

LIFE AT THE EDGE: IS FOOD QUALITY REALLY OF MINOR IMPORTANCE AT LOW QUANTITIES?

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Abstract. There is increasing evidence that the quality of nutrient-limited algae is suboptimal for zooplankton production. These effects of nutrient limitation are supposed to be important mainly at higher concentrations of food because at lower quantities the overall energetic limitation of body growth should hide the effect of mineral limitations. This has been hypothesized in a variety of papers, but experimental evidence is still weak. In the present study we carried out a set of growth experiments investigating the effect of food quality at very low food levels ranging from 30 $\mu\text{g C/L}$ up to 150 $\mu\text{g C/L}$. In all of the experiments, the growth rates of *Daphnia magna* neonates were lower when grown on P-limited *Scenedesmus obliquus*. This effect disappeared when phosphorus was added to the P-limited algae prior to feeding, indicating that mineral limitation can occur even at very low levels of food.

Neonates born to mothers raised on either high- or low-P *Scenedesmus* were analyzed for body mass and lipid content as well as mass-specific phosphorus content to check for possible differential investment into neonates in different environments. Although mass-specific phosphorus content was higher in animals born from mothers grown on high-P algae, when fed low quantities of P-limited algae, growth rates of neonates born under low-P conditions were higher than those of animals born under high-P conditions. This can be explained by an increased body lipid content of low-P neonates, even though there were no differences in neonate body mass between treatments.

These results illustrate the importance of the incorporation of low food concentrations in ecological stoichiometry models.

Key words: *Daphnia*; food concentration; nutrient; phosphorus; stoichiometry; threshold concentration.

INTRODUCTION

Secondary producers generally take up their food in packages and not as single nutrients. As prey items normally do not have the same nutrient requirements as their predators, nutritional imbalances of the food occur regularly. This imbalance is particularly clear at the primary producer–herbivore interface, as plants contain a surplus of carbon relative to nitrogen or phosphorus, to the extent that animals are thought to be limited by nitrogen in many terrestrial and marine environments (Roman 1983, White 1993) and by phosphorus in freshwater systems (DeMott and Gulati 1999). Obviously, the surplus carbon will dissipate through the food chain as it is needed as an energy source, but the implication is that many consumers will have more problems meeting their nutrient demands than their energy requirements.

Ecological stoichiometry, the study of the balance of energy and multiple chemical elements in ecology,

has been applied successfully to explain differences in quality between different food sources, especially in aquatic systems (Sterner et al. 1998, Elser and Urabe 1999, Sterner and Elser 2002), whereas there has been little comparable work on terrestrial communities (Elser et al. 2000). In terrestrial systems mostly single nutrients have been used as a measure of foraging success, with carbon (energy) as the main currency in studies on vertebrate feeding behavior (Schoener 1971), and nitrogen as the main limiting nutrient considered by insect nutritional ecologists (e.g., Joern and Behmer 1998). Stoichiometry theory predicts that animal growth and nutrient recycling should be tightly coupled with 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, under the assumption that the consumer will try to maintain homeostasis in its own tissue. This coupling implies that the quality of a certain resource is determined solely by its nutrient ratio compared to the requirements of the consumer, and that organisms should be highly efficient in the uptake and retention of the limiting nutrient (e.g., Raubenheimer and Simpson 1998). Thus, theoretically, excretion of the limiting nutrient should approach zero as the car-

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bon : nutrient ratio increases in the food (Anderson 1992, but see Andersen 1997). The evidence to back up this assumption of potential 100% nutrient utilization efficiencies is, however, weak. Olsen et al. (1986) implied such a relationship in a model study of the cladoceran *Daphnia*, and Thingstad (1987) did the same for bacteria. The experimental evidence, however, is less clear. Sugiura et al. (2000) observed that trout raised under phosphorus-limited conditions did not excrete phosphorus, but marine copepods use nitrogen with an efficiency of only 40%, even when supplied with high C:N food (LeBorgne 1986, Anderson and Hessen 1995). In addition, many terrestrial organisms continue to release the limiting nutrient even under severe limitation (e.g., Slansky and Feeny 1977, Partridge et al. 1989). One reason for this continued release of the limiting nutrient might be the increase in feeding activity that is very often observed to occur on food of low nutrient content, and which results in a decrease in retention efficiency (Tabashnik 1982, Grimm 1988, Reynolds 1990), but excretion of the limiting nutrient under severe limitation also occurs (DeMott et al. 1998).

This excretion and suboptimal use of nutrients even when consumers are severely limited has several consequences for our understanding of the processes that play a role in the producer-consumer interface. It is generally accepted that, whereas carbon is required for both new biomass and energy, nutrient elements fulfill primarily a structural role. This implies that at food levels below the maintenance threshold, the mineral content of the food should not play a role, as materials for biomass gain are irrelevant under low food quantity, and the animals only need carbon (energy) for their maintenance (Hessen 1992, Urabe and Watanabe 1993). This consequence of the stoichiometry models has not been tested experimentally very often, and only once formally (Sterner and Robinson 1994). Kilham et al. (1997) observed quality differences between different food sources for *Daphnia* even at low food concentrations, as did Lampert (1977). In contrast, Vos et al. (2000) observed that energy content of the food seemed to be more important at lower food concentrations than mineral content in early instar larvae of the midge *Chironomus riparius*. Sterner and Robinson (1994) reported no differences in growth between *Daphnia magna* individuals fed very low concentrations of phosphorus-limited and phosphorus-sufficient algae, as did Rothhaupt (1995) in a study with the rotifer *Brachionus rubens*. Based on this, Sterner and Robinson concluded that indeed differences in mineral content of the food do not play a role when food concentrations are low. However, as the premise of the 100% retention efficiency of the stoichiometry models seems to falter, and animals still excrete considerable amounts of the limiting nutrient, there is no real theoretical ground to expect that the mineral content of the food should not be of importance at low food quan-

ties. The significance of limiting nutrients at low food levels is of vital importance for our understanding of maintenance metabolism (e.g., Kooijman 2000). Therefore it is essential to reassess the importance of mineral content differences at low food levels, especially since the study of Sterner and Robinson (1994) was based on relatively small sample sizes. Hence, the hypothesis we examine is that mineral limitation is not important to growth when food is scarce because energy is more important under these conditions, and basic energy-driven metabolic needs must be satisfied first. We investigate this using the cladoceran *Daphnia magna* as a model system.

One of the other premises in ecological stoichiometry theory to date is the strict homeostasis of the consumers. This homeostasis introduces the linearity in stoichiometric models (Kooijman 1995), which makes them mathematically simpler (Sterner 1997). However, even in aquatic systems, where stoichiometric approaches have been primarily used (Elser and Hassett 1994, Anderson and Pond 2000, Touratier et al. 2001), the homeostasis assumption does not seem to hold completely. Cladocerans (DeMott et al. 1998, Plath and Boersma 2001), copepods (Walve and Larsson 1999), and fish (Sterner and George 2000) all change the relative composition of their body tissue more or less dramatically when fed different food sources. Moreover, allometric differences, i.e., differences in the stoichiometry between animals of different sizes and ages, have also been found (DeMott et al. 1998, Sterner and George 2000), which suggests that there might be differential investment in somatic and reproductive growth. Although the subject of allocation to reproduction has been a topic of interest for many years in many organisms (e.g., Lack 1947, Smith and Fretwell 1974, Stearns 1976, Congdon and Gibbons 1987, Boersma 1997a, Glazier 2000), most of these studies focused on energy as the only available resource. The main interest was to explain how the available energy was divided up between and among clutches, and although there is a wealth of information available on the effect of differences in food quality supplied to mothers on investment in offspring, this is usually restricted to the number of eggs produced or the mass of individual eggs (e.g., Nager et al. 1997, Joern and Behmer 1998, Urabe and Sterner 2001). However, given the relaxation of the assumption of perfect homeostasis, we set out to investigate whether mothers do indeed supply their offspring with different amounts of nutrients based on their own feeding history, and also to assess the consequences of these differences for the fitness of their offspring.

MATERIAL AND METHODS

Growth experiments

In the first part of this study we investigated the resource-dependent growth of *Daphnia magna* neo-

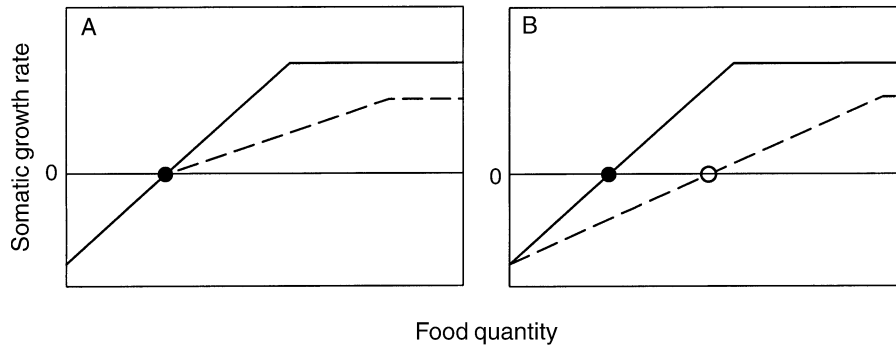


FIG. 1. Hypothetical dependence of body growth on food nutrient content and quantity (redrawn from Sterner and Robinson [1994]). Basic metabolic costs (threshold food concentrations; C_0) are indicated by circles (solid = high-nutrient food; open = low-nutrient food). (A) Identical values for C_0 should occur if only energy and no nutrients are required for maintenance metabolism. (B) Differences in threshold values are expected if the animals do require phosphorus even at very low food concentrations. Solid lines = high-nutrient food; dashed lines = low-nutrient food.

nates at very low levels of food of different specific phosphorus content. The daphniids originated from long-established stock cultures at the Max-Planck-Institute in Plön, Germany. During all phases of the experiments and the precultivation they were kept in an artificial, phosphorus-free medium (ADaM) (Klüttgen et al. 1994). Prior to each experiment, several females were raised separately in 200-mL jars from neonates to adults and fed P-sufficient green algae, *Scenedesmus obliquus* (formerly known as *S. acutus*), at concentrations >1 mg C/L. The daphniids were transferred to clean jars at regular intervals and third-brood neonates released within 24 h were used for the experiments. Neonates from several mothers were pooled and then distributed randomly to the experimental vessels, and a random subsample was taken to estimate initial mass. The experiments were carried out in a flow-through culture system similar to the one described by Lampert et al. (1988). Flow-through vessels with a volume of 120 mL were kept at 20°C in a water bath under continuous dim light. Fresh food suspensions were prepared daily and added to the vessels at a flow rate of 1 L/d. Food reservoirs were kept dark and stirred gently to avoid sedimentation of the algae. During the experiments, the animals were fed *S. obliquus* grown either on low- (83.7 $\mu\text{g P/L}$) or high- (1.395 mg P/L) phosphorus Z/4 medium (Zehnder and Gorham 1960) in semicontinuous chemostat cultures, with a dilution rate of ~ 0.1 d $^{-1}$. In order to establish the importance of the direct phosphorus limitation of algae, we had an additional treatment where we added dissolved phosphorus to the P-limited algae just before feeding them to the animals (DeMott 1998, Boersma 2000, Plath and Boersma 2001). Every morning, the particulate organic carbon (POC) content of the chemostat suspensions was measured by filtering an aliquot on GF/F filters. These filters were dried for 2 h at 100°C and afterwards analyzed in a LECO carbon analyzer (LECO, St. Louis, Missouri). An appropriate amount of algal suspension was then diluted with ADaM to the desired carbon

concentration. At the same time we also analyzed the particulate phosphorus concentration of the algal suspensions. The C:P ratios for high-phosphorus (200) and low-phosphorus (1200) algae were constant throughout the experiments.

Somatic growth rates were measured at five food levels ranging from 30 $\mu\text{g C/L}$ up to 150 $\mu\text{g C/L}$. The amounts of phosphorus pulsed to the different food emulsions ranged from 0.465 $\mu\text{g/L}$ (30 $\mu\text{g C/L}$) to 2.325 $\mu\text{g/L}$ (150 $\mu\text{g C/L}$) to ensure that the algal C:P ratios were similar for all different treatments. In each of the experiments, there were three replicate vessels per food concentration and mineral content; each of them contained five 1-d-old daphniids. After 3 d, the animals were removed from the vessels, and placed together in weighing boats. The boats were dried overnight at 60°C, and weighed to the nearest 0.1 μg . Dry masses were converted into somatic growth rates per day using the formula $g = (\ln[M_3] - \ln[M_0])/3$, where M_0 and M_3 are the masses of the animals at day zero and three, respectively, and 3 (days) is the duration of an experiment (Lampert and Trubetskova 1996).

Threshold food concentrations (C_0), food concentrations that support zero somatic growth, give an indication of food levels at which a population is sustainable (Kreutzer and Lampert 1999). C_0 is calculated from somatic growth rates determined at different food levels, and as such integrates all of the available information in an experiment (Lampert 1977, Gliwicz 1990). In their work, Sterner and Robinson (1994) contrasted two different possible scenarios when animals are fed low concentrations of food (Fig. 1). If, at very low food levels, only energy for maintenance and not the nutrient content for growth of the food is of importance, the growth rate of the daphniids should be similar on the different food types until the food concentration is so high that it will sustain net growth. Differences in growth rates between low- and high-nutrient food should be found only at food concentrations above C_0 , i.e., the consumer switches from energy

limitation to nutrient limitation (Fig. 1A). If the nutrient content of the food plays a role even if the energy content of the food is below the maintenance level, growth on the low-nutrient food should be lower, resulting in a higher threshold food concentration (Fig. 1B).

Two experiments were carried out, the only difference between them being that in the first experiment the neonates were born in the high-phosphorus food medium the mothers were kept in, and in the second experiment the mothers were transferred to food-free medium 24 h before the experiment was started. This was done to reduce potential P accumulation effects due to the feeding of the neonates on the high-P food during precultivation.

Reproduction

D. magna neonates were cultured in 1-L jars at high concentrations of high-P food during their juvenile phase at densities of 20 individuals/L. After the animals had released their second clutch of neonates, half of the adults were transferred to low-P food, whereas the other half still received the high-P algae. After 1 wk at the specific food conditions, mothers and neonates released within 24 h were analyzed for body mass and mass-specific P content. A series of 10×10 neonates and 10×2 mothers were pooled for the analysis; the entire experiment was carried out twice. In the second experiment we also determined body size and the area of visible body lipids for 10 neonates from each treatment using a video imaging system program AnalySIS 3.0 (Soft Imaging Systems, Münster, Germany) connected to a dissecting microscope.

To test for the effects of differential investment into reproduction, neonates born at the different food sources were used to assess somatic growth rates at either high (1.0 mg C/L) or low (0.1 mg C/L) quantities of low- or high-P *Scenedesmus* for 3 d. The experiments were carried out in a flow-through system as described in *Materials and Methods: Growth experiments*, and somatic growth rates were determined in the same way as in the growth experiments.

RESULTS

Growth experiments

We observed significant differences in growth rates between the different concentrations and different food types in both growth experiments (two-way ANOVA; $P < 0.001$). Animals fed high-P *Scenedesmus* mostly grew considerably better than those reared on low-P algae (Fig. 2). In the second experiment, the growth rates were lower than in the first one, which can be explained by the differences in the precultivation mode. As they were starved for 12 h on average, the animals in experiment 2 will have had lower energy reserves, leading to reduced overall growth rates, because they have to refill internal storage before further growth

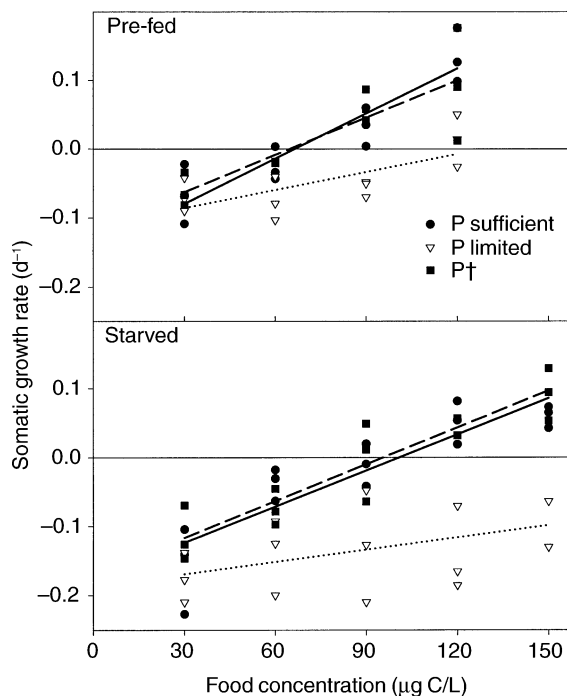


FIG. 2. Resource-dependent somatic growth rates (d^{-1}) of *Daphnia magna* neonates on high (P-sufficient), low (P-limited), or phosphorus-supplemented low-P (P†) *Scenedesmus obliquus* calculated for a 3-d period in experiment 1 (top panel), with pre-fed neonates; and experiment 2 (bottom panel) where the neonates were born in medium without food.

(McCauley et al. 1990, Bradley et al. 1991). A comparison of the growth rates under starvation conditions of pre-fed and starved animals over a period of 3 d supports this conclusion. The growth rates of pre-fed animals averaged $-0.0960 \pm 0.0370 d^{-1}$ (mean ± 1 SD), whereas the starved animals lost mass at a higher rate ($-0.170 \pm 0.0120 d^{-1}$). This difference was highly significant ($P = 0.004$, two-sample *t* test), and hence experiments one and two were not combined for further analysis.

Besides significant effects of both food concentration and mineral content, we also found a significant interaction of both effects ($P = 0.027$ for experiment 1 and $P = 0.05$ for experiment 2). Threshold food concentrations (Table 1) calculated from linear regression were two to three times lower at high-P food in both experiments ($64 \mu g C/L$, $104 \mu g C/L$) than those in the low-P treatments ($127 \mu g C/L$, $311 \mu g C/L$). As there were no overlaps of the 95% confidence intervals of the intersection point with the zero-growth axis, these results can be considered statistically significant. The addition of phosphorus to the low-P algae significantly increased growth of the animals compared to the nonsupplemented P-limited food (Tukey's hsd, $P < 0.006$ for experiment 1 and $P < 0.0001$ for experiment 2), resulting also in lower threshold values (Table 1). In fact, the increase in growth rates as a result of

TABLE 1. Threshold food concentrations (C_0) for somatic growth of *Daphnia magna* cultured with *Scenedesmus obliquus* of different mineral content (P sufficient, P limited, and P limited with pulses of dissolved P, just before feeding them to the daphniids [P pulsed]) in experiments 1 and 2.

Experiment	Treatment	Threshold food concentration ($\mu\text{g C/L}$)
1	P sufficient	64 (54–76)
	P limited	127 (99–252)
	P pulsed	65 (46–79)
2	P sufficient	104 (86–115)
	P limited	311 (195–ud)
	P pulsed	95 (83–105)

Note: Intersections of the 95% confidence intervals are given in parentheses (ud = undefined).

the P addition was such that we did not see any differences between the growth rates of the pulsed animals and those that were kept at high-P algae, suggesting that at these low food levels the difference in quality between low-P and high-P algae can be explained solely by the difference in P content (Table 1; Fig. 2). Hence, these results show that even at low food levels, quality differences of algae do play a role.

Because the interactions between food concentration and food type were significant, one could argue that as a result the growth rates at all food levels should be different, even though one might not be able to see this at the lower concentrations, because the growth rates were so close together. Alternatively, one could assess food quantity value at which significant differences between food types could be found. Post hoc tests (Tukey hsd) revealed that, compared to the low-P treatment, daphniids receiving high-P food grew significantly better at 90 $\mu\text{g C/L}$ ($P = 0.05$) and 120 $\mu\text{g C/L}$ ($P = 0.004$) in experiment 1, and at 120 $\mu\text{g C/L}$ ($P = 0.002$) and 150 $\mu\text{g C/L}$ ($P = 0.03$) in the second experiment.

Reproduction

As was the case in the growth experiments with neonates, we also found food-dependent differences between adult *D. magna* grown at high levels of either high- or low-P *S. obliquus*. The animals reared for 1 wk at high-P food were not only significantly heavier

than their sisters grown at P-limited algae, but also had an increased mass-specific P content (Table 2). Whereas the difference in body mass was a factor of two, differences in specific P content were less pronounced. In contrast to their mothers, neonates did not differ in body mass and had an average dry mass of nearly 8 μg in both treatments (Table 2). Interestingly, significant differences occurred in the mass-specific P content of the offspring (Table 2). Neonates born at low-P food had a mineral content of $0.007 \pm 0.001 \mu\text{g P}/\mu\text{g}$ dry mass, compared to the high-P treatment ($0.009 \pm 0.002 \mu\text{g P}/\mu\text{g}$ dry mass). Independent of food quality, neonates in both treatments had an increased specific P content compared to their mothers, and this trend was stronger in the low-P treatment. As we had to grow several mothers together to get sufficient neonates for analysis, we were not able to directly link daughters' P content with that of their mothers. By randomly combining neonate/adult values, however, we found that the mass-specific P content of the low-P neonates was increased by a factor of 2 relative to their mothers, whereas for the high-P offspring this concentration effect was 1.5. As was the case for the body masses, we did not find significant differences in the body size of neonates from the different treatments (Table 2). However, the area of visible lipid droplets, a measure of energy storage, showed differences. The amount of storage material of neonates born at low-P food was more than double that of the high-P animals (Table 2).

In the growth experiment, we observed a significant three-way interaction between precultivation P, actual P, and food concentration (Fig. 3). This was caused mainly by the animals cultured on the low food concentrations. Significant, precultivation-dependent differences in body growth could only be found at low concentrations of low-P food. Under these conditions, growth rates of neonates born at low P ($0.038 \pm 0.003 \text{ d}^{-1}$) were higher than those of animals born from animals fed high-P algae ($-0.14 \pm 0.036 \text{ d}^{-1}$) ($P = 0.005$, Tukey hsd post hoc test following three-way ANOVA). In all other combinations of food quality and quantity, no such differences between animals from different precultures could be found. The growth rates obtained

TABLE 2. Summary table of the differences between mothers and daughters in the reproduction experiment.

Trait	P–		P+		P^\dagger
	Mean	SD	Mean	SD	
Dry mass of mothers (μg)	275.0	45.6	526.2	80.0	<0.0001
Specific P content of mothers (% of dry mass)	0.40	0.12	0.64	0.27	<0.0004
Dry mass of neonates (μg)	7.74	0.98	7.76	1.40	NS
Specific P content of neonates (% of dry mass)	0.72	0.13	0.94	0.22	<0.004
Body size of neonates (mm)	0.88	0.01	0.86	0.03	NS
Lipid surface of neonates (μm^2)	36.4	9.3	15.3	3.7	<0.001

† Two-tailed t tests.

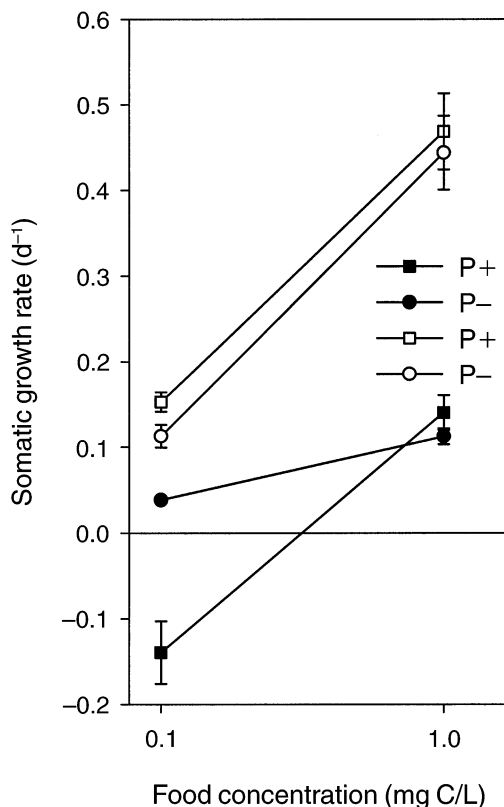


FIG. 3. Somatic growth rates of *Daphnia magna* neonates born from mothers that were cultured under high-phosphorus (squares) or low-phosphorus (circles) *S. obliquus* conditions, with the neonates cultured with low and high concentrations of high-P (open symbols) or low-P (solid symbols) *S. obliquus*, calculated for a 3-d period. Data are means of each three replicate vessels; error bars indicate \pm SD.

for the low-P/high-quantity treatment were nearly identical to those reached with low quantities of high-P food.

DISCUSSION

Ecological stoichiometry theory has been applied successfully to explain differences in quality between different food sources, especially in aquatic systems (Sterner and Elser 2002). Nevertheless, the theory hinges on several assumptions, which have been shown not to be completely accurate. In this study we investigated the consequences of these violations of the assumptions underlying the current stoichiometry theory, using the cladoceran *Daphnia magna* as test organism.

Nutrients in short supply should be taken up and retained with near to 100% efficiency. Although this was suggested using models (e.g., Olsen et al. 1986), several studies, using a range of different organisms, have shown this not to be the case in insects (Slansky and Feeny 1977, Tabashnik 1982, Reynolds 1990), mammals (Partridge et al. 1989), fish (Schindler and Eby 1997), birds (Al-Masri 1995), copepods (LeBorgne 1986, Anderson and Hessen 1995), and cla-

docerans (DeMott et al. 1998). The logical consequence of 100% nutrient retention is that at very low food levels only the energy content of the food should play a role, as this is needed for maintenance, but not the nutrient composition, as nutrients are needed for growth and production only, and all of the limiting nutrient is retained.

The basis for the stoichiometry models is a strict homeostasis in the consumer. However, even in aquatic systems where stoichiometric approaches have been primarily used, the homeostasis assumption does not seem to hold completely, and differences in the stoichiometry between animals of different sizes and ages have also been found (DeMott et al. 1998, Walve and Larsson 1999, Sterner and George 2000, Plath and Boersma 2001). This implies that animals might invest differentially in somatic and reproductive growth, and the consequences of this were investigated in this study.

Nutrient content at low food

Threshold food concentrations are an approximation of the basic metabolic demands of an animal derived from quantity-dependent growth rates, and consequently include costs of taking up and processing the food (specific dynamic action) (Gliwicz 1990, Kreutzer and Lampert 1999). Sterner and Robinson (1994) observed that threshold concentrations were independent of the mineral content of the food in *Daphnia magna*. In contrast, we found differences in threshold values between algae of different nutrient content as food for the same species. It is difficult to explain the differences between our results and those of Sterner and Robinson. There are several factors, however, that may have caused the high amount of unexplained variation in their experiments. First of all, the animals used in their study were not necessarily monoclonal. Secondly, the experiments were not standardized for the instar of the mother, yielding large differences in initial mass of the neonates. Thirdly, the lowest food levels were not considered as all of the animals died, which possibly caused an overestimation of the threshold values. As we avoided these difficulties, we conclude that even at low food levels not only is energy of importance, but the mineral content of the food plays a role as well. Mineral limitation in algae is normally accompanied by other changes apart from changes in nutrient content, such as the content of essential fatty acids (Müller-Navarra 1995), or structural changes reducing digestibility of the food (van Donk et al. 1997). These changes have been invoked to explain the differences in food quality, but the results of our supplementation treatment and of similar experiments reported in the literature (Urabe et al. 1997, 2002, DeMott et al. 1998, Boersma 2000, Elser et al. 2001, Plath and Boersma 2001) show that adding phosphorus to low-P algae increased growth rates up to levels reached with high-P food. Consequently, the 2–3 times higher C_0 values observed for

the low-P algae were a direct effect of mineral limitation.

The difference between the threshold food concentrations of animals grown on high-P food vs. low-P food can be interpreted as a measure of the energy that is needed to deal with low-P food. Many consumers show an increase in feeding activity when faced with food with a low mineral content (e.g., Slansky and Feeny 1977, Reynolds 1990, Plath and Boersma 2001). This results in a decrease in the assimilation efficiency for the limiting nutrient, but an increase in the total amount of the nutrient taken up, thus optimizing nutrient acquisition (Reynolds 1990). Unfortunately, the studies investigating behavioral responses to changes in the mineral content were carried out with high concentrations of food, and it is difficult to assess whether these responses could also occur at much lower food levels (see Philippova and Postnov 1988, Plath 1998). If such responses could occur, then this would provide us with a mechanistic explanation of the observed phenomenon. An increase in feeding activity would imply an increase in energy spent on food uptake, which would reduce the growth efficiency and secondary production (Sterner and Hessen 1994), and would be measurable as higher threshold food concentrations.

An increase in feeding costs with decreasing mineral content of the food has consequences for existing models of the interactions between food quality and quantity (e.g., Sterner 1997). In this model the boundary between energy and mineral limitation is a dynamical function dependent on both the amount and the nutrient content of the food supplied. At a given quantity of food of a specific C:P ratio, the homeostatic consumer should shift from energy (below this quantity) to mineral limitation. This quantity is expected to decrease with an increasing C:P ratio, i.e., to become lower with a lower mineral content of the food, reaching the asymptote of the threshold food concentration level, below which only energy limitation will play a role. We can use our data to investigate at which food concentration we can observe a significant difference between the growth rates of animals grown on high-P and low-P algae, and use these concentrations as an estimate of Sterner's (1997) quality threshold. Interestingly, these food concentrations are below the quantity threshold calculated for the low-P *Scenedesmus*. In Sterner's model equations this was not possible, but it can be explained by quality-dependent shifts in C_0 . Hence, we propose a more detailed separation of basal metabolic costs for further modeling of interactions between food quality and quantity. We assume that basic metabolic costs, such as the basal respiration rates (Bohrer and Lampert 1988), are unaffected by food quality. The total metabolic expenditure (C_0), however, should then be calculated by adding costs of taking up and processing the food (specific dynamic action) to these basal metabolic costs. With decreasing food mineral content, costs of feeding should become more and more

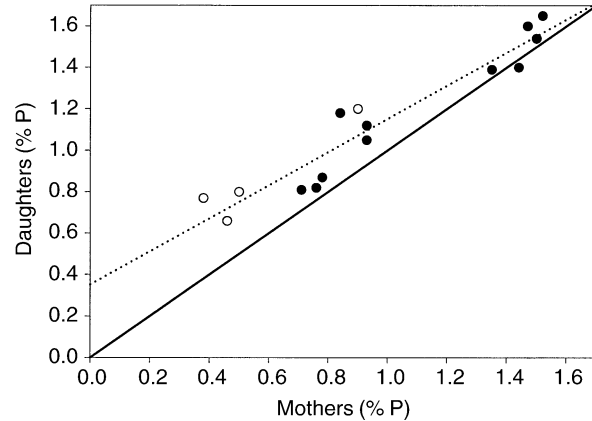


FIG. 4. Relationship between specific P content of adult *Daphnia magna* cultured at different qualities of food and their offspring. Data from our experiments (open circles) are plotted together with those given in DeMott et al. (1998) (solid circles). The solid line indicates a 1:1 ratio; the equation obtained from linear regression on all data points (dotted line) is: $Y = 0.8X + 0.35$ ($r^2 = 0.92$).

pronounced and C_0 higher and higher. In contrast to the assumptions made in the original model, this means that the boundary between the transition of C and P limitation must not necessarily become infinite at the threshold level, but metabolic costs can reach values well above this transition (see also Urabe and Watanabe 1992).

Investment in offspring

Many studies have considered the effects of environmental conditions on the allocation of energy to reproduction on the one hand, and the division of the available energy into individual offspring on the other hand (e.g., Smith and Fretwell 1974, Stearns 1976, Winkler and Wallin 1987, Boersma 1995). Most of these studies, however, only used energy as the currency of interest (but see DeMott et al. 1998, Urabe and Sterner 2001). Here, we focused on the effects of nutrient content of the food, with the potential energy intake of the mothers being constant. We observed that mothers produce offspring that are richer in P than they are themselves. The same was found by DeMott et al. (1998). Others also observed that smaller *Daphnia* individuals had a higher P body content than larger ones (e.g., Main et al. 1997). This is usually explained by the higher maximum growth rates during the juvenile phase, linked with higher RNA requirements, and as this is the most important pool of phosphorus (Vrede et al. 1999), with higher P requirements. Only Sterner and Schulz (1998) reported lower mass-specific phosphorus contents in eggs than in their mothers, the mechanism of which is difficult to explain, especially since the carbon (energy) contents were not significantly different. If we plot our data with those provided by DeMott et al. (1998) (Fig. 4) this shows a clear deviation of the relation between the specific P content of

daughters and their mothers from a proposed 1:1 ratio. Daughters generally have a higher mineral content than their mothers, and with decreasing mineral content of the food these differences become more and more pronounced. Daphniids grown on P-deficient food had a lower mass-specific P content, but they invested relatively more phosphorus into their offspring than the high-P animals. As high-P neonates still have a higher specific P content, this does not sufficiently explain the growth rate differences that we found at low concentrations of poor food (Fig. 3). Although mothers reared at low-P food were significantly lighter than their high-P sisters, their offspring did not differ in mass, and had more visible lipid droplets than the offspring produced by high-P mothers (Table 2). Whereas at low food quantities, additional energy will help the animals to overcome starvation periods (Boersma 1997b), at low P it could help neonates to cope with costs coming along with increased food uptake. In fact, in their recent study, Urabe and Sterner (2001) also observed that eggs produced by P-limited mothers contained more reserve substances. This increase in energy reserves could explain the differences in body growth rates that we found at low concentrations of poor food, as this might have provided the energy for an increased P uptake.

Conclusions

In this study, we have investigated the consequences of some violations of the assumptions underlying the current ecological stoichiometry theory. We have shown that even at very low food levels quality differences between different food sources influence growth rates for *Daphnia magna*. In contrast to the predictions of Sterner and Robinson (1994), we found quality-dependent shifts in threshold food concentrations. Moreover, we have also shown that even animals that show a large amount of homeostasis are capable of differentially investing nutrients between somatic growth and reproduction. Our results illustrate the importance of incorporating low levels of food into the current models.

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