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Phosphorus limitation of *Daphnia* growth: Is it real?

Jotaro Urabe,[1] *Jessica Clasen, and Robert W. Sterner*
Department of Ecology, Evolution, and Behavior, University of Minnesota,
1987 Upper Buford Circle, St. Paul, Minnesota 55108

Abstract

The possibility of P limitation for zooplankton growth has many implications for understanding changes in production efficiency and feedback dynamics between consumers and resources. However, there have been no direct tests to determine whether the putative P limitation is real. To answer this question, we directly supplied inorganic P to *Daphnia magna* apart from food algae, *Scenedesmus acutus,* and then examined changes in body mass. During the period from birth to age 6 d, *D. magna* were fed on live algae for 19 h and placed in water of high inorganic P (4 mM: P treatment) for 5 h each day. We used P-free water as a control treatment. Growth rate estimated from initial and final body mass during the 6-d incubation was significantly larger in the P treatment than in the control treatment when *Daphnia* fed on P-deficient algae, whereas a significant difference was not detected between the treatments for *Daphnia* fed on P-sufficient algae (C : P atomic < 300). The results clearly demonstrate that *Daphnia* growth is in fact limited by P itself when they feed on P-deficient algae.

Recently, controversy has sparked over the possibility of direct P limitation for zooplankton production. Several studies have demonstrated lower individual and population growth rates of *Daphnia* when they feed on algae with low P content relative to carbon, i.e. high C : P ratios (Sommer 1992; Sterner 1993; Sterner et al. 1993). Using mass-balance models, Olsen et al. (1986) and Urabe and Watanabe (1992) estimated the threshold food C : P ratio, above which net production of cladocerans is limited by P content rather than C in the food. In both these studies, the threshold was calculated to be ~300 (atomic ratio) for *Daphnia,* although the ratio changes according to food concentration and digestibility of carbon in the food (Hessen 1992; Urabe and Watanabe 1992) and because of changes in the proportion of metabolic cost (respiration) within assimilated carbon (Sterner and Robinson 1994; Sterner 1997). Seston has C : P ratios >300 in many north temperate lakes (Hecky et al. 1993; Elser and Hassett 1994; Sterner et al. 1997), implying that P limitation of *Daphnia* growth may be common.

Direct P limitation of zooplankton growth, however, has been called into question (Brett 1993; Müller-Navarra 1995*a, b*) in part because of the lack of direct evidence showing whether P is in fact the actual substance limiting the growth rate of *Daphnia.* Although direct demonstrations of the precise limiting factors for zooplankton feeding on low-quality foods have been lacking, several studies suggest that the growth rate of *Daphnia* is affected by the availability of individual long-chain unsaturated fatty acids such as EPA (eicosapentaenoic acid, 20 : 5ω3) and DHA (docosahexaenoic acid, 22 : 6ω3) (Ahlgren et al. 1989; Müller-Navarra 1995*b*). Fatty acids such as these are essential substances for most animals. In some specific instances (Müller-Navarra 1995*b*), but perhaps not in all, fatty acids may be correlated with P content in algae. Thus, the alternative hypothesis has been suggested that the observed lowered growth rate of *Daphnia* fed on P-limited algae is due to a correlated deficiency of these essential biochemicals rather than due to the P itself. Because elemental limitation of zooplankton production has many implications in ecological processes such as the success of a zooplankton species in a given environment, feedback dynamics between consumers and resources, nutrient cycling, and ecological transfer efficiency (Sterner and Hessen 1994; Urabe 1995; Urabe and Sterner 1996), it is important determine whether elemental limitation for zooplankton growth occurs. This study was designed to answer the question whether P limitation is real or not.

A variety of indirect approaches to testing for dietary limitation are possible and have been used in zooplankton and elsewhere. Indirect approaches include correlations between animal growth rate and concentration of chemical substances in the food (P: Sterner 1993; fatty acids: Müller-Navarra 1995*b*). A direct experimental approach is to control completely for diet but to arrange for an experimental group of animals to receive an additional supplement of only a single dietary substance hypothesized to limit growth. Differences in growth between this experimental group and a control group can then be taken to be direct evidence that a given single substance indeed limits the growth rate. This direct approach has been utilized for example by microencapsulation techniques used in studies of growth limitation in benthic invertebrates (Kreeger and Langdon 1994). Unfortunately, inorganic P is difficult to encapsulate successfully due to its high solubility in water. In the present study, we supplied inorganic P in solution apart from food algae directly to *Daphnia* feeding either on P-deficient or P-sufficient food. If the growth of *Daphnia* feeding on P-deficient food is stimulated by supplemental P additions, but animals feeding on P-sufficient food show no similar response, this should be taken as direct evidence for mineral P limitation of *Daphnia* growth.

[1] Present address: Center for Ecological Research, Kyoto University, Shimosakamoto 4-1-23, Otsu 520-01, Japan.

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To introduce supplemental P into the experimental *Daphnia,* we capitalized on the known ability of these animals to incorporate inorganic P directly into their bodies from the surrounding water when that P is in higher concentration than normally found in nature (Parker and Olsen 1966). Our design was such that animals fed on a cultured algal diet for most of the day, but for a period of several hours each day, animals were placed in treatment water either lacking or containing a high concentration of dissolved inorganic P. The precise mechanism of uptake of P from the dissolved phase in *Daphnia* is in some doubt, but may involve "anal drinking" (Peters 1987), uptake through thoracic limbs (Parker and Olsen 1966), or uptake by gut-resident bacteria and subsequent digestion. To ensure that feeding activities remained the same during the entire 24-h day, we supplied heat-killed algae that did not take up P while animals were in the treatment water. Thus, *Daphnia* maintained food collection and digestion throughout the experimental periods.

Methods

Food and animals—Scenedesmus acutus was cultured in chemostats using medium MPI (Sterner et al. 1993). Three type of *Scenedesmus* cells were used for experiments: moderately N limited (MON), severely N limited (LON), and severely P limited (LOP). These cells were produced by adjusting the N:P ratio in the influent medium together with the turnover rate of the chemostat reaction vessel. Details of these algal culture protocols and composition of these foods were described elsewhere (Sterner 1993; Sterner and Smith 1993; Sterner et al. 1993) and so are not repeated here. Most cells in this species occur as unicells in all growth conditions. Nevertheless, all colonies are within the size range of particles efficiently cleared by *Daphnia* (Sterner and Smith 1993). According to previous work (Sterner et al. 1993), MON algae are ranked as superior food for *Daphnia,* supporting high growth rate, while LOP algae are ranked as the lowest quality food.

In experiments, we used the artificial growth medium, COMBO (S. Kilham unpubl.), without any N and P compounds. We will call this P- and N-free growth medium basal combo medium (BCM). Because the pH of BCM as initially prepared was >10, we always adjusted its pH to <8 by titration with HCl.

For experiments, algae were harvested daily from the chemostat outflow vessel, centrifuged, rinsed with BCM, and then used as food. Cell concentration was determined by hemocytometer count. Heat-killed algae were prepared using LOP cells. To make heat-killed algae, LOP cells were concentrated into 15-ml test tubes with BCM. Test tubes were put into 70°C water for 15 min. The suspension was then cooled to room temperature, centrifuged, and rinsed by BCM. Microscopic observation indicated no morphological changes due to heat treatment. To check P uptake ability, dense concentrations (2×10^5 cells ml^{-1}) of live and heat-killed algae were placed into COMBO medium containing 3.1 μM P and changes in SRP concentration in the medium were monitored (Fig. 1). In contrast to live LOP cells, dissolved P concentration did not show a significant difference between time 0 and 21 h in the medium with heat-killed LOP cells (two-tailed *t*-test, $P < 0.05$), indicating that the heat-killed algae had no P uptake ability.

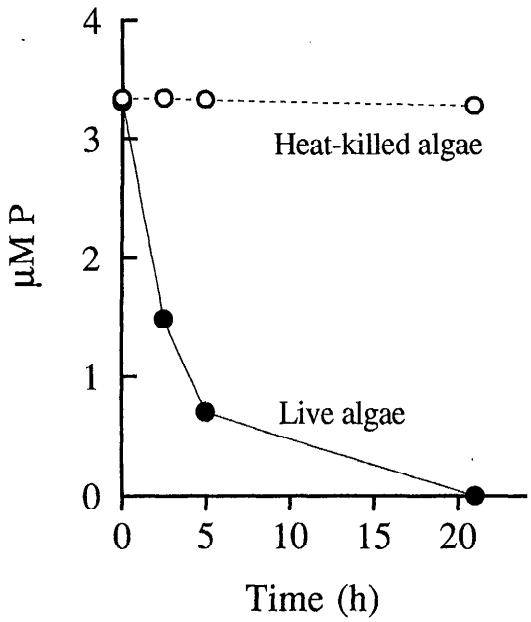

Fig. 1. Temporal changes in SRP concentrations in containers with live and heat-killed algae.

A strain of *Daphnia magna* kept under laboratory conditions in filtered lake water for >5 yr was used in experiments. A separate stock culture was established and renewed routinely by transferring neonates once every 1–2 weeks. Saturating amounts of MON algae were always supplied as food. One day before each experiment, 5–10 mature individuals carrying late-stage embryos in their brood pouch were transferred to 1-liter glass bottles containing BCM. Neonates born within 24 h from these mothers were used in the experiments.

Experimental protocol—Each experiment was carried out at 20°C with the same general procedure but with differences in the type of live algae used as food and in the type of treatment water (Table 1). To check repeatability, Exp. 1–4 were performed at different times in duplicate by different researchers (a and b). To start each experiment, neonates from the same mothers were randomly distributed to four 1-liter tissue roller bottles containing BCM (pH < 8) with appropriate concentration of live algae. Each bottle received 10 neonates. Another 10–20 neonates were used to estimate initial body mass (age 0 d). In each experiment except 2a, two bottles were used for P supplementation and the remaining two were used for control treatment (*see below*). Due to a temporary limitation of algal harvest, Exp. 2a was performed with only two bottles, one for each treatment. The experimental bottles were rotated horizontally at ~1 rpm to keep the algal suspension homogeneous.

During the 6-d incubations, *D. magna* were placed in beakers with 250 ml of treatment water with or without high P concentration for 5 h each day. We added 0.2 mg C $liter^{-1}$ of heat-killed *Scenedesmus* to the treatment water to stimu-

Table 1. Food source and combination of treatments in each experiment, and abundance and C:P ratio of algae in the bottle during experiments. For *Scenedesmus* cells used in experiments, LOP indicates severely P limited; MON, moderately N limited; and LON, severely N limited. HP indicates high P treatment, HC indicates control treatment with NaCl and KCl, LP indicates low P treatment, and BC indicates treatment without NaCl and KCl.

Exp.	Food source	Treatment	Algal abundance (mg C liter^{-1}) Bottle 1 $\bar{x} \pm$ SE	Algal abundance (mg C liter^{-1}) Bottle 2 $\bar{x} \pm$ SE	C:P atomic ratio Bottle 1 $\bar{x} \pm$ SE	C:P atomic ratio Bottle 2 $\bar{x} \pm$ SE
1a	LOP	HP	1.198±0.160	1.153±0.066	1,244±205	1,222±94
		HC	1.284±0.081	1.274±0.107	1,159±174	1,102±156
1b	LOP	HP	0.852±0.021	0.862±0.035	1,097±98	1,016±63
		HC	0.880±0.027	0.844±0.026	1,037±93	1,084±118
2a	LOP	HP	1.104±0.100		1,233±139	
		BC	1.182±0.130		1,360±175	
2b	LOP	HP	1.079±0.011	1.071±0.016	1,146±160	1,073±42
		BC	1.059±0.019	1.086±0.022	1,087±67	1,143±127
3a	MON	HP	0.670±0.138	0.590±0.076	104±37	94±5
		HC	0.699±0.209	0.592±0.040	103±31	92±22
3b	MON	HP	0.990±0.069	0.999±0.062	126±16	116±16
		HC	0.941±0.067	0.973±0.062	132±10	105±6
4a	MON	HP	0.661±0.051	0.665±0.054	98±14	102±6
		BC	0.629±0.055	0.751±0.108	114±9	113±13
4b	MON	HP	0.930±0.098	0.881±0.056	102±7	98±4
		BC	0.895±0.066	0.900±0.062	109±4	102±6
5	LOP	LP	1.312±0.211	1.256±0.207	1,249±183	1,375±122
		BC	1.342±0.208	1.279±0.279	1,201±159	1,393±201
6	LON	HP	0.787±0.047	0.725±0.253	233±8	238±27
		BC	0.788±0.072	0.782±0.228	220±15	240±37

late *Daphnia* to "drink" the treatment water. The treatment water was made with BCM. For the P treatment water, we dissolved NaH_2PO_4 and KH_2PO_4 (equimolar Na and K) into BCM to obtain the desired inorganic P concentration. The combination of these chemicals was chosen to keep the individual concentrations of Na and K as low as possible. To increase P uptake by *Daphnia,* it is desirable to use treatment water containing inorganic P as high as possible. Very high concentrations, however, may be toxic. Preliminary experiments indicated that all individuals survived without food for at least 24 h in the medium with ≤4 mM P. Therefore, we used BCM with 4 mM P (HP water) for P treatment except for Exp. 5 when BCM with 4 μM (LP water) was used for P treatment. As control water, NaCl and KCl (equimolar Na and K) was dissolved into BCM (HC water, total 4 mM Cl in Exp. 1 and 3; LC water, 0.4 μM Cl in Exp. 5). These chemicals were used to equalize Na and K concentrations and thus osmotic pressure to the P treatment water. To check for the effect of Cl^-, BCM without NaCl and KCl (BC water) was used as a control in Exp. 2 and 4. The pH of the treatment water was adjusted to ~7–8.

At each daily transfer, *Daphnia* were gently pipetted with <10 ml of water by a Komagome pipette (a wide-bore pipette) and rinsed in a series of four beakers containing 300 ml distilled water (P free) to remove live algae. *Daphnia* were then immersed in the beaker containing the treatment water. This procedure took less than 10 min and enabled us to reduce algal density to a negligible level without damaging the *Daphnia* individuals. After the 5-h treatment, *Daphnia* were rinsed with distilled water four times as before and replaced into the bottles containing fresh BCM and live algae. To check for the possibility of P contamination from the treatment water to the live algal suspension, we measured SRP concentration in the water used for rinsing. In every case, four rinses were enough to reduce dissolved P contamination from P treatment water to the algal feeding suspension to an undetectable level (Fig. 2).

No individuals released offspring during the 6-d incubations. The body growth rate (g; d^{-1}) of *Daphnia* was, therefore, estimated as follows:

$$g = \ln(B_6/B_0)/6,$$

where B_0 and B_6 are body mass at age 0 d and 6 d. Because the age of maternal individuals from which the neonates were obtained differed among the experiments, and because the body mass of neonates depends on the size and instar of maternal individuals, initial body mass (B_0) was not the same between the experiments, being ~6.8–14.3 μg ind.$^{-1}$.

Chemical content of algae and body mass of Daphnia—At each daily transfer, aliquots of the algal suspension were collected from the experimental bottles and filtered onto preignited acid-rinsed GF/F filters after first removing *Daphnia* individuals. Particulate C was measured with a Perkin-Elmer model 2400 CHN analyzer. Particulate P was determined by the ascorbate-reduced molybdenum-blue method

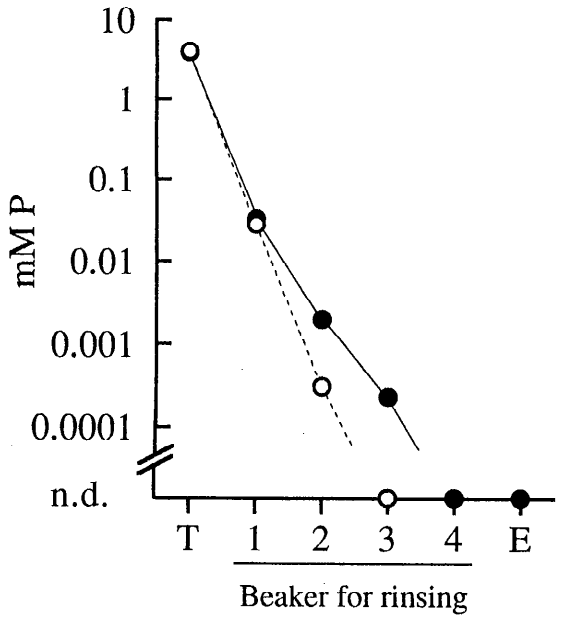

Fig. 2. Two examples of changes in SRP concentrations during the transfer process of *Daphnia* from P treatment water (T) to feeding suspension with live algae (E) through four serial rinses with P-free water. SRP concentration below detectable level is denoted by n.d.

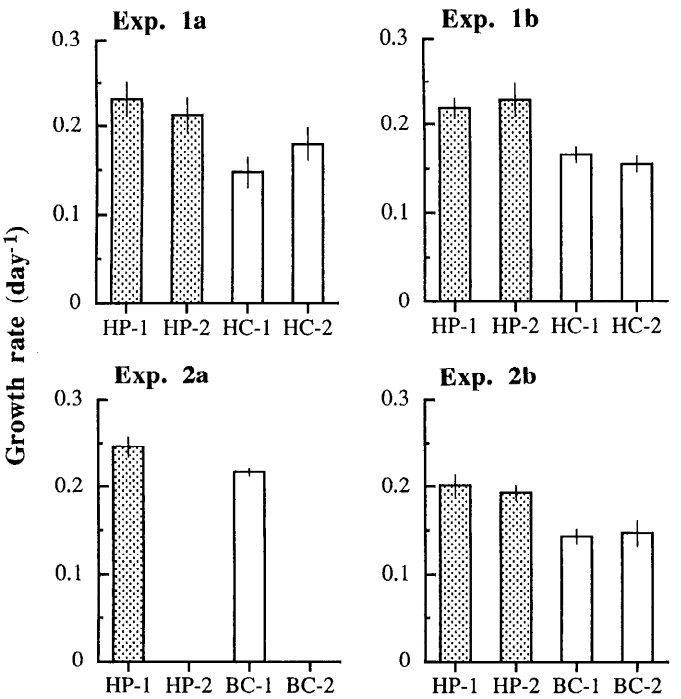

Fig. 3. Mean growth rate of *Daphnia* within bottles in the experiments with severely P-limited (LOP) algae. The vertical bar indicates 1 SE (across individuals within bottles). HP, high P treatment (4 mM P); HC, control treatment with NaCl and KCl; and BC, control treatment without NaCl and KCl. Statistical results are given in Table 2 and indicate significant differences in *Daphnia* growth in HP vs. control treatments. Furthermore, the type of control and the identity of researcher did not have a significant effect. Error bars indicate relatively little variation within bottles compared to between treatments.

after digestion with potassium persulfate at 120°C for 30 min. At the end of the experiment, *Daphnia* were removed and placed individually onto small squares of aluminum foil. Animals were dried at 60°C overnight, stored in a desiccator, and weighed (±0.2 μg) with a Mettler UMT2 microbalance equipped with an antistatic pad.

Data analysis—To overcome possible "pseudoreplication" error and to check the effects of researchers and type of control treatments (HC and BC), we performed three-way nested ANOVA both for experiments with LOP algae (Exp. 1 and 2) and MON algae (Exp. 3 and 4) using average body mass in each bottle as independent data. Here, effect of P amendment was nested within effect of the researchers, which was in turn nested within effect of the type of control treatments. Variability of individuals within bottles is presented graphically along with the means but is not used in hypothesis testing. Both abundance and C:P ratio of algae were also examined statistically by the nested ANOVA after log transformation to stabilize variances.

Results

Neither abundance nor C:P ratio of algae was significantly different within and between treatments in all experiments for a single food type ($P > 0.05$: Table 1), implying that any differences in body growth rate of *Daphnia* between treatments could not be attributable to food regimes. Although we planned to set algal abundance to ~1 mg C liter^{-1}, it was lower than this level in some experiments due to limited daily algal harvest from chemostat cultures when these experiments were conducted. As in the previous studies (Sterner et al. 1993), the atomic C:P ratio of LOP algae exceeded 1,000, while that of LON was ~230, and that of MON was near the Redfield ratio (106) (Table 1).

In experiments using LOP algae and high P treatment water (Exp. 1 and 2), a significant difference in growth rate was apparent between the P treatments (Fig. 3, Table 2). Growth was significantly larger in the P treatment than in control treatments regardless of osmotic pressure adjustment (i.e. the type of control treatment). The growth rate of *Daphnia* fed on LOP algae was improved 30% by immersing them into high P-treatment water for 5 h per day. In contrast, no significant difference was detected in the growth rate within and between treatments in the experiments with MON algae (Exp. 3 and 4) (Fig. 4, Table 2). For both experiments with LOP and MON algae, neither researchers nor the type of control treatments had a significant effect on growth rate. Phosphorus supplementation for 5 h per day did not entirely make up for the low quality of LOP algae. On average, the growth rate was two times higher in *Daphnia* fed on MON algae than in LOP algae (Table 3). The growth improvement with P amendments for individuals fed on P-deficient algae corresponds to 23% of the overall difference in the growth rate between P-deficient and P-sufficient algae.

In Exp. 5 when LOP algae were used but P concentration in the treatment water was reduced by a factor of 1,000 (4

Table 2. Results of nested ANOVA for average growth rate in experiments with severely P-limited (LOP) algae (Exp. 1 and 2) and moderately N-limited (MON) algae (Exp. 3 and 4). n.s. is probability larger than 0.05.

Source of variance	Experiments with LOP algae				Experiments with MON algae			
	df	MS	*F*	*P*	df	MS	*F*	*P*
Type of control	1	0.0002	0.07	n.s.	1	0.00035	2.98	n.s.
Researchers	2	0.0025	1.03	n.s.	2	0.00012	2.67	n.s.
Treatments	4	0.0025	12.5	<0.005	4	0.00004	0.14	n.s.
Within treatments	6	0.0002			8	0.00030		

μM P), *Daphnia* did not respond to the treatment water (Fig. 5). Likewise, in Exp. 6 when LON algae was used as food, *Daphnia* did not respond to P treatment water (Fig. 5), indicating that additional P supply had no effect on the growth rate of *Daphnia* fed on algae with C : P ratio of 230.

Discussion

The answer to the question posed in our title is "yes." *Daphnia* feeding on P-deficient algae do have growth stimulated by supplementing their intake of inorganic P, but animals feeding on algae with a C : P ratio <230 show no such response. Thus, the experiments clearly demonstrate that the growth rate of *Daphnia* feeding on algae with low P content was limited by P itself. In other words, phosphorus itself can determine food quality for *Daphnia*. Our results are in good agreement with the prediction from the estimated threshold C : P ratio (300) below which *Daphnia* growth is no longer limited by P in the food (Olsen et al. 1986; Urabe and Watanabe 1992).

In our experiments, NaCl and KCl were added to the control water in some experiments in order to equalize osmotic pressure to that in the P treatment. One may suppose that differences in the growth rate between the P and control treatments would be due to a deleterious effect of chloride ions rather than stimulation effect of P. However, in experiments using MON algae, a significant difference was not detected between the treatments even with chloride in the control water. In addition, we found that growth rate in the P treatment was significantly higher than in the control treatment with or without chloride whenever LOP algae was used as food. These results indicate that the difference in the growth rate of *Daphnia* between the treatments cannot be attributable to a potential deleterious effect of chloride ions.

Another error source would be changes in P content of algae. In the case of heat-killed algae, however, such a change in chemical composition was avoided, because they did not take up P even under high P supply (Fig. 1). In addition, we rinsed *Daphnia* individuals with P-free distilled water at every daily transfer. Thus, there was no chance for live algae to take up P during the incubation with *Daphnia*. Also note that elemental composition of live algae, which was measured using samples collected for daily replacement, did not significantly differ between the treatments in any experiment (Table 1).

Direct P uptake at high concentration by *Daphnia* is not surprising. One earlier study (Parker and Olsen 1966) described that *Daphnia* can directly take up ^{32}P-phosphate from water and locate it within ovaries and muscles within 30 min. The present study further indicates that sufficient inorganic P was taken up directly from surrounding water to

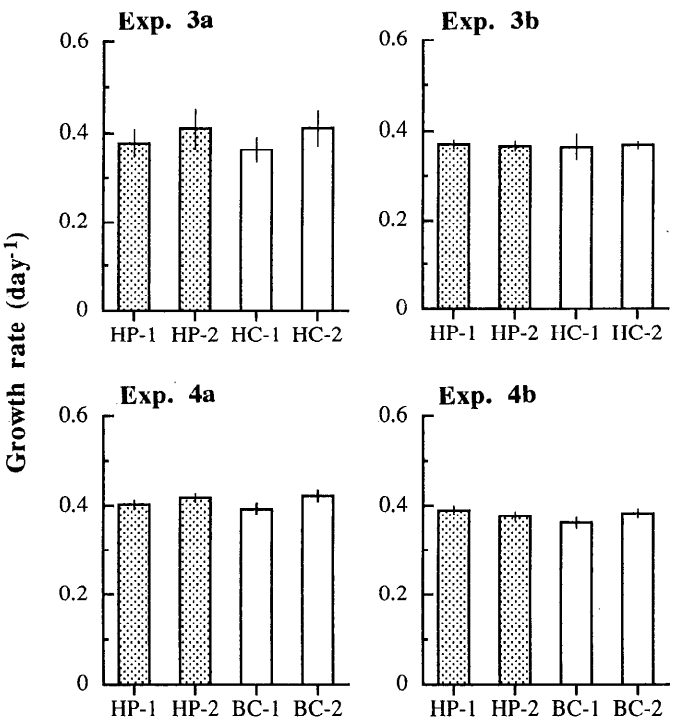

Fig. 4. Mean growth rate of *Daphnia* within bottles in the experiments with moderately N-limited (MON) algae. The vertical bar indicates 1 SE. Statistical results are given in Table 2 and indicate lack of significance of either type of control, researcher, or treatment. Abbreviations are as in Fig. 3.

Table 3. Mean growth rate of *Daphnia* in high P (HP) and control (HC and BC) treatments.

Exp.	Food source	Treatment: High P water $\bar{x} \pm$ SE	Treatment: Control $\bar{x} \pm$ SE
1 and 2	LOP	0.219±0.007	0.167±0.010
3 and 4	MON	0.390±0.004	0.385±0.006

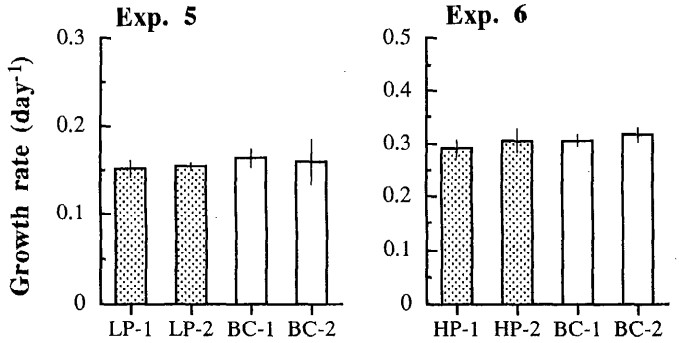

Fig. 5. Mean growth rate of *Daphnia* in the experiments with low P treatment (4 μM P, Exp. 5) and with severely N-limited (LON) algae (Exp. 6). The vertical bar indicates 1 SE. LP, low P treatment; HP, high P treatment; and BC, control treatment without NaCl and KCl.

lessen (but perhaps not remove) the growth limitation of *Daphnia*. In our experiments, heat-killed algae (LOP) were supplied to stimulate *Daphnia* to try to allow the animals to take up the treatment water together with food and by taking it through their gut wall. The gut wall, however, may or may not be the location where *Daphnia* can absorb phosphate. Parker and Olsen (1966) gave evidence that *Daphnia* can take up phosphate from thoracic limbs. We do not know whether any food particles are necessary for *Daphnia* to uptake inorganic P efficiently from water.

Although *Daphnia* can directly take up inorganic P when at high concentration, such an ability is of little if any use in nature at typical P concentrations. In our experiment using low P water (4 μM P, Exp. 5), growth rate was very similar in the P treatments compared to controls, indicating that direct P uptake depends on high P concentration, as demonstrated earlier by Parker and Olsen (1966). It should be noted that even our low P concentration in Exp. 5 is still higher than that in most natural lakes. Although it may be argued that *Daphnia* might respond to low concentrations of P if they were exposed to them for longer periods than used in our experiments, algae in nature have high C:P ratios when their growth is severely limited by P, where dissolved inorganic P is generally lower than the limit of detection by usual chemical analysis. It is therefore implausible that *Daphnia* compensate for P deficiency in their food by taking P directly from the lake water, as pointed out already (Urabe and Watanabe 1993).

Based on correlative evidence, Müller-Navarra (1995*b*) suggested that EPA (20:5ω3), one of several essential fatty acids, was determining the nutritional quality of P-limited algae for zooplankton, and concluded that direct P limitation of *Daphnia* growth was unlikely even when algal food had high C:P ratios. Müller-Navarra (1995*b*) attributed the difference in growth rate of *Daphnia* fed on P-sufficient and P-deficient *S. acutus* to relatively small differences in EPA content between these algal cells. Because we used the same algal strain as used in the experiments by Müller-Navarra (1995*b*), P-deficient algae (LOP) in the present study should also have low EPA. Nonetheless, supplemental supply of inorganic P promoted the growth rate of *Daphnia* fed on P-deficient algae, providing definite and direct evidence that P is in fact involved directly in the low food quality of LOP algae. The most direct method of determining which substances limit animal growth is to examine the response of animals when a target substance is separately supplied (as emphasized by Brett 1993). Comparative evidence of growth stimulation of *Daphnia* fed on P-deficient food by supplementation with a single fatty acid is lacking.

Although the growth rate of *Daphnia* fed on LOP algae was stimulated by inorganic P supplement, the improved rate was still lower than the growth rate in the experiments with MON algae (Table 3). This discrepancy opens up a set of possible explanations. One possibility is that "P deficiency" was not entirely overcome by our experimental design. Although phosphorus concentration in the high P-treatment water is much higher than that in natural lakes, it is still lower than P density in algal cells, which is ~0.0002 as the volume-specific P content (Urabe and Watanabe 1993). Thus, the treatment water in our experiments was a dilute P source for *Daphnia* compared with their normal algal P source. Furthermore, note that by necessity the period when animals were placed in the high P-treatment water was limited to only 5 h per day, leaving the bulk of the day to feed upon living algae. Even if given a large dose of P for part of a day, could the *Daphnia* make full use of it? We simply do not know if there is an animal-equivalent to "luxury uptake" seen in algae, and in fact the overall homeostasis of P content in metazoans argues that storage of P is slight at best. The growth improvement with P amendments in the experiments with LOP algae (23%, comparing the overall difference between P-stressed and P-saturated environments) was similar to the fraction of time spent in the high P-treatment water (21% of the day). This coincidence may suggest that the overall difference in the growth rate between animals fed on LOP and MON algae was due to the total availability of P in the 24-h cycle.

A second explanation is that the overall difference in the growth rate of animals fed on MON algae and LOP algae was not due to P deficiency alone. If animal growth is strictly Liegibian, only one substance at a time is truly limiting (i.e. the animal will respond to single additions of only one substance and not others). Under Liebigian growth, individual dietary substances are not substitutable. We can speculate that once P limitation is removed, some other dietary factors related with P deficiency, such as essential fatty acids, limit animal growth. For such a reason, *Daphnia* fed on LOP algae might not show the growth rate as high as those fed on MON algae even when P limitation is removed. Naturally, we can expect varying degrees of substitutability of different substances, but to what extent P and biochemicals are substitutable is not known.

A third possibility is that inorganic P and digestion resistance interact synergistically to determine food quality. Chlorophyte algae such as *Scenedesmus* produce very thickened cell walls under P limitation, and these thick cell walls inhibit digestion (van Donk and Hessen 1993; van Donk et al. 1997). Responses of *Daphnia* feeding on LOP algae, however, are not identical to those of animals feeding on low quantities of food because *Daphnia* feeding on LOP algae store larger quantities of lipid (Sterner et al. 1992). This finding implies that the digestion resistance alone is not

a sufficient explanation for low growth rate of *Daphnia* fed on P-deficient algae. It is possible, however, that digestion resistance adds to the unavailability of P in LOP algae or makes some other component(s) of the food less available (van Donk et al. 1997).

Our results should not be construed to mean that P is always limiting *Daphnia* growth. The most important point in stoichiometric models (*see* e.g. Kooijman 1995) is to provide criteria predicting where and when limiting factors are important. When the algal C:P ratio is below ~300 (as in MON food in the present study), P content should be unimportant in determining nutritional quality (Olsen et al. 1986; Urabe and Watanabe 1992). Under P sufficiency, the nutritional quality of algae may depend on the relative biochemical composition such as fatty acids and amino acids. In the Schöhsee, *Daphnia* growth rate was found to be more tightly correlated with fatty acids than P (Müller-Navarra 1995*a*). Notably, algal production in this lake is only weakly limited by P, and the C:P ratio of edible seston is always <300 (Sommer 1988). Unimportance of P as a dietary factor in this lake is consistent with stoichiometric growth models. The light environment may also affect food quality. For example, we found a significantly lower growth rate of *Daphnia* when they fed on P-sufficient *S. acutus* (C:P ratio, 100) cultured under low light compared to *S. acutus* with the same C:P ratio but grown under high-light condition (Urabe and Sterner 1996).

Another criticism regarding the possibility of P limitation of zooplankton has been that it is not common in nature for the C:P ratio of the resource to be higher than the threshold elemental ratio of consumer (Brett 1993). Evidence has accumulated, however, that a C:P ratio of seston >300 is in fact common in the epilimnion in many lakes (Hecky et al. 1993). Elser and Hassett (1994) found that sestonic C:P ratios were >300 in more than 70% of 36 temperate lakes they visited. In Canadian shield lakes, *Daphnia* growth rate was much lower in lakes with sestonic C:P ratio >300 (Mackay and Elser in prep.) and expected increases of *Daphnia* population following a decrease in planktivorous fish were not found in a lake with a high sestonic C:P ratio (Elser et al. unpubl.). These results further suggest that P limitation is one of the factors in determining the success of a zooplankton species in a given environment. It remains, however, to make direct field tests on the possibility of P limitation for zooplankton growth.

In conclusion, our results provide the first direct experimental demonstration of the importance of a single dietary factor in determining the quality of P-deficient algae. As we have seen, though, many more questions remain about the role of other factors and about food quality for herbivorous zooplankton in nature.

References

AHLGREN, G., L. LUNDSTEDT, M. BRETT, AND C. FORSBERG. 1989. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. J. Plankton Res. **12:** 809–818.

BRETT, M. T. 1993. Comment on "Possibility of N or P limitation for planktonic cladocerans: An experimental test" (Urabe and Watanabe) and "Nutrient element limitation of zooplankton production." Limnol. Oceanogr. **38:** 1333–1337.

ELSER, J. J., AND R. P. HASSETT. 1994. A stoichiometric analysis of the zooplankton–phytoplankton interaction in marine and freshwater ecosystems. Nature **370:** 211–213.

HECKY, R. E., P. CAMPBELL, AND L. L. HENDZEL. 1993. The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. Limnol. Oceanogr. **38:** 709–724.

HESSEN, D. 1992. Nutrient element limitation of zooplankton production. Am. Nat. **140:** 799–814.

KOOIJMAN, S. A. L. M. 1995. The stoichiometry of animal energetics. J. Theor. Biol. **177:** 139–149.

KREEGER, D. A., AND C. J. LANGDON. 1994. Digestion and assimilation of protein by *Mytilus trossulus* (Bivalvia: Mollusca) fed mixed carbohydrate/protein microcapsules. Mar. Biol. **118:** 479–488.

MÜLLER-NAVARRA, D. 1995*a*. Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. Arch. Hydrobiol. **132:** 297–307.

———. 1995*b*. Biochemical versus mineral limitation in *Daphnia*. Limnol. Oceanogr. **40:** 1209–1214.

OLSEN, Y., K. M. VARUM, AND A. JENSEN. 1986. Dependence of the rate of release of phosphorous by zooplankton on the C:P ratio in the food supply, as calculated by a recycling model. Limnol. Oceanogr. **31:** 34–44.

PARKER, R. A., AND M. I. OLSEN. 1966. The uptake of inorganic phosphate by *Daphnia schødleri* Sars. Physiol. Zool. **39:** 53–65.

PETERS, R. H. 1987. Metabolism in *Daphnia*, p. 193–243. *In* R. H. Peters and R. DeBernardi [eds.], *Daphnia*. Memorie Dell'istituto Italiano di Idrobiologia. Consiglio Nazionale Delle Richerche.

SOMMER, U. 1988. Does nutrient competition among phytoplankton occur in situ? Int. Ver. Theor. Angew. Limnol. Verh. **23:** 707–712.

———. 1992. Phosphorus-limited *Daphnia:* Intraspecific facilitation instead of competition. Limnol. Oceanogr. **37:** 966–973.

STERNER, R. W. 1993. *Daphnia* growth on varying quality of *Scenedesmus:* Mineral limitation of zooplankton. Ecology **74:** 235–2360.

———. 1997. Modeling interactions of food quality and quantity in homeostatic consumers. Freshwater Biol. **38:** in press.

———, J. J. ELSER, E. J. FEE, S. J. GUILDFORD, AND T. H. CHRZANOWSKI. 1997. The light:nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and function. Am. Nat. In press.

———, D. D. HAGEMEIER, R. F. SMITH, AND W. L. SMITH. 1992. Lipid-ovary indices in food-limited *Daphnia*. J. Plankton Res. **14:** 1449–1460.

———, ———, ———, AND ———. 1993. Phytoplankton nutrient limitation and food quality for *Daphnia*. Limnol. Oceanogr. **38:** 857–871.

———, AND D. O. HESSEN. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annu. Rev. Ecol. Syst. **25:** 1–29.

———, AND J. L. ROBINSON. 1994. Thresholds for growth in *Daphnia magna* with high and low phosphorus diets. Limnol. Oceanogr. **39:** 1228–1232.

———, AND R. F. SMITH. 1993. Clearance, ingestion and release of N and P by *Daphnia obtusa* feeding on *Scenedesmus acutus* of varying nutritional quality. Bull. Mar. Sci. **53:** 228–239.

URABE, J. 1995. Direct and indirect effects of zooplankton on seston stoichiometry. Ecoscience **2:** 286–296.

———, AND R. W. STERNER. 1996. Regulation of herbivore growth by the balance of light and nutrients. Proc. Natl. Acad. Sci. USA **93:** 8465–8469.

———, AND Y. WATANABE. 1992. Possibility of N or P limitation

for planktonic cladocerans: An experimental test. Limnol. Oceanogr. **37:** 244–251.

———, AND ———. 1993. Implications of sestonic elemental ratio in zooplankton ecology; reply to the comment by Brett. Limnol. Oceanogr. **38:** 1337–1340.

VAN DONK, E., AND D. O. HESSEN. 1993. Grazing resistance in nutrient-stressed phytoplankton. Oecologia **93:** 508–511.

———, LÜRLING, D. O. HESSEN, AND G. M. LOKHORST. 1997. Altered cell wall morphology in nutrient deficient phytoplankton and its impact on grazers. Limnol. Oceanogr. **42:** 357–364.

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