeding in the filament-rich seston of the Loosdrecht Lakes. We consider whether size-specific feeding inhibition, caused by filaments of cyanobacteria, can account for the absence of large-bodied *Daphnia* species in the Loosdrecht Lakes. An analysis of the effects of dietary P deficiency on the population dynamics, growth, and P balance of *D. cucullata* in all three lakes is presented elsewhere (DeMott et al. in press). That study describes the methods for collecting field samples and includes data on the seasonal dynamics of seston abundance and the seston C:P ratio in all three lakes.

Our growth and feeding experiments used *D. cucullata* isolated from Lake Breukeleveen and clonal cultures of three larger taxa from the Center of Limnology culture collection, including *D. magna*, *D. galeata*, and a *D. galeata* × *D. cucullata* hybrid. The hybrid clone was the product of a cross between the two parental species performed in the laboratory (K. Schwenk pers. comm.). Laboratory clones were grown in filtered water from Lake Maarsseveen and fed a high concentration (1–2 mg C  $\text{L}^{-1}$ ) from P-sufficient cultures (molar C:P = 80) of the green alga *Scenedesmus obliquus*. The *Daphnia* culturing, the growth assays, and the feeding experiments were carried out in the same temperature-controlled room at 19°C under dim light. Methods for culturing algae and measuring their concentration followed DeMott et al. (1998).

We conducted a total of 21 growth assays between 7 June and 16 July 1999, including separate experiments with seston from each of the three lakes. Growth assays tested the ability of juvenile *Daphnia* to grow on natural seston or natural seston supplemented with phosphate or with the P-rich cyanobacterium *Synechococcus*. In addition, we measured the growth of each cohort with a high concentration (2 mg C  $\text{L}^{-1}$ ) of the green alga *Scenedesmus* in filtered (GF/F) water from Lake Breukeleveen. *Scenedesmus* alone was expected to support high growth rates. The 4-d growth assays were run in beakers, with food and medium exchanged after 2 d. Growth assays for the three larger taxa were begun with a cohort of 1–3-d-old animals born during a 24-h period. We did not have a laboratory culture of *D. cucullata*. Therefore, we collected *D. cucullata* from Lake Breukeleveen and isolated cohorts of field-collected small juveniles under a dissecting microscope using a pipette.

Growth rates were calculated from changes in mass during the 4-d experiments. Three samples were used to estimate the initial mass, with each sample containing 3 *D. magna*, 5–8 *D. galeata*, 6–8 *D. galeata* × *cucullata*, or 10–15 *D. cucullata*. The number of animals and the size of the experimental beakers were adjusted to reflect the large differences in animal size and expected feeding rates. Beakers contained 3 *D. magna* in 500 ml of medium, 6–8 *D. galeata* or *D. galeata* × *cucullata* in 300 ml, or 10–12 *D. cucullata* in 150 ml. Each experimental treatment included three replicate beakers. The number of survivors, their lengths, and the number of eggs were recorded at the end of each 4-d experiment. Methods for processing the samples and calculating growth followed DeMott et al. (1998) (see DeMott et al. [2001] for further details on the growth assay methods).

The *Daphnia* growth assays included four treatments: (1) natural seston, (2) natural seston + 100 µg P L<sup>-1</sup> as phosphate, (3) natural seston + 2 mg C L<sup>-1</sup> of *Synechococcus*, and (4) filtered lake water with 2 mg C L<sup>-1</sup> of *Scenedesmus*. One series of experiments also included an additional treatment with seston supplemented with 2 mg C L<sup>-1</sup> of *Scenedesmus*. Natural seston was filtered through a 33-µm screen to remove zooplankton. The phosphate and *Synechococcus* treatments contained the same P concentration, and both were designed to reduce the C:P of the total diet to <200 (molar), below the threshold for P limitation in *Daphnia* (Sternner and Hessen 1994).

Two series of feeding experiments were carried out between 21 and 27 July 1999. Feeding experiments tested the clearance rates of each taxon with *Scenedesmus* (1 mg C L<sup>-1</sup>) in lake water filtered to remove grazable particles (GF/F filter) and in coarsely filtered seston (33 µm) from Lake Breukeleveen. The concentration of filaments in the coarsely filtered lake seston was estimated using an inverted microscope. Clearance rates were estimated using polystyrene beads (DeMott 1986). Animals of the three larger taxa were grown in cohorts in the laboratory. We used two or three size classes of each taxon, including 1-d-old juveniles, older juveniles, and primiparous adults. *D. cucullata* included groups of early instar juveniles and adults collected from Lake Breukeleveen. The mean individual mass for each size class was estimated by weighing a subset of animals isolated about 3 h before each feeding experiment.

Animals were acclimated in the feeding suspensions for 2–3 h before each feeding trial. A new stock solution of beads (6 µm; Duke Scientific) was prepared each day and counted with a Coulter Multisizer II electronic particle counter. Beads were mixed into the feeding suspension, and the animals were allowed to feed for 6 min. At the end of each feeding trial, the animals were filtered onto a screen, anesthetized with carbonated water, and preserved in 95% ethanol. The concentration of beads was varied with animal size and food concentration to reduce variation in the numbers of beads ingested between species and treatments. For trials with *Scenedesmus* (1 mg C L<sup>-1</sup>), the bead concentration ranged from 1,000 beads ml<sup>-1</sup> for *D. magna* to 3,000 beads ml<sup>-1</sup> for *D. cucullata*. For animals feeding in seston from Lake Breukeleveen (seston concentration about 8 mg C L<sup>-1</sup>), the bead concentration ranged from 4,000 beads ml<sup>-1</sup> for *D. magna* to 10,000 beads ml<sup>-1</sup> for *D. cucullata*. The volumes

Table 1. Ranges of length and mass of *Daphnia* spp. in growth assays. Final mass is from the *Scenedesmus* treatment, a mixture of the last juvenile and the first adult instars. Adult length is from measurements of egg-bearing females. Data are for 5 experiments with each of *D. magna* and *D. galeata*, 1 experiment with a *D. galeata* × *cucullata* hybrid (*D. g* × *c*), and 10 experiments with *D. cucullata*.

	Mass (µg)		Length (mm)	
	Initial	Final	Initial	Adult
<i>D. magna</i>	13–45	140–332	0.98–1.70	2.68–3.00
<i>D. galeata</i>	3.6–5.2	21–31	0.73–0.90	1.46–1.71
<i>D. g</i> × <i>c</i>	3.3–3.8	20–21	0.67–0.69	1.12–1.44
<i>D. cucullata</i>	0.7–0.9	2.7–4.1	0.42–0.46	0.59–0.73

of the feeding suspensions were the same as used in the growth assays. Three replicate beakers were used for each species, size class, and treatment.

To estimate clearance rates for beads, animals from a feeding trial were placed on a microscope slide, and body tissues were dissolved using several drops of a tissue solubilizer (TS-1; Research Products International). The number of beads in each animal was counted using a compound microscope (×100 and ×400 magnification), and the length of each animal was measured with an ocular micrometer. We predicted that clogging of the feeding mechanism and filament rejection would cause an increase in the proportion of beads that were collected in the feeding chamber but not swallowed. We therefore distinguished beads in the feeding chamber and food grove from beads in the gut. Mean body length and clearance rates were calculated for each beaker by calculating averages across individuals. Some animals failed to feed or fed at very low rates. Data from individuals with feeding rates <10% of the mean for a trial were excluded from further calculations.

The *Daphnia* species differed markedly in body size, ranging from juvenile *D. cucullata* weighing <1 µg dry mass per individual to adult *D. magna* >300 µg (Table 1). The *D. galeata* × *cucullata* hybrid was intermediate in size between the two parental species but was more similar to the larger parent, *D. galeata*. The largest *D. cucullata* encountered in field samples was about 0.80 mm in total length.

Periodic observations with an inverted microscope showed that filamentous prokaryotes dominated the phytoplankton throughout the study period. The concentration of filamentous prokaryotes was estimated for two coarsely filtered (33 µm) samples from each lake taken during July. Estimates ranged from 1.2 to 2.2 × 10<sup>5</sup> filaments ml<sup>-1</sup>. Most filaments were thin (1.5–3 µm in diameter), and the mean length was about 80 µm. Visual observations and microscope counts showed that the 33-µm screen retained negligible numbers of filaments. Weekly estimates of seston passing through a 33-µm screen from each lake ranged from 5.7 to 8.9 mg C L<sup>-1</sup> (DeMott et al. 2001). The seston C:P ratio ranged from about 350 to 500 for all three lakes. Estimates of the filament concentration in the coarsely filtered seston used in the feeding experiments ranged from 1.9 to 2.2 × 10<sup>5</sup> filaments ml<sup>-1</sup>.

Figure 1 shows the results of the first growth experiment,

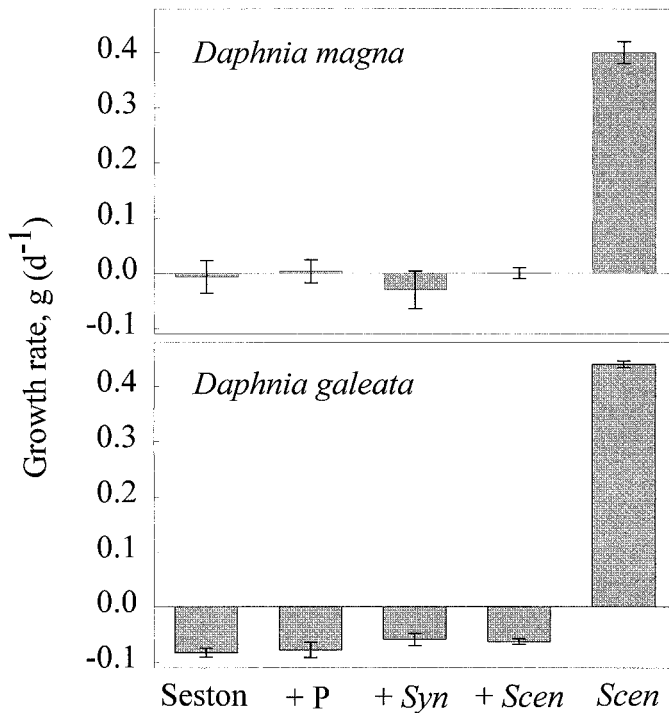


Fig. 1. The growth (per day) of *D. magna* and *D. galeata* in seston from Lake Vuntus, in seston supplemented with phosphate (+P), *Synechococcus* (+Syn), or *Scenedesmus* (+Scen), and in filtered lake water with *Scenedesmus* (Scen). Data show means  $\pm$  standard error for three replicate beakers for an experiment run from 7 to 11 June 1999.

with *D. magna* and *D. galeata* feeding in seston from Lake Vuntus, in seston supplemented with phosphate, *Synechococcus*, or *Scenedesmus* ( $2 \text{ mg C L}^{-1}$ ), and in filtered lake water with the same concentration of *Scenedesmus* alone. Growth was high with *Scenedesmus* alone but was zero or negative in each treatment with natural seston. The remaining growth experiments used the same experimental design as the first experiment (Fig. 1), except that they lacked a treatment with seston plus *Scenedesmus*. Since the results for the larger taxa did not vary over time or between lakes, we summarized all 21 growth assays in Fig. 2. All four taxa exhibited high growth rates with *Scenedesmus* alone. However, growth in treatments with natural seston varied with body size. *D. magna* and *D. galeata* lost mass in natural seston, the hybrid showed low growth, and *D. cucullata* showed moderate growth. The three larger taxa showed no response to the supplements (one-way ANOVA, each of 11 tests,  $P > 0.05$ ). In contrast, *D. cucullata* exhibited improved growth in response to the phosphate and *Synechococcus* supplements in each experiment (one-way ANOVA,  $P < 0.05$  for each of 10 tests). For *D. cucullata*, mean growth in natural seston supplemented with *Synechococcus* was 85% of the growth rate in *Scenedesmus* alone.

*D. magna* and *D. galeata* growing in treatments with natural seston experienced variable mortality (0–60%), with at least one *D. magna* or two *D. galeata* surviving in each beaker. At the end of the experiment, survivors of these two species in the seston treatments were often found at the bot-

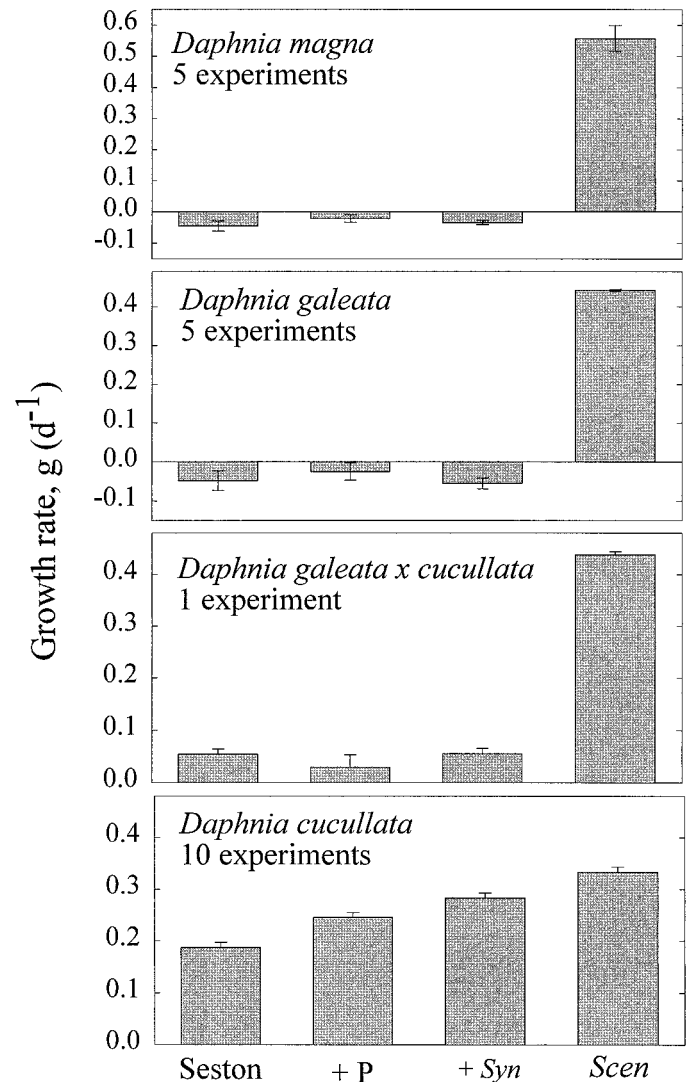


Fig. 2. The growth (per day) of four *Daphnia* taxa in seston, in seston supplemented with phosphate (+P) or *Synechococcus* (+Syn), and in filtered lake water with *Scenedesmus* (Scen). Data show overall means  $\pm$  standard error for five experiments each with *D. magna* and *D. galeata* and 10 experiments with *D. cucullata*. Data for *D. galeata*  $\times$  *cucullata* show means  $\pm$  standard error for three replicate beakers for a single experiment conducted with seston from Lake Breukeleveen. Data for each of the other three taxa include separate experiments conducted with seston from each of the three Loosdrecht Lakes. Note differences in scale between species.

tom of the beakers, barely moving. Lower survival may have created biases, if smaller individuals were more likely to die. Thus, growth on natural seston by these large taxa may have been overestimated. However, the key point is the lack of growth by the two larger *Daphnia* species in each treatment with natural seston. Survivorship was high (>90%) for all four taxa feeding in *Scenedesmus* alone and for the hybrid and *D. cucullata* in all treatments.

Feeding data are expressed as functions of body length and carbon. Carbon was assumed to equal 50% of body mass. The proportion of beads in the gut was about 90% for animals feeding on *Scenedesmus* alone and showed little or

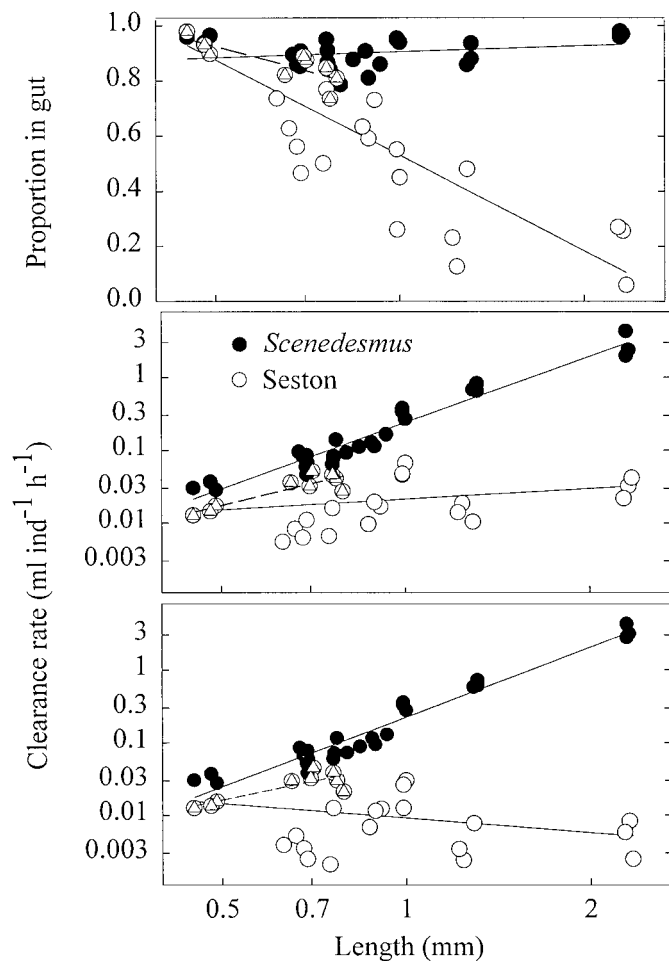


Fig. 3. Relationships between body length and feeding rates on 6- $\mu\text{m}$  beads for four taxa of *Daphnia* feeding in seston from Lake Breukeleveen or in filtered lake water with *Scenedesmus* (1 mg C L<sup>-1</sup>). Regressions were calculated for all four taxa combined (circles and solid lines) and for *D. cucullata* alone (triangles and dashed line). The upper panel shows the proportion of beads in the gut of the total number collected. Clearance rates (milliliters per individual per day) were calculated for all beads collected (middle panel) and for beads in the gut (lower panel). Each data point is the mean for 3–30 animals from a beaker.

no variation with body length (Fig. 3, upper panel;  $r^2 = 0.07$ ,  $P = 0.18$ ). In contrast, the proportion of beads in the gut declined with increasing body size for all species feeding in natural seston (Fig. 3, upper panel;  $r^2 = 0.69$ ,  $P < 0.001$ ). The proportion of beads in the gut also declined with increasing body size for *D. cucullata* feeding in seston ( $r^2 = 0.66$ ,  $P < 0.001$ ). However, adult *D. cucullata* tended to have a higher proportion of beads in their guts than did juvenile *D. galeata* and *D. galeata*  $\times$  *cucullata* similar in size to the adult *D. cucullata*.

As expected, the clearance rate on *Scenedesmus* alone increased strongly with increasing body size (Fig. 3, middle panel; Table 2). By contrast, for animals feeding in natural seston, the relationship between clearance rate and body size was weak, even when beads that were collected but not ingested are included (Fig. 3, middle panel; Table 2). When

only beads found in the gut are included, the absolute clearance rate declined with increasing body size, although the slope is not significant (Fig. 3, lower panel; Table 2). In contrast to the overall regressions, separate regressions for *D. cucullata* exhibited positive slopes (Fig. 3; Table 2). Adult *D. cucullata* tended to show a higher clearance rate than juvenile *D. galeata* and *D. galeata*  $\times$  *cucullata* similar in size to the adult *D. cucullata*. No species-specific responses were noted among the three larger taxa. Clearance rates for juvenile *D. magna* overlapped with values for adults of *D. galeata* and the hybrid of similar size.

The mass-specific clearance rate (MSCR; ml mg C<sup>-1</sup> d<sup>-1</sup>) on *Scenedesmus* showed little or no change over a 100-fold change in body mass (MSCR = 1,104 Mass<sup>0.040</sup>,  $r^2 = 0.03$ ). Thus, the mean daily ration of *Scenedesmus* across all four taxa was about 110% of body carbon. In contrast to data for *Scenedesmus*, the MSCR in natural seston declined sharply with increasing size (MSCR = 619 M<sup>-0.775</sup>,  $r^2 = 0.68$ ,  $P < 0.001$ ). The predicted MSCR of juvenile *D. cucullata* (about 0.5  $\mu\text{g}$  C per individual) was about 40 times higher than that for a juvenile *D. magna* 100-fold greater in mass.

Our experiments show a clear distinction between the small native *D. cucullata* and three larger *Daphnia* taxa in their abilities to feed and grow on natural seston from the three Loosdrecht Lakes. In support of the P-limitation hypothesis, *D. cucullata* showed improved growth with phosphate and *Synechococcus* supplements. The role of P limitation in constraining the growth and abundance of *D. cucullata* in the Loosdrecht Lakes gains further support from a 9-yr field study (DeMott and Gulati 1999) and from a study that includes data on the population dynamics and P balance of *D. cucullata*, as well as a detailed analysis of the growth assays for that species (DeMott et al. 2001).

In contrast to *D. cucullata*, *D. magna* and *D. galeata* failed to grow in natural seston even when P, *Synechococcus*, or *Scenedesmus* was added. The lack of response to the supplements is strong evidence that the poor growth of these taxa was not due to a nutritional deficiency. P limitation can be ruled out by the lack of response to phosphate and P-rich *Synechococcus*. Moreover, since *Scenedesmus* is a good food by itself, the lack of growth when seston was supplemented with *Scenedesmus* further demonstrates that poor growth in seston alone was due to the presence of an inhibiting factor rather than to a deficiency in nutrients or energy per se. Excellent growth in filtered water from Lake Breukeleveen with *Scenedesmus* provides evidence against any dissolved poisons or inhibitors. Our findings differ with those of Müller-Navarra et al. (2000), who argued that the poor growth of *D. magna* in a lake dominated by *Oscillatoria* was due to a deficiency in essential fatty acids.

Although all four *Daphnia* species fed in seston above the C:P threshold for P limitation, only *D. cucullata* shows evidence of P-limited growth. As noted in the introduction, strong energy limitation can prevent P limitation (Sterner 1997). Thus, these results are consistent with stoichiometric theory, as long as one assumes that the three larger taxa experienced strong energy limitation.

Our feeding experiments support the hypothesis that the three larger taxa experienced strong energy limitation due to feeding interference. The feeding rate on natural seston did

Table 2. The results of regressions, where clearance rate (CR, ml ind<sup>-1</sup> h<sup>-1</sup>) is expressed as a power function of length (mm) of the form CR =  $a$ Length <sup>$b$</sup> . Animals were fed in *Scenedesmus* alone (1 mg C L<sup>-1</sup>) or in seston from Lake Breukeleveen. Regressions were calculated separately for all beads and for beads found in the gut. Regressions were run for all four species combined and for *D. cucullata* alone. Each sample ( $n$ ) is the mean length and clearance rate for a beaker of animals.

	All beads					Beads in gut			
	$n$	$a$	$b$	$r^2$	$P$	$a$	$b$	$r^2$	$P$
<i>Scenedesmus</i>									
All species	27	0.238	3.02	0.95	0.001	0.223	3.19	0.94	0.001
<i>D. cucullata</i>	9	0.144	2.00	0.95	0.001	0.113	1.69	0.90	0.001
Seston									
All species	27	0.021	0.48	0.09	0.13	0.009	-0.67	0.09	0.12
<i>D. cucullata</i>	9	0.071	2.03	0.78	0.002	0.054	1.73	0.068	0.006

not increase with increasing length, even though the four species represent a wide range in length and mass. This lack of a relationship between body size and feeding rate means that the energy demands of the larger species would not be met even if assimilation efficiency were high. In contrast, all four species showed high feeding rates and excellent growth when feeding on *Scenedesmus* in filtered lake water. When all four species were included in the feeding rate versus body length regression, the exponent  $b$  was about 3 for *Scenedesmus* (Table 2). This value is within the range for various sizes of *Daphnia* species feeding on readily ingested resources (Lampert 1987b; DeMott 1995).

Comparison of the proportion of beads in the gut provides further support for the notion that the three larger species suffered from clogging of their feeding mechanism and/or increased rejection rates when feeding on natural seston. Although a previous study noted differences between the collection and ingestion of large beads (Bern 1990), our study is the first of which we are aware that shows how beads can be used as an indicator of interfering particles.

Many laboratory experiments have documented size-specific inhibition of feeding and growth in *Daphnia* by filamentous algae (reviewed by Gliwicz 1990a). Studies by Gliwicz (1990a,b) and Gliwicz and Lampert (1990) are of special interest because they include *D. cucullata* in their experiments. The growth of two larger *Daphnia* species was inhibited by the presence of filaments, while *D. cucullata* showed no growth inhibition and even benefited from the presence of filaments when the *Scenedesmus* concentration was low (Gliwicz and Lampert 1990). Another study was designed to test the concentration of *Aphanizomenon* filaments where the growth rate of each of four *Daphnia* species approached zero, despite a high concentration of *Scenedesmus* (Gliwicz 1990b). *D. magna* reached a zero-growth threshold at about 5,000 filaments ml<sup>-1</sup>, whereas *D. cucullata* approached zero growth at about 70,000 filaments ml<sup>-1</sup>.

Our feeding and growth experiments together suggest that filament concentrations in the Loosdrecht Lakes were above the zero-growth threshold for both *D. magna* and *D. galeata*. That is, weight loss occurred in natural seston, even when seston was supplemented with phosphate, *Synechococcus*, or *Scenedesmus*. Since the *D. galeata* × *cucullata* hybrid showed weak growth in natural seston, the filament concentration was apparently a little below its zero threshold for growth.

In contrast to the three larger taxa, the growth of *D. cu-*

*cullata* in seston supplemented with *Synechococcus* was 85% of the value for *Scenedesmus* alone. Three factors may contribute to the low or negligible negative impact of filaments on the feeding and growth of *D. cucullata* from the Loosdrecht Lakes. First, *D. cucullata* from the Loosdrecht Lakes is very small, smaller than that grown in the laboratory by Gliwicz (1990b) and Gliwicz and Lampert (1990). A previous study commented on the presence of “dwarf” *D. cucullata* in lakes with dense fish populations (Hrbacek and Hrbackova-Essova 1960). Second, our feeding studies suggest that adult *D. cucullata* from Lake Breukeleveen may be better at coping with filamentous cyanobacteria than other *Daphnia* of similar size. However, our feeding and growth experiments were not designed to distinguish between species differences, clonal differences, and possible effects of long-term acclimation. Finally, the condition and size of the filaments may affect the zero threshold. For example, Gliwicz (1990b) showed that filaments of *Aphanizomenon* in a deteriorating physiological state were better tolerated by *Daphnia* than were filaments in a good physiological state (see also Gulati et al. 2001). Gliwicz (1990a) also suggested that shorter filaments cause weaker feeding interference than longer filaments. Thus, the thin, relatively short filaments of the Loosdrecht Lakes may be better tolerated than longer, thicker filaments.

Lake Loosdrecht makes an interesting comparison with another well-studied, shallow, highly eutrophic Dutch lake, Lake Tjeukemeer. Both lakes have similar fish communities, and the phytoplankton of both lakes is dominated by *Oscillatoria* during summer. However, in contrast to Lake Loosdrecht, Lake Tjeukemeer has abundant populations of *D. galeata* and *D. galeata* × *cucullata* hybrids, as well as *D. cucullata* (Boersma and Vijverberg 1994). Although the *Oscillatoria* biomass is similar, the filament density is about five times higher in Lake Loosdrecht than in Lake Tjeukemeer, which is dominated by a species (*Oscillatoria aghardii*) that produces thicker, longer filaments (Lammens et al. 1992). Thus, a difference in the species composition of the filamentous cyanobacteria may account for the differences in *Daphnia* species composition between the two lakes. This assumes that the higher density of filaments in the Loosdrecht Lakes more than offsets the effects of thicker, longer filaments in Lake Tjeukemeer.

Frankly, we were surprised by the strength and consistency of the feeding and growth inhibition experienced by the

larger *Daphnia* taxa in natural seston from the Loosdrecht Lakes. These lakes provide an example in which poor individual growth seems sufficient to explain the absence of *Daphnia* species larger than *D. cucullata*. Although we did not study fish predation, evidence reviewed in the introductory paragraphs indicates that fish predation is also intense in these lakes. DeMott and Gulati (1999) suggested that the summer disappearance of *D. cucullata* from individual lakes during certain years was a consequence of consistently strong fish predation and variable P limitation. Clearly, zooplankton may experience strong fish predation and poor food quality simultaneously.

Our results show that *Daphnia* species differing in size face different food quality constraints. The possibility that *D. cucullata* from the Loosdrecht Lakes has adaptations for dealing with high filament concentrations, in addition to small size, may merit further study. Our results provide strong support for earlier studies suggesting that the abundance of *D. cucullata* in the Loosdrecht Lakes is constrained by P limitation (DeMott and Gulati 1999), whereas large-bodied *Daphnia* species are excluded by the interfering effects of filamentous cyanobacteria (Davidowicz et al. 1988). Our results also illustrate the importance of simultaneously testing alternative food quality mechanisms and for taking into account interactions between energy limitation and nutritional deficiencies.

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