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MINERAL LIMITATION OF ZOOPLANKTON: STOICHIOMETRIC CONSTRAINTS AND OPTIMAL FORAGING

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Abstract. Nutritional deficiencies are a very common phenomenon, and consumers generally face food that is not optimally suited for their needs. Especially herbivores are habitually confronted with food of inferior quality, usually a result of too-low nutrient concentrations in plant material. Waterfleas of the genus *Daphnia* are good model organisms to study the effect of inferior quality food, and how animals deal with this. We tested the effect of algae to which we had given different phosphorus contents on both life history and feeding parameters of *Daphnia magna*. Phosphorus content of the algae strongly affected both the growth rate and the feeding activity of the daphniids. Feeding activity increased with declining food quality (increase in C:P ratio of the algae), whereas growth rates were maximal at intermediate C:P levels. We conclude that the direct limitation of phosphorus is a very important factor determining food quality for zooplankters. Daphniids counterbalanced lower P content of their food by spending more C (energy) on acquiring this limiting resource. This implies that when *Daphnia* are given phosphorus-limited food both the addition of phosphorus and the addition of carbon (energy) should increase the growth rate of the animals (co-limitation). The influence of the phosphorus content of the food on the feeding activity of *Daphnia* offers a mechanistic explanation for the observed homeostasis in daphniids.

Key words: co-limitation, nutrient; Daphnia; *food quality; model; nutritional deficiency; optimal foraging; stoichiometry; trade-off.*

INTRODUCTION

All secondary producers face the problem of nutritional imbalances. Food is mostly taken up in packages and not as single nutrients. Since prey items in general do not have the same nutrient requirements as their predators, food is regularly of sub-optimal quality. More specifically, in the relationship between plants and herbivores, nutrients such as nitrogen and phosphorus are often limiting, as there is a surplus of carbon in plants (White 1993). Obviously, this carbon is needed as an energy source, and hence it will dissipate through the food chain, but it implies that many consumers will have more problems meeting their nutrient requirements than their energy requirements. Moreover, the variation in the specific nutrient content of autotrophs is generally larger than the variation in herbivores (e.g., Sterner et al. 1998), a variation arising from differences in the relative balance between light and nutrients (Sterner et al. 1997), or the nutrient richness of the habitat (Vitousek 1982).

In aquatic systems, the issue of nutritional quality and its effect on consumers has received a lot of attention in recent years. Especially the link between primary producers and herbivores is particularly well studied, focusing mainly on algae and microcrustaceans (see Hessen 1992, Sterner and Smith 1993, Gulati and DeMott 1997 [and papers therein], Urabe et al. 1997, van Donk et al. 1997, DeMott 1998, DeMott et al. 1998, Sterner and Schulz, 1998). In most freshwater systems, phosphorus is the limiting nutrient for phytoplankton growth, and hence most studies focused on the effect of limitation of this element (e.g., Elser and Hassett 1994), but also nitrogen has received attention (Butler et al. 1989, Groeger et al. 1991). Freshwater systems show most of the characteristics described above. The carbon to phosphorus (C:P) ratio of algae (seston) in lakes shows considerable variation (Sterner et al. 1998), whereas the C:P ratio of zooplankters is lower (i.e., they have more P relative to C), and much more constrained (Hessen 1990, Hessen and Lyche 1991). Consequently, the zooplankton—phytoplankton interface is very well suited for the study of the effects of nutrients on food quality. Many aspects of phosphorus limitation in zooplankton were investigated using the waterflea, *Daphnia*, as a model organism. Most researchers observed that growth of *Daphnia* fed P-limited food sources was lower compared to *Daphnia* with high-phosphorus food. As true filterfeeders, daphniids have only limited potential to select for higher quality food (DeMott 1986, Butler et al. 1989). This makes them very susceptible to potential changes in the ambient food quality. Nevertheless, daphniids maintain a lower and more constant C:P ratio than other zooplankters (Hessen and Lyche 1991). Although a number of researchers have tried to establish why different zooplankton species have different specific C:P

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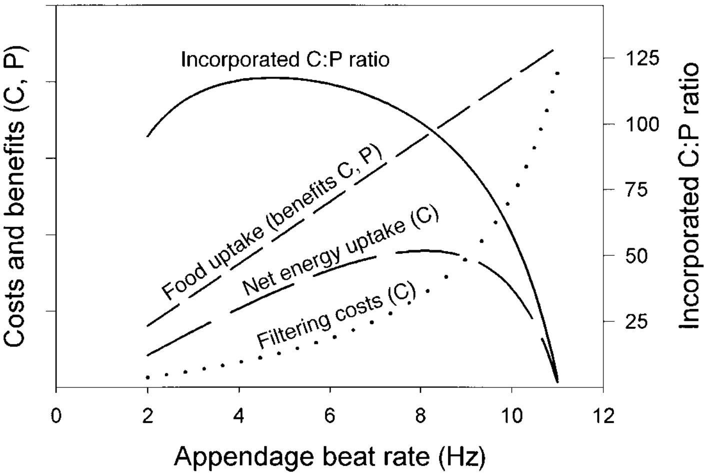

FIG. 1. Theoretical model, describing the dependence of food uptake in *Daphnia* and the costs to take up this food as a function of the appendage beat rate. The net C uptake can be computed as the difference between these two curves. Under the assumption that the P-uptake efficiency does not change, this results in different incorporated C:P ratios for the animal. Hence, surplus energy is channeled into taking up the limiting resource, P, thus reducing the C:P ratio of the animal.

ratios, to our knowledge no reports exist explaining the mechanisms of selective uptake of different elements by *Daphnia* and the resulting internal state of homeostasis.

Traditionally, an animal's internal state is believed to control its behavior, but an animal could also use its behavioral repertoire to control its internal state (Krebs and Davies 1993). Many consumers selectively feed on high energy or high nutrient food particles or a specific combination thereof (e.g., Belovsky 1978, DeMott 1993). Since daphniids do not have the possibility of such selective feeding, they can only change the intensity of their feeding behavior, by changing their filtration rate. Most studies on feeding behavior of *Daphnia* have reported maximum filtration rates at low food levels, while ingestion rates were believed to be constant and maximal at higher food levels (Rigler 1961, Muck and Lampert 1980). Obviously, this leaves no room for behavioral variation of feeding activity. Recently, however, it was shown that feeding behavior of *Daphnia* is not congruent with simple maximal feeding in responses to food quantity (Plath 1998). Daphniids actively altered their feeding activity, adapting to changes in their food supply. We postulate that feeding behavior in *Daphnia* may be sensitive not only to food quantity but also to food quality. Therefore, changes in feeding activity may potentially influence the homeostasis of the daphniids.

Stoichiometry theory predicts that zooplankton growth and nutrient recycling are tightly coupled with the resource nutrient ratios (Sterner and Hessen 1994, Elser et al. 1996). Consumers should release much of the nutrients present in excess, while retaining most of the limiting nutrient. This implies that the quality of a certain resource is determined solely by its nutrient ratio compared to the requirements of the consumer, and that the homeostasis of consumers presents a useful approach in understanding growth rates under various food quality conditions encountered in natural habitats. In this paper, we investigated whether stoichiometry theory can explain the differences in growth under different nutrient ratios of the food, by establishing the growth of *Daphnia magna* fed algae with different C:P ratios. Moreover, we investigated how behaviorally mediated changes in feeding activity may serve as mechanisms adjusting the C:P ratio of ingested food and thus offer a possible mechanistic explanation for homeostasis in *Daphnia.* As food is gathered with filter screens of the 3rd and 4th trunk appendages, feeding activity of a daphniid can be quantified by measuring the beat rate of the filtering appendages (Fryer 1991), which has been used by many authors (McMahon and Rigler 1963, Burns 1968, Haney et al. 1995, Lampert and Brendelberger 1996, Plath 1998). Unlike the measurement of ingestion rate, which is an interaction of food concentration and feeding activity, appendage beat rate is directly linked to the feeding effort of single animals.

The potential influence of the feeding activity on the incorporation ratio of C:P can be visualized by a simple model (Fig. 1) and is based on a few assumptions, which are described in detail by Lehman (1976) and Plath (1998). The first assumption is a linear increase in food uptake with increased feeding activity. Secondly, we assume a nonlinear increase in energy cost with increasing feeding activity (Lehman 1976). As long as these assumptions are met, there will be an optimal type curve for the net C uptake, independent of the exact shape of the curves. In the example (Fig. 1), the maximal net C uptake is at an appendage beat rate of about 8 Hz (maximum difference between food uptake and the filtering costs). P uptake is correlated with food uptake. The relation between net C uptake and P uptake results in the relative C:P incorporation ratio, which, in this example, is set to 100 at the specific feeding activity of 8 Hz. Starting from this point, an increase in feeding activity would lead to an increase in food ingestion but in return to a decrease in net C uptake. Provided that P uptake remains coupled with food uptake, relatively more phosphorus would be ac-

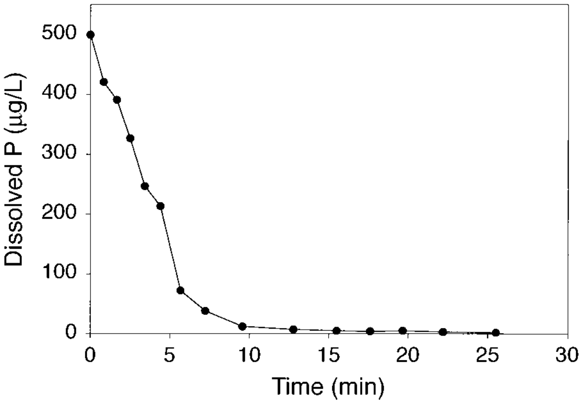

FIG. 2. Dissolved P in aquatic medium containing phosphorus-limited *Scenedesmus obliquus.* P is taken up rapidly and almost absent in the medium after 15 min.

quired, i.e., the C:P incorporation ratio would decrease. If phosphorus is the limiting resource this increased feeding activity could lead to an enhanced growth, by allocating surplus energy, i.e., carbon, to foraging. Hence, from this model we predict that when supplied with food of inferior quality (high C:P ratio) daphniids should increase their appendage beat rate to be able to take up more phosphorus.

MATERIALS AND METHODS

The *Daphnia magna* clone used in this study, originally collected from a pond in Frankfurt, Germany, was kept in the laboratory for many years. All experiments, except for the behavioral experiments, were carried out in an artificial, phosphorus-free medium (ADaM) (Klüttgen et al. 1994). Behavioral experiments were carried out in filtered Schöhsee lake water (0.45-μm filter). Juvenile animals were collected from a stock culture, and placed individually in 200-mL containers. They were fed a suspension of non-phosphorus-limited *Scenedesmus obliquus* (1 mg C/L at 20°C). Media were changed daily. The day–night cycle consisted of 16 h light and 8 h of darkness. Third brood juveniles of these animals were collected and served as the experimental animals.

When grown under different nutrient conditions, not only the nutrient ratio of the algae but also their biochemical composition changes (Müller-Navarra 1995*a*, Boersma 2000). Moreover, for some green algae it has been demonstrated that they also changed their morphology (van Donk et al. 1997). Therefore, when investigating the direct effect of P limitation it does not suffice to culture algae under different nutrient limitations, and feed them to the daphniids. Hence, we cultured all of our algae under the same conditions, and shortly before these algae were supplied to the daphniids, they were pulsed with different amounts of phosphorus (DeMott 1998, Boersma 2000). This approach enabled us to investigate the sole effect of phosphorus limitation on *Daphnia. S. obliquus* was cultured in Z/4 Medium (Zehnder and Gorham 1960), with reduced phosphorus content (from 1.395 mg P/L to 83.7 μg P/L) in semicontinuous cultures. Phosphorus-limited algae took up phosphorus very rapidly (Fig. 2) (see also Rothhaupt 1995, DeMott 1998). Therefore, we added the phosphorus-limited algae to the *Daphnia* media, and these media were subsequently enriched with different concentrations of a K_2HPO_4 solution. Algal media were kept in the dark to avoid photosynthesis and growth of the algae in the food media. Algal media were changed daily. Previous experiments (Boersma 2000) have shown that the C:P ratio of phosphorus-limited *Scenedesmus* changed rapidly as a consequence of the P uptake, but that the biochemical composition, measured as the fatty acid spectrum of the algae, changed much more slowly. Hence, the only measurable difference between algae that did vs. did not receive a phosphorus pulse was their P content. The phosphorus-limited algae were supplied with six different P pulses: 0, 4, 8, 16, 32, and 280 μg/L. Fig. 3 shows the resulting C:P ratios of the algae after 24 h. Even in the treatment that did not receive any dissolved phosphorus, we observed a decrease in the C:P ratio of the algae, thus creating algae as food for the daphniids, with C:P ratios ranging from >600 to 30. All C:P ratios in this paper are given as molar ratios.

In order to investigate at which C:P levels phosphorus was actually limiting growth, we added an emulsion rich in highly unsaturated fatty acids (HUFAs), especially EPA (eicosapentenoic acid) and DHA (docosahexenoic acid), which are also believed to be limiting under certain conditions (Müller-Navarra 1995*b*). This emulsion was a standard emulsion made available through the International Council for the Exploration of the Sea (ICES) working group on mass rearing juvenile fish. Every day fresh emulsions of ICES 30/0.6/C, supplied by INVE Technologies (Gent, Belgium)

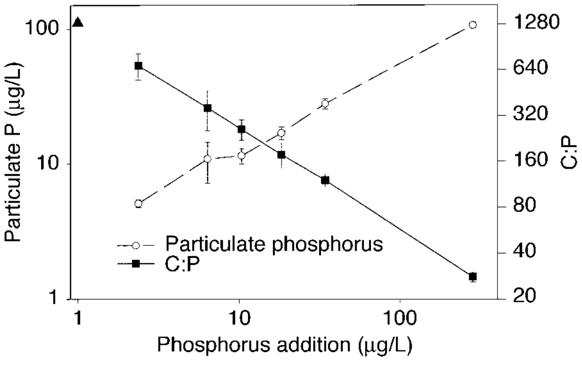

FIG. 3. Particulate P (open circles) and algal C:P ratios (solid squares) of phosphorus-limited *Scenedesmus obliquus,* exposed to different concentrations of dissolved phosphorus (K_2HPO_4), and kept overnight in *Daphnia* medium. All the axes are on a log scale, and the added phosphorus includes the particulate P originally in the algae. The solid triangle indicates the C:P ratio of the original P-limited cells, before exposure to the *Daphnia* medium.

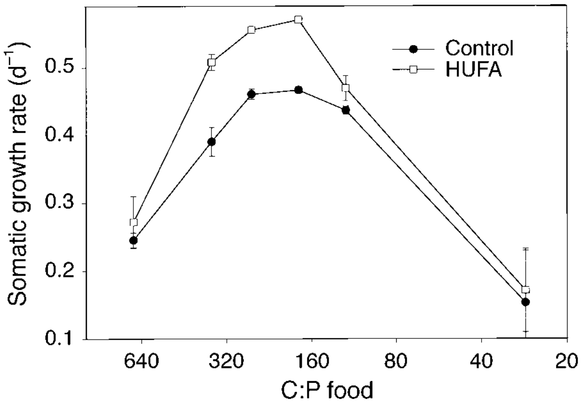

FIG. 4. Growth rates of *Daphnia magna* fed algae with different C:P ratios, and with (open squares) and without (filled circles) the addition of emulsions of highly unsaturated fatty acids (HUFAs). Error bars indicate ±1 SE. Duncan's multiple-range test indicated that differences between the HUFA treatments and the controls are significant for the C:P ratios between 150 and 400 only.

(Coutteau et al. 1996, Boersma 2000) were prepared by adding 0.5 mg wet mass of emulsion (0.4 mg C) per liter of *Daphnia* feeding suspension. Analysis of the particle size showed that the emulsion particles were in the range of 1–2 μm, but that they were readily taken up by daphniids (see also Weers and Gulati 1997). The six different C:P treatments were crossed in a full factorial design with the emulsion treatments (controls and emulsions added), yielding twelve different treatments. The carbon content of the algal cultures was checked daily before preparing the feeding suspensions: 5–10 mL were filtered through precombusted GF/C filters, and analyzed for carbon using a LECO carbon analyzer (LECO Corporation, St. Joseph, Michigan). Total algal carbon content of the media supplied to the daphniids was 1 mg C/L. For details of the fatty acid content of the emulsions and of the algae see Boersma (2000). All treatments were carried out with seven replicates.

Third-brood neonates produced by the mothers of the experimental animals were collected within 12 h of birth and placed in 120-mL flow-through vessels, with a flow rate of 1 L/d. Every flow-through vessel contained three animals, from which one was harvested after 6 d for determining the growth rates, and one could be used for the analysis of the appendage beat rate. Measurement of feeding activity was only carried out for animals without the addition of the fatty acid emulsions. Initial body mass of the animals was established by grouping four neonates from each clutch at the start of the experiment, while 6-d-old animals were analyzed individually. For the analysis of dry mass, animals were transferred to small aluminum weighing boats, dried for 24 h at 60°C, stored in a desiccator, and weighed to the nearest 0.1 μg using an electronic microbalance. Somatic growth rates were computed and analyzed in a two-way analysis of variance with algal phosphorus content and emulsion treatment as the independent variables.

The feeding behavior of the daphniids was established by measuring the appendage beat rate of animals taken from the different C:P treatments. Where possible, two animals randomly chosen from different flow-through vessels were analyzed for each phosphorus treatment. They were transferred to a beaker containing 200 mL of particle-free filtered water (<0.45 μm), to ensure that animals displayed maximal beat rates during subsequent measurement. After 20 min, individuals were fixed by the back of their carapace to a cover slide with a drop of Vaseline white petroleum jelly, transferred into an observation chamber, and placed in an upright position as described in Plath (1998). Infrared light from behind was used for observation of the beating trunk appendages. A through flow of particle-free filtered water was established and the appendage beat rate was recorded for 20 min. Following the first measurement without food, appendage beat rate was recorded for another 20 min at a food concentration of 0.5 mg C/L of non-P-limited *S. obliquus.* All animals received the same treatment in order to compare the effect of growing conditions on the animals only. Nonlimited *Scenedesmus* were used to ensure that all of the experimental animals were exposed to different conditions than those in the growth experiments.

RESULTS

Daphnia showed a strong response to algae with different C:P ratios. Growth was low when the P content of algae was low (C:P > 500). Moreover, also at the very low C:P ratios we observed low growth of daphniids (Fig. 4). An increase in growth rates with the addition of the fatty-acid emulsions was significant in the intermediate range of algal C:P (Duncan's multiple-range tests). There was no significant interaction between P treatment and the addition of the fat emulsions (Table 1, Fig. 4).

Not only the growth of the daphniids was affected by the algae, but also the C:P ratio of the animals changed. In fact, the C:P ratio of the animals decreased monotonically over the range of C:P ratios we tested (Fig. 5). No significant effect of the emulsion treatment on the C:P ratio of the animals was observed.

We observed large differences in appendage beat rate

TABLE 1. Analysis of variance for somatic growth rate of *Daphnia magna* with fatty-acid emulsion type and phosphorus content of algal food as independent factors.

Source	MS	df	*F*	*P*
P treatment	0.2495	5	57.4	<0.001
Emulsion	0.0812	1	18.7	<0.001
Emulsion × P	0.0060	5	1.4	0.241
Error	0.0043	66		

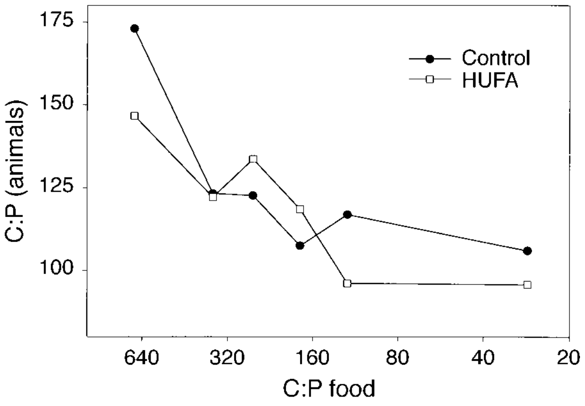

FIG. 5. C:P ratios of *Daphnia* cultured under different conditions, with (open squares) and without (solid circles) the addition of emulsions of highly unsaturated fatty acids (HUFAs).

for animals, which were fed with algae of different quality. While daphniid growth rates were highest at intermediate C:P ratios, highest appendage beat rates could be observed at the lowest food quality, i.e., the highest C:P ratios (Fig. 6). With the exception of the single animal at the lowest C:P ratio, the mean appendage beat rates measured after food addition were all lower than without food (Wilcoxon matched-pairs test, $P < 0.001$). This indicates that the feeding activity measured without food represented the maximal beat rates for each animal (see also Plath 1998). Feeding activity, estimated by maximal beat rates, was not correlated with body size or growth rate but was rather negatively correlated with food quality. This implies that with decreasing food quality daphniids spent more energy on filtering.

DISCUSSION

The effect of nutrient limited food on zooplankters was intensively studied in the past (Hessen 1992, Sterner and Smith 1993, Elser et al. 1996, Urabe et al. 1997, DeMott et al. 1998). However, to date, investigations have been hampered by the fact that changes in the nutrient availability of the algae alter a whole suite of characteristics at the same time. This study provides the first test of the effect of different sestonic C:P ratios on zooplankton growth, where exclusively the phosphorus content of the algae was changed. Only the approaches of DeMott (1998), Urabe et al. (1997), and Boersma (2000) tried to discriminate between direct and indirect phosphorus-limitation effects as well, but these studies were carried out with only two different treatments, low and high phosphorus availability. Essentially, all of the published reports (see Sterner and Schulz 1998) found similar results: daphniids cultured under P-limited conditions showed poor growth. It has been argued that these lower growth rates on P-limited algae are a result of other characteristics of the algae, such as an increase in cell wall thickness and an ensuing increase in digestion resistance (van Donk et al. 1997), or a decrease in the content of highly unsaturated fatty acids (Müller-Navarra 1995*a*). Our results convincingly show that direct limitation of mineral phosphorus plays an essential role in the determination of food quality (Fig. 4). In our experiments, growth was limited by phosphorus, a nonsubstitutable resource. By changing only the P concentration, one would expect growth to increase linearly with increasing P content of algae. In accordance with Liebig's Law of Minimum (von Liebig 1855), growth should level off after a certain P concentration is reached. At that point, growth should be limited by another resource and additional phosphorus would no longer enhance growth. Our data are, however, not consistent with this prediction. At the lower phosphorus concentrations, both the addition of phosphorus and the addition of fatty acids increased the growth rates of the daphniids. Moreover, growth of *D. magna* individuals did not level off, but was max-

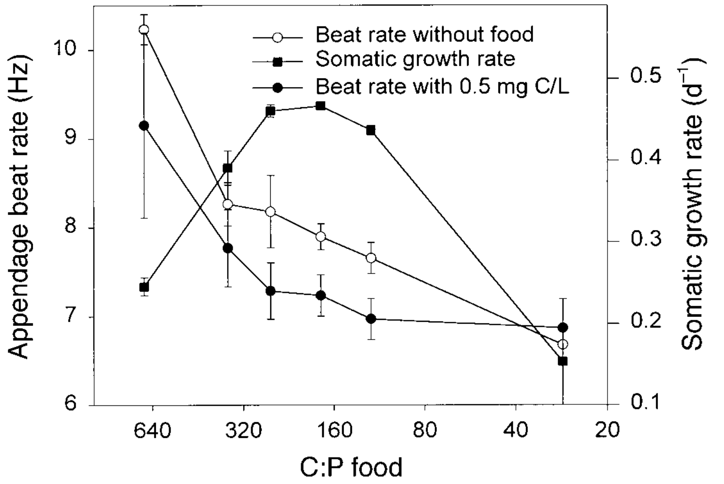

FIG. 6. Feeding-appendage beat rates of *Daphnia magna* cultured under different phosphorus limitations, and somatic growth rates (solid squares) of the animals cultured without the addition of fatty acid emulsions. Displayed are maximal *Daphnia* feeding-appendage beat rates measured without food (open circles) and beat rates in the presence of *Scenedesmus* algal food constituting 0.5 mg C/L (solid circles).

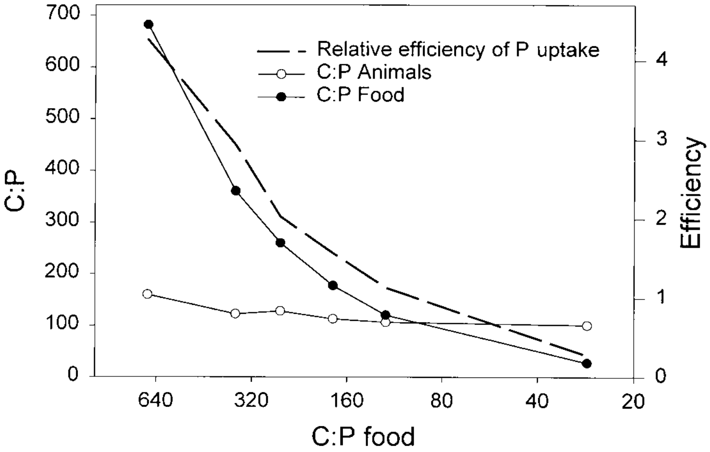

FIG. 7. C:P of the *Scenedesmus* algal food (solid circles), C:P of the *Daphnia* (open circles), and the relative efficiency of the P uptake. At the highest C:P ratio of the food the C:P ratio of the animals is >4× lower than the food; at the lowest C:P ratio of the food the C:P ratio of the *Daphnia* is actually higher.

imal at intermediate P levels and declined at higher P concentrations.

A decrease in growth at very high nutrient levels is not unusual. Brewer et al. (1985, 1987) found in experiments with the western budworm (*Choristoneura occidentalis*) feeding on white fir (*Abies concolor*) and Douglas-fir (*Pseudotsuga menziesii*) that the level of nitrogen available is a major influence on the success of the larvae, but that larval performance fell significantly when they were fed needles with very high levels of nitrogen. Similar patterns were observed for grasshoppers (Joern and Behmer 1998), mites (Toko et al. 1996), and corn borers (Saito 1996). Possibly, aberrant growth of the host plants (Brewer et al. 1985, 1987) can explain this phenomenon. In our study, we can exclude this, however, since all the algae were grown under the same nutrient conditions, but several alternative explanations are possible. The addition of the P salts could have caused osmotic stress, although this is highly unlikely. The added salts represented less than 1% of the total salt content in the medium and a change in conductivity of the media could not be observed. Furthermore, *Daphnia magna* as a common inhabitant of saline rock-pools (Bengtsson 1986, Teschner 1995) is reasonably salt tolerant (Teschner 1995). Alternatively, high P levels could be toxic to daphniids. This also is rather unlikely, since growth already started to decrease at an intermediate P level, and Urabe et al. (1997) added even higher quantities of dissolved phosphorus without observing any negative effect. Moreover, equivalent high P levels are regularly found in eutrophic lakes, which nevertheless contain high densities of daphniids (e.g., Jeppesen et al. 1997).

An alternative explanation for the decrease in growth rates at high phosphorus concentrations could be that the level of phosphorus was above a presumed optimum for daphniids, an explanation suggested by Archer et al. (1982) for aphids feeding on sorghum. In fact, growth rates started to decline when the C:P ratio of animals was actually higher than that of their food (Fig. 7). Since daphniids normally live in an environment where the C:P ratio of the food is higher than their own, it seems unlikely that they will ever encounter P-saturated food. In a persistently P-limited environment, adjusting the feeding effort directly to the phosphorus contained in the food could be a fast and efficient way to optimize the uptake of this limiting resource (c. f. Slansky and Feeny 1977). In that case, carbon content of the food would only play a minor role in adjusting feeding, and the available phosphorus could directly control the animals feeding effort. However, this mechanism would fail if phosphorus were present in excess. At high phosphorus levels, it could lead to a reduction in net C incorporation, by the reduction of feeding effort (i.e., shifting appendage beat rate left of the optimum in Fig. 1). Indeed, the observed appendage beat rate frequency of animals adapted to high-P-content algae supports the hypothesis. Appendage beat rates were comparable to those of animals adjusted to low food concentrations of P-sufficient *Scenedesmus obliquus* (7 Hz at 0.1 mg C/L, typical C:P ratio of 250), much lower than the 10 Hz observed for animals acclimated to 1 mg C/L of P-sufficient *Scenedesmus* (Plath 1998). Hence, when feeding on food with high phosphorus concentrations, animals would take up less food than possible.

Independent of the explanation, these results demonstrate that excessive phosphorus addition could lead to a reduction in growth. We expect that the ecological importance of this reduction is low, since in limnetic habitats food is usually phosphorus limited, with C:P ratios usually between 150 and 1000 (Hecky et al. 1993, Elser and Hassett 1994, Sterner et al. 1998). Only Hessen and Faafeng (2000) and de Lange and Arts (1999) reported lower values, but they gave no further information on zooplankters present in this lake. Since these extreme values are rarely observed, further discussion will focus on the lower P concentrations, which seems to be the predominant situation encountered by zooplankters.

In the literature, zooplankters are often presumed to be homeostatic, i.e., they have almost unchanging nutrient ratios irrespective of the nutrient ratios of their food (e.g., Hessen 1990). A growing body of evidence exists, however, that this is not the case (Lehman and Naumoski 1985, Sterner et al. 1993), although most of these studies were not very conclusive. Recently, DeMott et al. (1998) showed that a change in C:P ratio of the food caused a change in the C:P ratio of the daphniids, ranging from ~80 to 120. In this study, we also observed a change in the specific phosphorus content of the daphniids, with C:P ratios in the animals ranging from 100 to 150 (Fig 5). However, as was argued by DeMott et al. (1998), the range of C:P values in the animals is still much smaller than in their food. Hence, the foundations of the stoichiometry models are still supported. Since the nutrient composition of the daphniids is relatively constant (Fig. 6), the effective incorporation rates of carbon and phosphorus must change with different food quality. The increase in effective relative P incorporation efficiency could entirely result from an increased efficiency in P uptake from the food. This, however, would be in contradiction with the results of DeMott et al. (1998), who observed a slight decrease in the P-assimilation efficiency with an increase in C:P ratio of the food. In any case, if an increase in P-uptake efficiency would be responsible for the relatively constant C:P ratio of the animals, then growth rates should remain constant, as animals would take up the same amounts of the limiting nutrient P, while C was constant in all treatments. Since this is clearly not the case, it is more likely that there are costs involved in maintaining homeostasis. Any type of metabolic cost would lead to a higher relative P-incorporation efficiency, because the net C-incorporation efficiency would decrease.

When supplied with food of high C:P ratios, daphniids displayed a high feeding activity (Fig. 6). This will have led to higher ingestion rates, resulting in decreased gut passage times. Feeding more quickly on food of inferior quality seems a common phenomenon (e.g., Reynolds 1990). For example, the larvae of the white butterfly, *Pieris rapae,* increased feeding when reared on plants with lower levels of nitrogen (Slansky and Feeny 1977). While this means a reduced efficiency at extracting nutrients, because not all of the available nitrogen in the food is fully digested, it is counteracted by passing more food through the gut in the same amount of time. The increase in feeding activity, which we observed for *Daphnia,* can thus explain the decrease in P-assimilation efficiency and in gross growth efficiency, as observed by DeMott et al. (1998), when daphniids were fed algae with very high C:P ratio. It could also explain why daphniids continue to excrete phosphorus, even when severely limited by phosphorus (Olsen et al. 1986, DeMott et al. 1998). It is, therefore, unlikely that the observed homeostasis in *Daphnia* is due to an increased P-uptake efficiency. Hence, the higher relative incorporation rate of P must be the result of a decreased net carbon incorporation. This decrease could originate from either lower carbon uptake, higher C-excretion, or higher respiration rates (see also DeMott et al. 1998). A decrease in net carbon incorporation could well be accounted for by the behavioral response, predicted by the model (Fig. 1), which we observed in the animals under different feeding conditions. Although several authors argued that the energy (carbon) expended on filtration is low (Brendelberger et al. 1986, Bohrer and Lampert 1988, Urabe and Watanabe 1990), others reported higher values (Gerritsen et al. 1988, Philippova and Postnov 1988, Fryer 1991). In any case, the fact that Philippova and Postnov (1988), Plath (1998), and others observed decreasing feeding activity of daphniids at low food concentrations evinces that there are considerable metabolic costs attached to filtering activity. Apart from the higher respiratory losses from the increased feeding activity (Kersting and van de Leeuw-Leegwater 1976), additional energy costs may arise from a greater loss of digestive enzymes into the gut (cf. Slansky and Feeny 1977).

Further support for the claim that the increased feeding activity causes the decrease in relative carbon incorporation comes from the treatments where the fatty acids were added. Although growth was clearly limited by phosphorus, it was higher in all treatments with fatty acids (Fig. 4). Highly unsaturated fatty acids are often essential resources themselves, when added to a food source that is poor in HUFAs. It is difficult to differentiate between these two effects, but the fact that we found poor growth on P-limited algae even in the presence of essential fatty acids suggests that the phosphorus content of the algae was more important than the effect of the fatty acid composition (see also Boersma 2000). Most likely, the added fatty acids served as an additional, easily accessible carbon source. This additional energy could have been used in the behavioral response, the higher appendage beat rates, and thus a higher uptake of phosphorus. It could explain why we found an increase in growth rates when we added phosphorus, as well as when we added fatty acids, and it further explains why the nonsubstitutable resource phosphorus seems to be substitutable in our experiments.

Even though the conceptual model (Fig. 1) did not incorporate any possible life history changes connected to the different food qualities, which may lead to differences in filter screens and mesh sizes (Lampert and Brendelberger 1996), it did predict the observed changes in feeding behavior of the daphniids. The model points out certain mechanisms that could explain both feeding parameters and growth rates of the daphniids. As such, it endorses the role of behavior in controlling the internal state of an animal. Behavioral adjustment to food quality could explain the fact that DeMott et al. (1998) observed a decrease in C-assimilation efficiency within 30 min of exposing daphniids to a P-deficient diet.

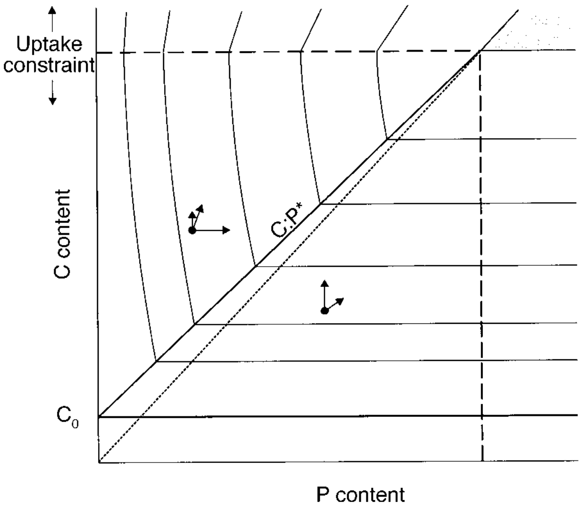

FIG. 8. Schematic shape of the *Daphnia* growth rate isoclines for different food P content and C content estimated from the model (Fig. 1). The C:P* line denotes the optimal C:P ratio for uptake. Below this line, growth is C limited. Above this line growth is co-limited by C and P. The shaded area marks maximal growth rates. C_0 is the threshold food concentration, which is equal to zero growth. Above the uptake constraint, growth rates are constant. The uptake constraint consists of a maximal uptake capacity for either volume or concentration and is therefore variable with the food type. The solid dots indicate a food concentration at the specific combination of C and P content, while the arrows indicate the expected change in growth rate by the addition of C (vertical arrow), P (horizontal arrow), or food quantity (diagonal arrow)

In the case of *Daphnia* feeding on P-limited food, we observed a trade-off between maximizing P and C uptake. As a combination of stoichiometry theory and optimal foraging theory, the model predicts that maximizing fitness would not be reached through a maximized uptake of either C or P but would rather lead to a behavioral feeding investment that optimizes the incorporation ratio. Thus, the valid currency of an optimal foraging model for *Daphnia* should be maximizing ingestion at an optimal C:P incorporation ratio (see also Reynolds 1990).

In the aquatic realm, many studies implied that there is a boundary of seston C:P ratio for transition between C and P limitation (e.g., Andersen 1997, Sterner 1997). However, a variable behavioral response will lead to a possible trading of surplus C (energy) for additional P uptake (resource). With increasing food concentration this co-limitation should occur over an increasing range of food quality (i.e., C:P ratio), thus leading to a plane of co-limitation. Within this plane, the addition of either C or P would lead to an increase in growth (Fig. 8). In our experiments, we observed an effect as it is expected by co-limitation. Growth of daphniids increased with the addition of P as well as with addition of energy (fatty acids). Our results suggest that P limitation is not necessarily more important at the higher food levels (Sterner 1997). Co-limitation of P and C will be of interest also at low food concentrations if food quality is severely limited by P content. The lower boundary of the plane and the impact of a behavioral response will depend on surplus energy, which will not be available at food levels around the threshold food concentration. At very high food concentrations, the effect of P limitation will be an increased ingestion rate leading to a decrease in gut passage time to optimize the assimilation efficiency of phosphorus. However, the gut passage time can only be decreased to a certain extent. After that, feeding activity can no longer be increased and surplus energy cannot be employed to increase P ingestion. This will lead to conditions where compensation for P limitation will no longer be effective, the upper boundary of the plane. At this point the animal's growth rate will truly be limited by P. The relevance of pure P limitation is difficult to determine. Since this scenario will only be encountered at high food quantities, it is likely that during most of their life cycle the animals will not encounter such high abundance of low-quality food.

In conclusion, we have shown that the growth rates of daphniids are strongly affected by the P content of the food and that daphniids change their feeding behavior in response to differences in food quality. We suggest that direct limitation by phosphorus is a very important factor determining food quality for zooplankters and the amount of available phosphorus could have a direct influence on the feeding effort. Daphniids could counterbalance at least partly the lower P content of their food by spending more C (energy) on acquiring this limiting resource. This implies that when given low amounts of phosphorus-limited food the addition of either phosphorus (resource) or carbon (energy) would increase the growth rate of the animals.

Our findings have implications for our understanding of nutrient cycling in lakes. As the excess carbon most likely mainly dissipates as carbon dioxide, it will not be available for other trophic levels, and hence we would not expect a higher bacterial biomass in phosphorus-limited systems, as hypothesized by Sterner et al. (1998). On the other hand, contrary to the prediction of Sterner and coworkers (1997), the higher feeding rates of zooplankters on poor-quality food would cause higher turnover rates of both phosphorus and carbon, because of the decreased efficiency of the consumers. Further analysis on the interaction of these two processes is needed to truly understand nutrient cycling in aquatic systems.

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