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Supplementation of cyanobacterial food with polyunsaturated fatty acids does not improve growth of *Daphnia*

Abstract—The hypothesis that the low food quality of nontoxic, ingestible cyanobacteria for *Daphnia* is due to the absence of long-chained polyunsaturated fatty acids (PUFAs) was tested. *Synechococcus elongatus,* which is well assimilated by *Daphnia* and is deficient in PUFAs, was chosen as a model food organism. A newly devised method was used to load single fatty acids and mixtures of fatty acids on beads of bovine serum albumin, which then were added as supplements to cyanobacterial suspensions. Growth rates of juvenile *Daphnia galeata* on *Synechococcus elongatus* were low and were not enhanced by the addition of C_{18}-PUFAs and C_{20}-PUFAs singly or together on beads. Supplementation of lipids from the green alga *Scenedesmus obliquus* significantly enhanced growth of *D. galeata,* indicating that the low quality of cyanobacterial carbon is due to a deficiency in a lipid other than PUFAs. The low food quality of *Synechococcus elongatus* was

partly mitigated when the animals first fed on *Scenedesmus obliquus,* suggesting that the storage of algal lipids provides a mechanism to cushion the effect of short episodes of cyanobacterial dietary deficiencies on the growth of *D. galeata.*

The transfer of energy at the primary producer/herbivore interface is a crucial parameter determining the trophic structure of aquatic food webs. A decoupling of primary and secondary production often is due to a low transfer efficiency from phytoplankton to grazers, which leads to both low biomass of herbivores and an accumulation of phytoplankton biomass. This is a frequently encountered problem in biomanipulation when the manipulation of piscivorous fish is intended to enhance zooplankton grazing and thus reduce phytoplankton abundances but instead often leads to high biomass of cyanobacteria (Oliver and Ganf 2000). Cyanobacteria are well known to be of low food quality for zooplankton due to toxicity (Lampert 1981), mechanical interference (Porter and McDonough 1984), or nutritive deficiencies. The importance of dietary deficiencies in cyanobacteria for the growth of *Daphnia* has recently been articulated in a correlative study by Müller-Navarra et al. (2000), who found low food quality in summer when cyanobacteria strongly dominated the hypereutrophic pond and higher food quality in winter when diatoms were dominating the phytoplankton. Of all sestonic parameters tested, the content of the long-chained ($>C_{18}$) polyunsaturated fatty acid (fatty acid with two or more unsaturated bonds, PUFA) eicosapentaenoic acid (EPA, 20:5n-3) has shown the highest correlation with growth of *Daphnia*. This suggests that the food quality is constrained not by C, N, and P stoichiometry, but rather by a shortage of essential biochemicals in the diet. PUFAs are essential for many vertebrates and invertebrates (Stanley-Samuelson et al. 1988), and the importance of PUFAs in freshwater zooplankton nutrition has recently been discussed (Brett and Müller-Navarra 1997). Since cyanobacteria in general lack long-chained PUFAs (Cobelas and Lechardo 1988), the low food quality of cyanobacterial carbon in the hypereutrophic pond is ascribed to the lack of EPA in these prokaryotes, and Müller-Navarra et al. (2000) have suggested that EPA deficiency is the main reason for the decoupling of primary and secondary production commonly observed during cyanobacterial blooms.

These correlative evidences for a dietary deficiency of cyanobacteria in essential nutrients are supported by experiments in which poor growth at high carbon concentrations is shown not to be due to low ingestion, reduced digestibility, or toxin content. Lampert (1977a,b) found high rates of carbon assimilation by *Daphnia pulicaria* feeding on *Synechococcus elongatus,* but no somatic growth on a pure diet of this cyanobacterium. Additional experiments (Lampert 1981) ruled out toxicity, indicating that the low quality of carbon probably was due to a nutritional deficiency in an essential compound. *Synechococcus* contains only traces of long-chained PUFAs, and DeMott and Müller-Navarra (1997) recently showed that growth and reproduction of *Daphnia* improves when *Synechococcus* is supplemented with PUFA-rich fish oil emulsion and concluded that the low carbon transfer efficiency at the *Synechococcus–Daphnia* interface was probably due to a deficiency in long-chained PUFAs.

However, fish oil contains fatty acids and other lipids; therefore, the ameliorating effect of fish oil does not provide direct experimental proof of a PUFA-limitation of *Daphnia* when growing on cyanobacteria. The aim of this study was to test the hypothesis that the poor quality of the dietary carbon of cyanobacteria is due to the absence of EPA in particular or of PUFAs in general in these prokaryotes. In order to ensure that toxicity and mechanical interference were not constraining food quality, we used the same strain of *Synechococcus elongatus* as Lampert (1977a) and DeMott and Müller-Navarra (1997). Growth of *D. galeata* on *Synechococcus elongatus* supplemented with single PUFAs or mixtures of fatty acids was studied to assess changes in food quality. Growth of *D. galeata* on the green alga *Scenedesmus obliquus,* which is of fairly good food quality for *Daphnia* (DeMott and Müller-Navarra 1997), was used as a reference.

Growth of cyanobacteria and algae—Synechococcus elongatus (strain SAG 89.79, Stammsammlung für Algen, SAG) and *Scenedesmus obliquus* (strain SAG 276-3a) were grown as continuous cultures. *Synechococcus elongatus* was grown in Cyano medium (Jüttner et al. 1983) at a dilution rate of 0.25 d^{-1} at 20°C with illumination at 40 μmol m^{-2} s^{-1}. *Scenedesmus obliquus* was grown in modified WC medium (Guillard 1975) at a dilution rate of 0.5 d^{-1} at 20°C with illumination at 120 μmol m^{-2} s^{-1}. *Synechococcus elongatus* had a molar C:N:P ratio of 84:16:1. Chemostat-grown cells were concentrated by centrifugation and resuspended in WC medium. Carbon concentrations of these stock solutions of organisms were estimated from photometric light extinction (800 nm) using carbon-extinction equations.

Supplementation of fatty acids and lipids—The fatty acid content of the green alga *Scenedesmus obliquus,* which supports fairly good growth of *Daphnia* and of the cyanobacterium *Synechococcus elongates,* was compared using the method described by Von Elert and Stampfl (2000). In *Synechococcus elongatus,* neither C_{18}-PUFAs nor long-chained PUFAs were detectable (Table 1); a deficiency of the cyanobacterium in any of the fatty acids present in the green alga could account for a limitation of the growth of *D. galeata* on the cyanobacterium. A putative limitation can be tested straightforwardly by supplementation of the lacking compound. We therefore devised a method to supplement cyanobacterial suspensions with proteinaceous beads made from bovine serum albumin (BSA) loaded with fatty acids. To load the beads with single fatty acids (obtained from Sigma), 1 mg of a free fatty acid was dissolved in 50 ml of ethanol, and 200 μl of an ethanolic suspension (20 mg ml^{-1}) of BSA-beads (Micromod) was added. This suspension was gently evaporated to dryness with a rotatory evaporator and resuspended by ultrasonification in 40 ml of WC medium. The resulting aqueous suspension was passed through a mesh (30-μm pore size), and 10 ml of the suspension was added as a supplement to 0.5 liters of a suspension of *Synechococcus elongatus* (2 mg C L^{-1}). The mean diameter of the beads was 2.61 μm and that of cells of *Synechococcus*

Table 1. Fatty acid content and composition of *Synechococcus elongatus* and *Scenedesmus obliquus.* Values for fatty acid content are means (SE) for $n = 3$. n.d. (not detected).

	Synechococcus elongatus		*Scenedesmus obliquus*	
Fatty acid	Fatty acid content (μg mg C^{-1})	Fatty acid composition (%)	Fatty acid content (μg mg C^{-1})	Fatty acid composition (%)
14 : 0	22.65 (0.56)	19.11	2.91 (0.00)	0.99
14 : 1n-5	1.00 (0.02)	0.85	n.d.	0.00
15 : 0	0.68 (0.01)	0.57	n.d.	0.00
16 : 0	31.41 (0.64)	26.50	64.39 (0.01)	22.01
16 : 1n-7	58.05 (1.17)	48.98	1.14 (0.01)	0.39
18 : 0	1.82 (0.08)	1.53	8.11 (0.02)	2.77
18 : 1n-12/n-9	0.78 (0.01)	0.66	84.91 (0.07)	29.02
18 : 1n-7	2.11 (0.06)	1.78	1.40 (0.01)	0.48
18 : 2n-6	n.d.	0.00	34.40 (0.00)	11.76
18 : 3n-6	n.d.	0.00	1.45 (0.00)	0.49
18 : 3n-3	n.d.	0.00	80.16 (0.06)	27.40
18 : 4n-3	n.d.	0.00	10.93 (0.04)	3.73
20 : 1n-9	n.d.	0.00	1.60 (0.01)	0.55
20 : 5n-3	n.d.	0.00	n.d.	0.00
22 : 0	n.d.	0.00	1.21 (0.09)	0.41
Total	118.50 (2.24)	100.00	292.59 (0.09)	100.00

elongatus was 1.65 μm. In the resulting suspension, the beads constituted 12% of all particles (total: 4.5×10^6 ml^{-1}), 69% of the volume of all particles (total: 4.49×10^6 nl ml^{-1}), and 14% of the total particulate carbon.

Pure beads alone already contained saturated fatty acids and C_{18}-PUFAs (Table 2); therefore, the total fatty acid content of the food suspension (cyanobacterial suspension plus pure beads) was 20% higher than that of the cyanobacterial suspension alone. Beads loaded with the C_{18}-PUFAs α-linolenic acid (α-LA, 18 : 3n-3) or stearidonic acid (SA, 18 : 4n-3) or with the long-chained PUFA EPA (20 : 5n-3) contained substantial amounts of these PUFAs (Table 2). When the beads were loaded with a single PUFA, the amount of the other fatty acids did not change, which indicated that this method would allow a specific single compound to be supplemented. An exception was observed only with SA (18 : 4n-3); the concomitant increase in EPA content noted (Table 2) was due to the lower purity (90%) of this fatty acid compared to the higher purity (>99%) of all other fatty acids used. In order to load the beads with a mixture of fatty acids so that the overall fatty acid content of the food suspension would resemble that of *Scenedesmus obliquus* and to test simultaneously for a limitation by EPA, various fatty acids were added in different amounts to 50 ml ethanol prior to the addition of beads (500 μg each of 18 : 3n-3 and 20 : 5n-3, 250 μg of 18 : 2n-6, 75 μg of 18 : 4n-3, 15 μg each of 18 : 3n-6 and 18 : 1n-7) (Table 2). Additionally, beads were loaded with lipids from *Scenedesmus obliquus* cells; cells (1 mg C) were extracted with a mixture of dichloromethane/methanol (2 : 1, v/v), and the cell-free extract was evaporated with nitrogen to dryness and resuspended in 50 ml of ethanol before beads were added as described above.

Table 2. Amount of fatty acids (μg) supplemented with the addition of bovine serum albumin beads to 1 mg particulate carbon of *Synechococcus elongatus.* Beads were treated with various fatty acids; pure beads were treated in the same way, but without fatty acids being added.

	Amounts of fatty acids (μg)				
Fatty acid	Pure beads	Beads + 18 : 3n-3	Beads + 18 : 4n-3	Beads + 20 : 5n-3	Beads + fatty acid mixture
16 : 0	5.50	4.68	3.88	5.11	4.79
18 : 0	2.41	2.25	1.96	2.27	2.11
18 : 1n-12/n-9	3.48	2.81	2.35	3.10	2.48
18 : 1n-7	0.00	0.00	0.00	0.00	0.03
18 : 2n-6	9.90	8.26	6.07	8.83	22.53
18 : 3n-6	0.00	0.00	0.00	0.00	0.39
18 : 3n-3	2.90	84.86	4.87	4.30	13.77
18 : 4n-3	0.00	0.00	110.48	0.00	6.07
20 : 1n-9	0.00	0.00	0.00	0.00	0.35
20 : 5n-3	0.00	0.00	7.64	104.57	14.58
Total	24.20	102.86	137.26	128.19	67.11

When 2-d old juveniles of *D. galeata* were fed for 4 d on *Synechococcus elongatus* cells supplemented with beads loaded with EPA, this long-chained PUFA was detectable in chromatograms of fatty acid methyl esters. EPA was absent in the chromatograms when animals were fed only the cyanobacterium (Fig. 1). These results indicated that fatty acids loaded onto the beads were ingested and assimilated by *D. galeata.*

Daphnia growth experiments—Growth experiments were carried out at 20°C with third-brood juveniles of a clone of *D. galeata,* originally isolated from Lake Constance (Stich and Lampert 1984). Juveniles were collected within 8 h of birth and grown until the age of 48 h in a flow-through system on *Scenedesmus obliquus* (2 mg C L^{-1}) or *Synechococcus elongatus* (2 mg C L^{-1}). In one experiment, juveniles were grown in the flow-through system to the age of only

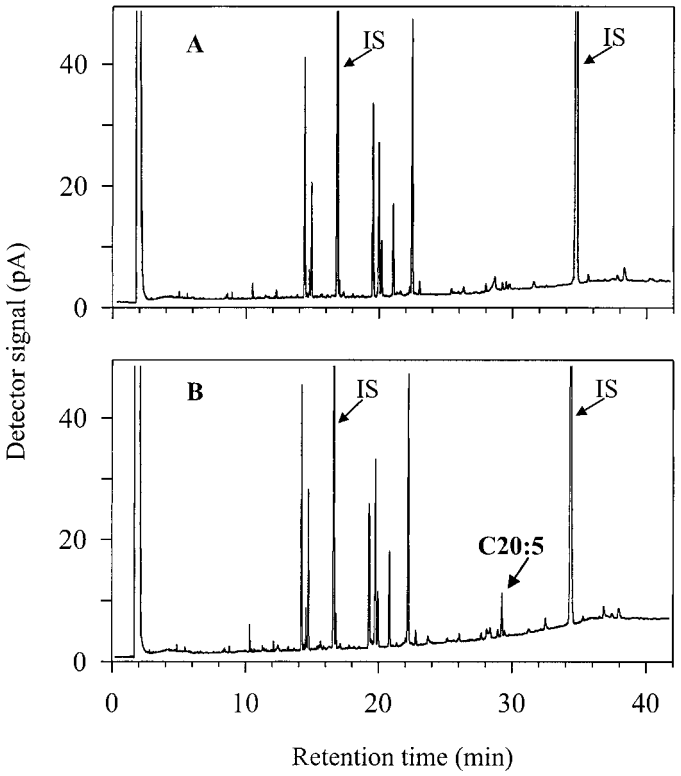

Fig. 1. Gas chromatogram of fatty acid methyl esters of *Daphnia galeata* grown (A) on only *Synechococcus elongatus* and (B) on *Synechococcus elongatus* supplemented with beads loaded with eicosapentaenoic acid (20 : 5n-3). IS = internal standard.

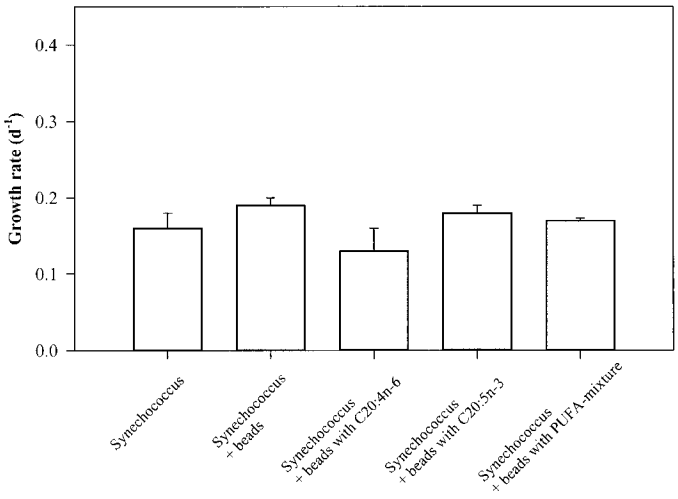

Fig. 2. Growth of *Daphnia galeata* on *Synechococcus elongatus* with and without supplements of beads enriched with single long-chained polyunsaturated fatty acids or with a mixture of fatty acids. Data points are means ($n = 3$); error bars indicate standard errors. Supplementation did not significantly affect growth (one-way ANOVA: $F_{4,10} = 1.84$; $P = 0.2$). Prior to the experiment, animals were prefed on *Scenedesmus obliquus* for 48 h.

38 h. After growth in the flow-through system, the animals were transferred to fresh medium, and the subsequent growth experiments lasted for 4 d. Experiments were carried out in 0.5 liters of filtered (0.45-μm pore size) lake water containing algal or cyanobacterial food (1 or 2 mg C L^{-1}); the food suspensions were renewed daily. Somatic growth rates (g, d^{-1}) were calculated according to Wacker and Von Elert (2001) from the mean individual dry weight of a subsample of the animals at the beginning of the experiment and from the mean individual dry weight of the daphnids at the end of the experiment. Mean individual dry weights were mean values of 10 individuals weighed on an electronic balance (Mettler UMT 2) that recorded to the nearest 0.1 μg. Each treatment consisted of three replicates with 10 animals each, and the growth rate was calculated from the mean of each treatment. The data were analyzed by one-way ANOVA and post hoc comparisons (Tukey).

Growth on *Synechococcus elongatus* alone was poor ($g = 0.16$ d^{-1}) and was not significantly affected by the addition of pure beads, despite the presence of traces of C_{18}-PUFAs in the beads (Fig. 2). A C : N : P molar ratio of 84 : 16 : 1 of *Synechococcus elongatus* excludes phosphorus limitation of *D. galeata* growth as the underlying mechanism for the low food quality of the cyanobacterium (Sterner 1993). Although the C : P molar ratios were not determined for the BSA-beads, the addition of pure beads obviously did not reduce the food quality, and hence we conclude that, when feeding on *Synechococcus elongatus* and BSA-beads, *D galeata* was also not limited by the availability of phosphorus. The addition of a long-chained n-6 or n-3 PUFA also had no ameliorating effect on growth (Fig. 2), which indicated that these long-chained PUFAs did not constrain the food quality. Even the addition of a PUFA mixture, which provided an overall fatty acid content of the food suspension resembling that of *Scenedesmus obliquus* supplemented with EPA, did not affect growth (Fig. 2); this suggested that neither the lack of C_{18}-PUFAs nor the lack of C_{20}-PUFAs was the reason for the low food quality of *Synechococcus elongatus*. The monounsaturated fatty acid oleic acid (18 : 1n-7) is found in large amounts in *Scenedesmus obliquus* and is lacking in *Synechococcus elongatus*; since DeMott and Müller-Navarra (1997) found no enhancement of growth of *Daphnia* by addition of oleic acid to *Synechococcus elongatus*, we did not test for a possible limitation of this fatty acid.

In the standard growth experiments, juvenile daphnids were fed on *Scenedesmus obliquus* for 48 h prior to the start of the growth experiment. Since this prefeeding on this high-quality food might reduce the susceptibility of *D. galeata* to the PUFA deficiency in *Synechococcus elongatus*, another experiment was carried out in which juveniles were fed on the green alga for only 38 h. Growth on *Synechococcus elongatus* alone ($g = 0.1$) was significantly lower (t-test, $p < 0.05$) than in the standard growth experiment, which suggested that the animals indeed were more susceptible to the dietary deficiency of *Synechococcus elongatus* (Fig. 3) after shorter prefeeding on the green alga. However, this did not lead to more pronounced effects of supplementary fatty acids: the addition of either of the two C_{18}-PUFAs, α-LA (18 : 3n-3) or SA (18 : 4n-3) present in *Scenedesmus obliquus* did not affect growth (Fig. 3). Therefore, α-LA, which is found in high amounts in *Scenedesmus obliquus* and is lacking in

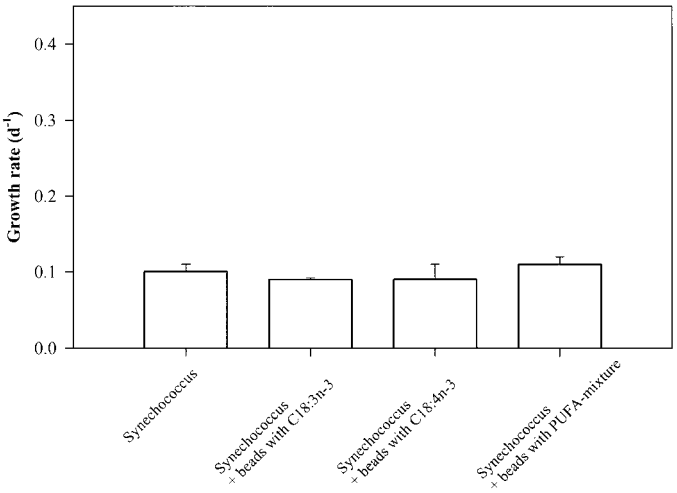

Fig. 3. Growth of *Daphnia galeata* on *Synechococcus elongatus* with and without supplements of beads enriched with single polyunsaturated fatty acids or with a mixture of fatty acids. Data points are means ($n = 3$); error bars indicate standard errors. Supplementation did not significantly affect growth (one-way ANOVA: $F_{3,8} = 0.61$; $P = 0.63$). Prior to the experiment, animals were prefed on *Scenedesmus* for 38 h.

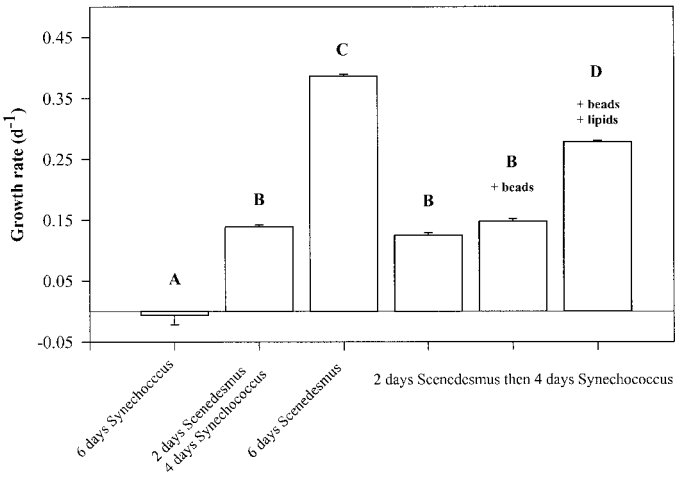

Fig. 4. Growth rates of neonates of *Daphnia galeata* determined from day 2 to day 6. Growth on food (2 mg C L^{-1}) without supplements (not shaded) indicates that constituents previously accumulated from *Scenedesmus obliquus* partly mitigated the dietary deficiency of *Synechococcus elongatus*. Growth of animals that had fed on *Scenedesmus obliquus* (2 mg C L^{-1}) for the first 48 h and that were subsequently fed on *Synechococcus elongatus* (1 mg C L^{-1}) (shaded) increased when beads loaded with lipids extracted from *Scenedesmus obliquus* (1 mg C) were added. Data points are means ($n = 3$); error bars indicate standard errors. Data points marked with the same letter are not significantly different (Tukey's post hoc test).

Synechococcus elongatus, can be ruled out as the limiting resource in the cyanobacterium. Supplementation with a mixture of PUFAs did not improve the food quality; therefore, even colimitation by PUFAs can be excluded as a reason for the nutritional deficiency of the cyanobacterium.

DeMott and Müller-Navarra (1997) found that *Daphnia* had high growth rates during the first 2–3 d on *Synechococcus elongatus* cells alone, but exhibited reduced growth or even negative growth rates during the remainder of the 6-d experiment. The authors suggested that this decline in growth over time might be due to a gradual depletion of a reserve that had been present at the start of the experiment. In order to investigate whether these reserves are of maternal origin or are assimilated from *Scenedesmus obliquus* during the 48-h feeding prior to the growth experiment, we split a cohort of freshly hatched neonates and subjected them to three different food regimes for the following 6 d. Animals feeding on only *Synechococcus elongatus* exhibited slightly negative growth rates ($g = -0.007$ d^{-1}) from day 2 to day 6 (Fig. 4). Those animals that grew on *Scenedesmus obliquus* for 2 d prior to the growth experiment with *Synechococcus elongatus* had a growth rate of 0.14 d^{-1}, which indicated that the reserves that accumulated during the feeding on *Scenedesmus obliquus* partly mitigated the dietary deficiency of *Synechococcus*. However, the reserves were not sufficient to alleviate the cyanobacterial inadequacy completely, as is indicated by the high growth rate (0.39 d^{-1}) of animals that were fed only on *Scenedesmus obliquus* (Fig. 4). These results indicate that the constituents from *Scenedesmus obliquus* accumulated and stored may supplement the dietary deficiency of *Synechococcus elongatus* when *D. galeata* later feeds only on the cyanobacterium. In order to investigate whether this supplementary effect of *Scenedesmus obliquus* was due to a lipid, a growth experiment with reduced food concentrations (*Synechococcus elongatus* at 1 mg C L^{-1}) was carried out. The growth rate was the same as that when grown with *Synechococcus elongatus* at 2 mg C L^{-1} (Fig. 4). Addition of beads had no significant effect, but when lipids extracted from *Scenedesmus obliquus* (1 mg C) were loaded onto the beads, the food quality was substantially improved (Fig. 4). Although the growth rates were lower than with only *Scenedesmus obliquus* at 2 mg C L^{-1}, it is evident that at least a part of the deficiency of *Synechococcus elongatus* can be complemented by lipids from the green alga.

Like many other strains of *Synechococcus*, the one used here contains little or no C_{18}-PUFAs, while strains of *Anabaena*, *Microcystis*, and some strains of *Gleocapsa* contain substantial quantities of C_{18}-PUFAs (Kenyon and Stanier 1970; Kenyon 1972). However, these strains are not known to be of better food quality than *Synechococcus*, thus confirming our observation that the lack of C_{18}-PUFAs did not determine the food quality of the cyanobacterium used in this study.

In the light of the nonsignificant effects of PUFA additions, the growth-ameliorating effect of the lipids from *Scenedesmus obliquus* indicates that lipids others than PUFAs must be the limiting resource for *D. galeata* feeding on *Synechococcus elongatus*. Cyanobacteria are prokaryotes and hence not only lack long-chained PUFAs (Phillips 1984), but furthermore do not contain sterols (Urich 1990), which are present in *Scenedesmus* (Rzama et al. 1994). Sterols are indispensable for vital functions such as membrane fluidity, growth, and reproduction (Ederington et al. 1995; Crockett

1998). In crustaceans, cholesterol is the dominant sterol (Goad 1981) and serves as a precursor for ecdysteroids, which are involved in the regulation of moulting. In arthropods, sterols must be obtained from the food (Goad 1981), and isotope-ratio GC/MS analyses confirm that copepods derive their sterols from algal food, either directly or as precursors for the production of other sterols (Grice et al. 1998). Recently the poor quality of *Dunaliella* for the ontogenetic development of the marine copepods *Temora longicoris* and *Pseudocalanus elongatus* has been suggested to be due to a sterol deficiency of the alga (Klein Breteler et al. 1999). Since the fish oil used by DeMott and Müller-Navarra (1997) contains cholesterol (2–3 mg g^{-1}, pers. comm. O. Thorstad), the ameliorating effect of this oil on the quality of *Synechococcus* is due to the cyanobacterial deficiency in sterols rather than a deficiency in long-chained PUFAs. The growth-enhancing effect of lipids from *Scenedesmus obliquus* indicates that lipids from different food algae might be supplementary in their nutritional value for *Daphnia*. However, blooms of cyanobacteria under highly eutrophic conditions are poor in eukaryotic algae; therefore, the dietary deficiency of cyanobacteria can hardly be complemented by eukaryotic algal taxa under these field conditions. Correlative studies have suggested growth limitation by sestonic PUFAs in *Daphnia* in a mesotrophic (Müller-Navarra 1995) and in an oligotrophic lake (Wacker and Von Elert 2001). The presence of significant abundances of cyanobacteria in the natural seston implies lower levels or even the absence of both PUFAs and sterols, which improves the correlation of growth of *Daphnia* with sestonic PUFA levels, thereby suggesting a general importance of PUFAs at the algae/*Daphnia* interface (Müller-Navarra et al. 2000). Testing this with the model cyanobacterium *Synechococcus elongatus* strongly indicated that it is not a cyanobacterial deficiency in PUFAs, but rather a shortage in another lipid that might cause the weak control of cyanobacterial biomass by grazers. It remains to be seen whether this is due to the absence of sterols in cyanobacteria.

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Calanoid copepods versus cladocerans: Consumer effects on protozoa in lakes of different trophic status

Abstract—Through their consumption of protozoa, cladocerans and copepods link classical food chains and microbial food webs in aquatic ecosystems. Published results of studies of the effects of these metazooplankton on protozoa in lakes allow few generalizations to be made. To determine if general patterns exist along a trophic gradient, we measured the effects of cladocerans and calanoid copepods on heterotrophic nanoflagellates (HNF) and ciliates in four lakes that ranged from ultraoligotrophic to eutrophic using the same methodology. Copepods (*Boeckella* spp.), and to a lesser extent cladocerans (*Daphnia, Ceriodaphnia*), had significant negative effects on the growth of protozoa, and the rates at which both groups cleared protozoa from the water were higher in nutrient-poor conditions than in nutrient-rich conditions. In two oligotrophic lakes, calanoid copepods ingested HNF at biomass-specific rates that were 2.2 times higher than those of cladocerans. Rates of ciliate ingestion by copepods, relative to cladocerans (top-down effects on the ciliate community), increased with lake productivity from 2.5 times higher in an ultraoligotrophic lake to 9.5 times higher in a mesotrophic lake. Our study shows that copepods are more effective consumers of protozoa than cladocerans, particularly in eutrophic conditions.

Protozoa are integral components of the microbial food webs in lakes and the sea where they provide trophic links between primary producers, bacteria, and the metazoan zooplankton. The major protozoa in these aquatic systems are generally ciliates and heterotrophic flagellates (HNF). It is now well established that cladocerans and calanoid copepods will consume HNF and ciliates (e.g., Stoecker and Capuzzo 1990; Burns and Gilbert 1993; Pace and Vaqué 1994), but most studies of the top-down effects of metazooplankton on microbial food webs have focused only on one lake (e.g., Carrick et al. 1991; Wiackowski et al. 1994; Havens and Beaver 1997; Wickham 1998; Adrian and Schneider-Olt 1999; Jürgens et al. 1999), only on one group of protozoa (e.g., Wiackowski et al. 1994; Gasol et al. 1995; Havens and Beaver 1997; Herbst 1998), or only on *Daphnia* as consumers (e.g., Pace and Funke 1991; Jürgens et al. 1994; Pace et al. 1998).

The effect of cladocerans and copepods on populations of protozoa in lakes in these studies is inconsistent. For example, cladocerans appeared to control HNF in some eutrophic and mesotrophic lakes (Jürgens et al. 1994; Pace and Vaqué 1994; Herbst 1998) but had no detectable effect on the growth of HNF in other lakes (Pace and Vaqué 1994; Burns and Schallenberg 1998). In mesotrophic Schöhsee, North Germany, *Daphnia* reduced the ciliate community significantly in early summer, but had no effect on ciliates 1 month earlier (Wickham 1998). Calanoid copepods reduced the growth of HNF in eutrophic Lake Biwa, Japan (Nagata et al. 1996), and in some oligotrophic lakes (Carrick et al. 1991; Burns and Schallenberg 1998), but not in other oligotrophic and mesotrophic lakes (Jürgens et al. 1994; Burns and Schallenberg 1996; Carrias et al. 1998). Cyclopoid copepods had little or no effect on ciliates in eutrophic Lake Okeechobee (Havens and Beaver 1997) but had strong effects in mesotrophic Schöhsee (Wickham 1998) and a hypertrophic lake in Denmark (Jürgens et al. 1999). Differences in methods, experimental design, and data analysis reduce the extent to which the results of these studies can be compared and the conclusions generalized.

Although calanoid copepods and cladocerans occur in most lakes, there have been only a few comparisons of their consumer effects on microbial food webs. In studies of the short-term effects of *Daphnia* and calanoid copepods on protozoa in mesotrophic lakes in New Zealand and Germany, the negative effect of copepods on ciliates was much stronger than that of *Daphnia* (Burns and Schallenberg 1996; Adrian and Schneider-Olt 1999). In contrast, *Daphnia rosea* was as effective as the copepod, *Diaptomus novamexicanus* in depressing ciliate growth in a short-term study in Castle Lake, California (Brett et al. 1994; Wiackowski et al. 1994).

Numerous factors have the potential to influence the effect of cladocerans and copepods on planktonic microbial food webs. Among them are the diversity, the biomasses and production of microorganisms, and the feeding behaviors and biomasses of the crustacean zooplankton. Our aim was to compare the consumer effects of cladocerans and calanoid copepods on protozoa in lakes of different trophic state to determine if general patterns exist that might be related to microbial or phytoplankton biomass. Our study developed from a more intensive investigation of the effects of nutrients and crustacean zooplankton on microbial food webs of lakes