

Resource quality effects on life histories of *Daphnia*

Claes Becker

Max-Planck-Institut für Limnologie, Postfach 165, D-24302 Plön, Germany

Maarten Boersma

Max-Planck-Institut für Limnologie, Postfach 165, D-24302 Plön; and Alfred-Wegener-Institut für Polar- und Meeresforschung, Biologische Anstalt Helgoland, Postfach 180, 27483 Helgoland, Germany

Abstract

We investigated the changes in life histories imposed on the water flea, *Daphnia magna*, due to biochemical and mineral limitations. Phosphorus-deficient *Scenedesmus obliquus* were incubated with or without a single essential fatty acid, eicosapentaenoic acid (EPA). Additionally, the algae were spiked with dissolved phosphorus to create a range of C:P ratios from 600 to 200. This procedure created the possibility to study the importance of different essential resources. We found that somatic growth is retarded until a C:P ratio (molar) ~ 350 is reached. Adding more phosphorus did not further increase growth. At the same time, at high C:P ratios the addition of EPA did not make a difference in growth, whereas below the nutrient threshold (C:P = 350), the fatty acid had a strong positive impact on growth. In a second experiment we studied how the food conditions (with regard to EPA) affected the growth and investment in reproduction and whether this effect was passed on to the next generation. We found that animals fed EPA made an earlier and larger investment in reproduction. Also, the EPA-enriched animals had a higher mortality. The juveniles from mothers fed EPA-enriched algae had a higher growth rate than neonates from control mothers.

In recent years, numerous studies on the effect of food quality differences on herbivorous zooplankton have appeared (e.g., Hessen 1992; Sterner et al. 1993; Müller-Navarra 1995b; DeMott et al. 1998; Boersma 2000; Boersma and Stelzer 2000; Brett et al. 2000; Elser et al. 2000; Urabe and Sterner 2001). Despite the considerable interest in this subject, the increase in our knowledge on the factors that determine the quality of the food for herbivorous zooplankton has been relatively slow. Obviously, the important characteristics determining the quality of the food have been recognized some time ago, but the importance of these factors relative to one another has not yet been clarified. On one hand, this is probably caused by the fact that different characteristics of the food play a dominant role in different systems, but, on the other hand, different researchers have focused mainly on different aspects of food quality. As a result, different schools advocate different “most important” factors.

Four factors determining the nutritional quality of seston for zooplankters have been identified. First, size and morphology of the algae are of importance. If the food particles are too small (Brendelberger 1991) or too large (Bern 1994) or if the cells have protective structures, such as thick cell walls (van Donk et al. 1997) or gelatinous sheaths (Porter 1976), the quality of the algae as a food source is low (DeMott and Tessier 2002). Second, certain compounds of

algae may be actually toxic for zooplankters (Turner and Tester 1997). Third, the mineral composition of the algae/ seston can influence its nutritional quality. If the phosphorus concentration of algae is low, the nutritional quality of the food is low (Urabe et al. 1997; DeMott 1998), and, last, biochemical features of the food, such as the fatty acid content determine the quality of the food. Especially highly unsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid, are essential for many consumers, and several studies have indicated the importance of these fatty acids for herbivorous zooplankton (Müller-Navarra 1995a,b; Brett and Müller-Navarra 1997; Sundbom and Vrede 1997; Weers and Gulati 1997; Wacker and von Elert 2001).

Not only have most of the studies cited above concentrated on one aspect of the food, they have also done so using a variety of different techniques. Whereas the researchers advocating mineral limitation mainly used experimental evidence to substantiate their claim, much of the evidence from those supporting biochemical limitations as the main factor of importance comes from field and correlative evidence (*but see* von Elert and Wolffrom 2001). In previous studies on the effect of different feeding conditions of *Daphnia*, and more specifically on the effect of differences in phosphorus availability, the algae were grown under different phosphorus conditions (Sterner et al. 1993; DeMott 1998). Unfortunately, this cultivation procedure not only affects the phosphorus content of the algae but also their morphology and biochemical composition. The cell wall becomes thicker (Tillberg et al. 1984), thus making the algae harder to digest for the zooplankton (van Donk et al. 1997). Previous work has shown that P-deficient algae have a higher total content of fatty acids (Müller-Navarra 1995a; Boersma 2000) but that the content of highly unsaturated fatty

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acids is lower. Therefore, it is unclear whether the decrease in growth of *Daphnia* when fed P-limited algae is in fact the direct consequence of phosphorus limitation or of concurrent changes in the algae. Several experimental approaches have, in the mean time, been developed to overcome the problems mentioned above. Urabe et al. (1997) observed that daphniids can take up dissolved nutrients from the surrounding water. DeMott (1998) worked with variable mixtures of P-deficient *Scenedesmus* and P-rich *Synechococcus*, and DeMott (1998), Boersma (2000), Elser et al. (2001), and Plath and Boersma (2001) used the fact that algae rapidly can take up dissolved phosphorus from the water, resulting in a direct change of algal phosphorus content without changes in morphology or biochemistry.

Only fairly recently, there have been some attempts to study more than one aspect of factors determining food quality simultaneously (Boersma 2000; Elser et al. 2001; DeMott and Tessier 2002). Elser et al. (2001) observed that, in a lake with a very high C:P ratio, adding phosphorus to the seston improved the quality as food for *Daphnia*, which suggests that, when the phosphorus content of the food is very low, this is the primary factor determining food quality. Boersma (2000) came to the same conclusion using laboratory cultured algae with roughly the same C:P ratio as the seston of the field study by Elser and coworkers. Plath and Boersma (2001) studied a range of different C:P ratios of the food and combined these with different fatty acid treatments. They concluded that, when daphniids were fed severely limited algae, the animals seemed to be colimited by both biochemical as well as mineral factors, and they explained this by changes in feeding activity. Plath and Boersma (2001) argued that the fatty acids might not be used primarily as a nonsubstitutable resource but more as an extra energy source (*but see* DeMott and Tessier 2002). Although this result is intriguing, the weakness of the study of Plath and Boersma (2001) was that they supplied EPA in emulsions with many saturated fatty acids present and hence could not distinguish between the energy effect of the emulsion additions and the effect of EPA as an essential resource. Hence, in the present study, we set out to investigate whether EPA and phosphorus can really colimit growth of *Daphnia magna* individuals by supplying the animals with EPA only, using the method described by von Elert and Stampfl (2000).

Even though it has been suggested that different factors might be important during different life phases, or more specifically that, during the juvenile phase, phosphorus limitations are more important, whereas after maturity other factors should become the quality determining factors (Urabe and Sterner 2001), most research to date has focused almost exclusively on somatic growth (*but see* Brett 1993; Sterner and Schulz 1998). Only recently, Urabe and Sterner (2001) included reproduction in a study on dietary effects. Hence, the second aim of the present study was to investigate the effects of different dietary components on growth and reproduction, also taking into account the fate of juveniles produced under different conditions.

Materials and methods

The *D. magna* clone used in the present study was originally collected from a pond in Frankfurt, Germany, and had

been kept in the laboratory for many years. Phosphorus-free medium was used (ADaM) (Klüttgen et al. 1994) in all experiments. Juvenile animals were collected from a stock culture and placed individually in 200-ml jars. They were fed phosphorus-sufficient *Scenedesmus obliquus* (1 mgC L⁻¹ at 18°C) every day, and the medium was renewed twice per week. Third-brood juveniles from these animals were used as experimental animals. All experiments were carried out at constant dimmed light at 18°C.

A semicontinuous culture of *S. obliquus* was used as experimental food source. The algae were grown in Z/4 medium (Zehnder and Gorham 1960) with reduced phosphorus content (from 1.391 mg P L⁻¹ to 83.5 µg P L⁻¹). The algae were cultivated in a 10-liter bottle with an exchange rate of 1.8 liter Z/4 P-medium d⁻¹. An aliquot equivalent to 60 mg carbon was harvested each day. Two fatty-acid treatments were prepared by dividing the harvested algae over two bottles and supplementing one of the algae suspensions with EPA dissolved in ethanol (Williams et al. 1990; von Elert and Stampfl 2000), such that the total EPA content in the incubation was 5% of the algal dry weight. Thereafter, the algal suspensions were incubated in the light for 3 h. The incubation was ended with centrifugation (3,400 g for 5 min), and the pellets were resuspended in ADaM to achieve an algal concentration of 1 mgC L⁻¹. This resulted in an increase of EPA in (or on) the algae from below the detection limit to 7.6 µg mgC⁻¹, whereas there was no significant change in any of the other fatty acids (*see*, for analytical methods of fatty acid analysis, Wiltshire et al. 2000). To investigate potential P limitation, the algal suspensions were subsequently enriched with five different amounts of a K₂HPO₄ solution (Plath and Boersma 2001). The additions were 0, 2.5, 5, 10, and 20 µgP L⁻¹, which gave a range of C:P ratios from 600 to 230 (molar). For comparison, we also had one treatment where we fed P-sufficient algae to the animals. This gives a total of 11 different treatments. All treatments were carried out in quintuplicate, thus yielding a total of 55 experimental vessels. The food suspensions were renewed daily, kept in the dark in 5-liter bottles, and the suspensions were gently stirred to prevent sedimentation.

The experimental animals were collected within 24 h of birth and randomly divided in 120-ml flow-through vessels, with a flow rate of 1 liter d⁻¹. Every vessel contained four individuals. Six randomly chosen individuals were put together in an aluminum weighing boat to determine the initial dry mass. After 3 d, two animals were harvested from each experimental vessel and put together in aluminum weighing boats. These were then dried at 60°C for 24 h and stored in an exicator until weighed on an electronic microbalance to the nearest 0.1 µg. Somatic growth rates were computed. Once the animals reached maturity, another animal was harvested to determine the weight at first reproduction, and the somatic growth rate over the whole juvenile period was computed (Lampert et al. 1994). The one remaining animal was followed until it reached the third adult instar, the number of viable offspring was established for the first and second adult instar, and the average mass of these offspring was measured.

To investigate whether the effect of an essential fatty acid is passed on to the next generation, we carried out a second

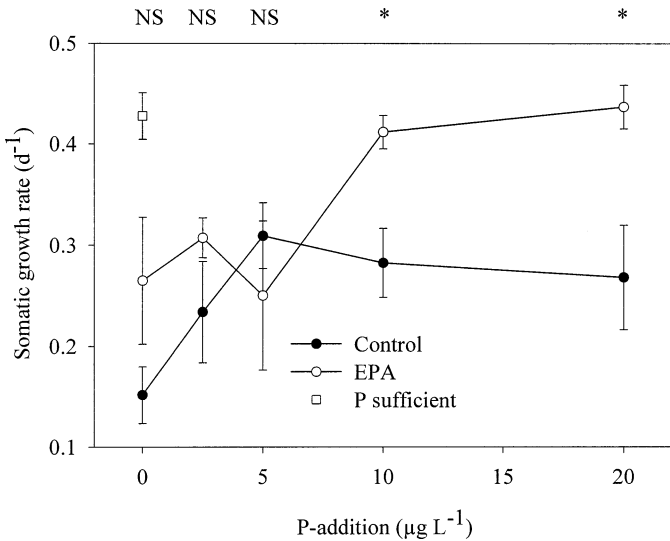


Fig. 1. Somatic growth rate over the first 3 d for *D. magna* fed either P-sufficient or -limited *Scenedesmus*. The P-limited algae were supplemented with a range of P additions and with or without the addition of EPA. Error bars indicate \pm SE. NS, not significant; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ indicate the significance of the differences between the control and the EPA treatment (Duncan's multiple range test).

experiment using the same set-up as above but with only one P-addition of $5 \mu\text{g P L}^{-1}$. This gives a C:P molar ratio ~ 350 , and the algae should only differ in the EPA content. Third-brood *D. magna* juveniles, <24 h old, were used as experimental mothers. They were kept solitary in 200-ml jars. Every day the animals were transferred into new glasses with fresh medium. From these animals, the time of reproduction and the size of the successive broods were determined. The animals were cultivated until death or until they hatched their third brood of juveniles. Third-brood juveniles of these experimental mothers were cross-tested for the different growth conditions. For comparison, we also added third-brood juveniles from P-sufficient mothers. We added a starvation treatment (fed only ADaM), to study how the growth rate declines. The experimental setup was identical to the one described above for the first experiment, with five individuals in each flow-through vessel, but all of them were harvested after 3 d, and somatic growth rates determined.

Results

Juvenile growth rates were positively affected by both the addition of phosphorus as well as the addition of EPA (Table 1). The interaction between the two factors was marginally significant. Animals fed P-limited *Scenedesmus* spiked with a range of P additions grew considerably better at the higher P concentrations than at the lower ones (Fig. 1). Duncan's multiple range tests showed that the difference between control and EPA additions were significant at the two highest P additions only (10 and $20 \mu\text{g P L}^{-1}$).

We observed a similar pattern in the somatic growth rates until maturity. As was the case with the 3-d growth rates, both the addition of phosphorus as well as the addition of

Table 1. Summary table of differences in life-history traits due to phosphorus and EPA additions— P values of two-way ANOVAs.

Trait	P treatment	EPA treatment	Interaction
Somatic growth rate (3 d)	0.005	0.0024	0.09
Somatic growth rate until maturity	<0.0001	0.0015	0.0044
Reproduction investment	<0.0001	0.0048	0.2971

EPA had a positive impact on the growth rates. Also, however, the interaction between these two factors explained a significant part of the variation in the growth rates (Table 1; Fig. 2). Nevertheless, as was the case with the growth rates over the first 3 d, the differences between the controls and the EPA additions were significant for the two highest P additions only (Duncan's multiple range test). Hence, we conclude that when the algae are severely P-limited, this is the main food quality determining factor: adding P increased growth rates and adding EPA did not. After P-addition, the addition of the EPA increased growth further. This switch from P limitation to EPA limitation happens around the addition of $5 \mu\text{g P L}^{-1}$, which corresponds to a C:P ratio ~ 350 .

The investment in reproduction, measured as the number of offspring in the first clutch, was larger with added phosphorus (Table 1). The EPA addition also enhanced the investment in reproduction over all P additions. (Table 1; Fig. 3). The increase in the number of viable offspring produced after the EPA addition seemed more pronounced than the increase in somatic growth rates (somatic growth rates at the highest two P additions only increased by 23% and 36%,

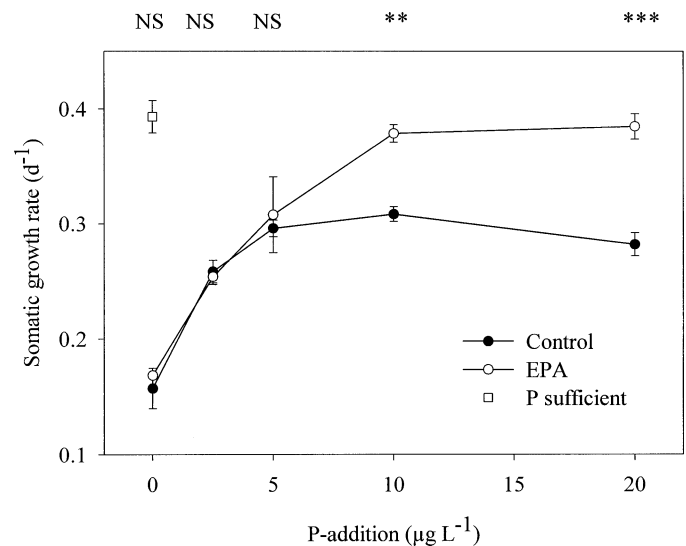


Fig. 2. Somatic growth rates of the entire juvenile growth phase of *D. magna* fed either P-sufficient *Scenedesmus* or P-limited *Scenedesmus* with different P additions, with and without the addition of EPA. Error bars denote \pm SE for five replicates. NS, not significant; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ indicate the significance of the differences between the control and the EPA treatment (Duncan's multiple range test).

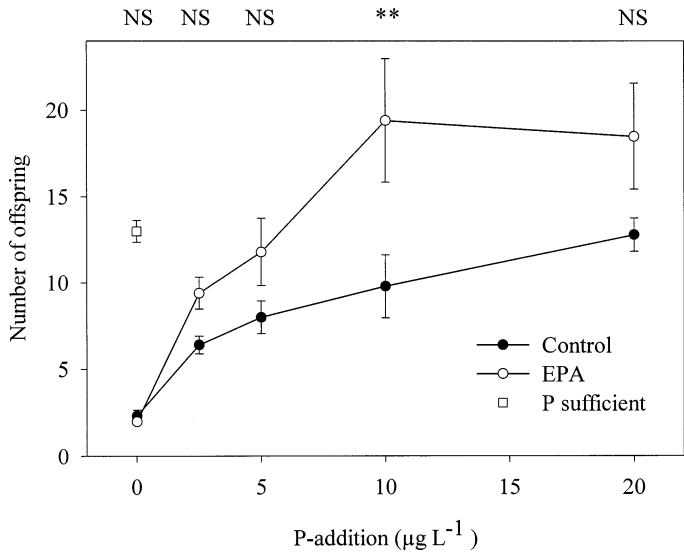


Fig. 3. Number of offspring in the first clutch of *D. magna* fed either P-sufficient or -limited *Scenedesmus*. The P-limited algae were spiked with a range of P additions and also supplemented with or without EPA. Error bars denote \pm SE. NS, not significant; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ indicate the significance of the differences between the control and the EPA treatment (Duncan's multiple range test).

whereas the number of produced juveniles increased by 97% and 45%). The weight of the offspring was significantly affected only by the instar number of the mother (first vs. second brood; repeated measurement analysis, $F_{1,28} = 10.3$; $P = 0.003$), and by the three-way interaction among instar number, P, and fatty-acid treatment. The effect of phosphorus was marginally significant ($F_{3,28} = 2.7$; $P = 0.06$), with a tendency of animals that received more phosphorus producing offspring of a higher mass.

In the second experiment, we found that daphniids that were fed moderately P-limited *Scenedesmus* (C:P = 350) enriched with EPA made a significantly earlier (ANOVA; $P < 0.005$) and larger (ANOVA; $P = 0.002$) investment in reproduction (Fig. 4), which suggests better feeding conditions for the daphniids when EPA was added. However, survival of the animals fed EPA-supplemented algae was significantly lower than the survival of the animals fed control algae (Gehan's Wilcoxon test $P = 0.02$; Fig. 5). To test whether the population growth rate differed between the two treatments, we computed the intrinsic rate of population increase, r , using the Euler equation. The animals from each treatment were randomly divided into three replicate populations. This procedure ensured the variation needed to test significance. We found that, even though mortality is much higher in the EPA treatment, the r for this treatment was 0.192 d^{-1} (SE ± 0.009), which is significantly higher (t -test; $P = 0.036$) compared with the control 0.162 d^{-1} (SE ± 0.002).

Mothers that had been fed different foods produced offspring of different quality (Fig. 6), even though the maternal growth conditions did not have a significant effect on the initial neonate size (ANOVA; $P = 0.085$). However, the maternal growth condition did have an impact on the growth

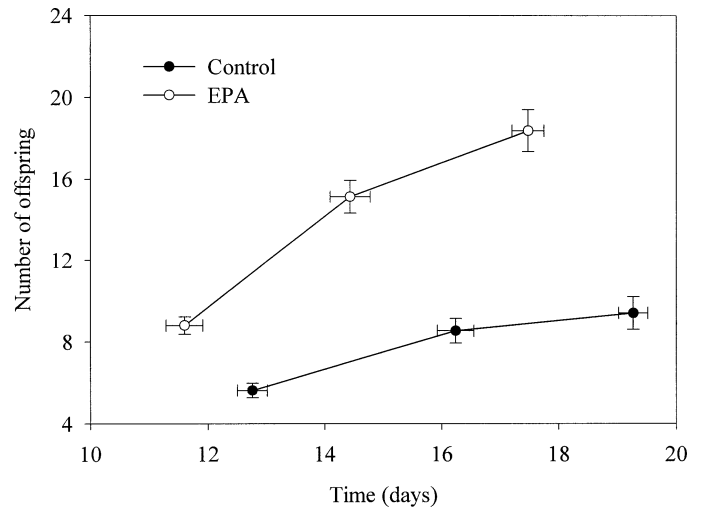


Fig. 4. Time and size of reproduction for *D. magna* fed on P-limited *Scenedesmus* spiked with $5 \mu\text{g P L}^{-1}$ (C:P 350) and also supplemented with or without EPA. Error bars are \pm SE.

rate of the juveniles (two-way ANOVA; $P < 0.001$) (Fig. 6). The neonates from EPA-enriched mothers grew considerably better than juveniles from both of the other two treatments (control and P-sufficient mothers), but only when the neonates were fed. No significant difference was found between the growth rates in animals that were born in EPA-rich medium and then transplanted to EPA-poor medium, and those that were born in EPA-poor medium and were transplanted to EPA-rich medium.

Discussion

Liebig's law of minimum (von Liebig 1855) states that, at any one point, only one factor can be actually limiting growth or reproduction. This translates to the prediction that increases in growth rate should level off with increasing

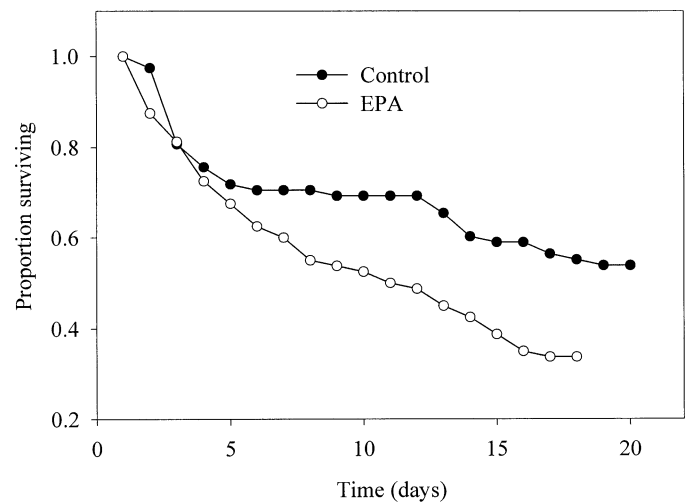


Fig. 5. Survival of *D. magna* fed P-limited *Scenedesmus* spiked with $5 \mu\text{g P L}^{-1}$ (C:P 350) and also supplemented with or without EPA.

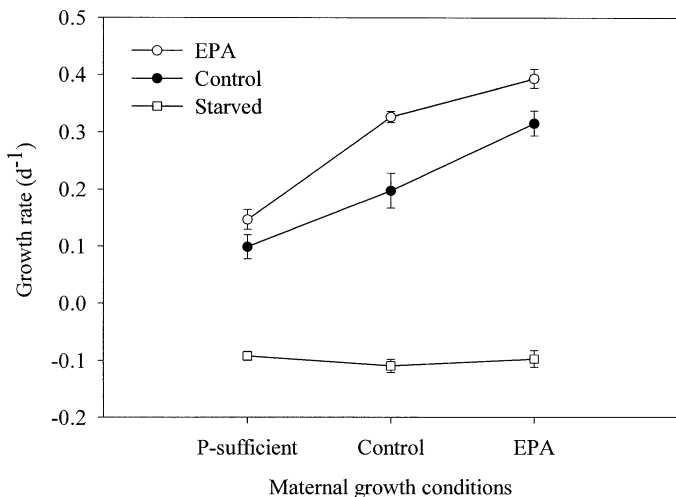


Fig. 6. Somatic growth rate for *D. magna* over 3 d, dependent on the maternal growth conditions. The juveniles were either starved or fed P-limited *Scenedesmus* enriched with $5 \mu\text{g P L}^{-1}$ (C:P 350) and either supplemented with or without EPA. The mothers had previously been fed P-sufficient or -limited *Scenedesmus* enriched in the same way as the juveniles. Error bars are $\pm\text{SE}$.

phosphorus concentrations if phosphorus is the limiting nutrient. Above the concentration of phosphorus at which growth no longer increases, another resource should become the limiting factor, and the addition of this resource should enhance growth further. Our results are consistent with this prediction. P-deficient algae are a poor food source for daphniids, resulting in low somatic growth rates. When we added phosphorus to these algae, the growth rates increased, until reaching a plateau $\sim 5 \mu\text{g P L}^{-1}$. Above this concentration, supplementation with a highly unsaturated fatty acid (EPA) increased growth further. Both somatic growth rates over the first 3 d as well as those for the whole juvenile period showed these patterns, even though there seems to be some (nonsignificant) tendency that in the 3-d measurements also at the lower P concentrations the EPA additions had a positive effect on the growth of the animals. This could have been a result of the maternal growth conditions, because the experimental animals all were born from mothers fed P-sufficient *Scenedesmus*, but this is not very likely, because DeMott (in press) showed that the depletion of the phosphorus stores is very rapid. Plath and Boersma (2001) observed that, when using emulsions of fatty acids instead of single fatty acids, as was done here, these additions had a positive affect on the growth of the animals even when phosphorus was limiting. They argued that, at the lower phosphorus levels, the emulsions might have acted as a rapidly degradable energy source, enabling higher uptake rates of the limiting nutrient. Because we did not observe significantly higher growth rates at the lower P additions, and we fed EPA only, the explanation of Plath and Boersma might be correct, but a more parsimonious explanation might be that as from their second lowest P treatment (adding $4 \mu\text{g P L}^{-1}$, resulting in a C:P ratio of the algae ~ 350) P was actually no longer limiting *Daphnia* growth, thus creating other limitations.

We conclude that above a C:P ratio ~ 350 , mineral phos-

phorus is really limiting growth of the daphniids, and adding phosphorus directly increases the growth rates of the animals. When the C:P ratios were lower, phosphorus seemed to be present in ample supply for somatic growth, and, in our experiments, the essential fatty acid EPA became the limiting factor for growth. The C:P threshold of 350 for *Daphnia* growth is in the same range that Brett et al. (2000) found in the field but higher than what Vrede et al. (2002) showed in their laboratory study. However, the exact level of the threshold is probably very dependent on the kind of food used, and animal species could also play a role. DeMott and Tessier (2002) argued that digestion resistance might play a more important role than previously accepted, because they observed no significant increases in growth when adding phosphorus or essential fatty acids to natural seston from different lakes in southern Michigan, but they observed a strong increase in growth rates when adding a readily digestible alga, even though the C:P ratio in some of the lakes should indicate phosphorus limitation. In contrast, Elser et al. (2001) observed very strong responses to the addition of phosphorus to high C:P seston of a set of different lakes, whereas Boersma et al. (2001) observed weak responses for seston with a C:P ratio ~ 300 . Most likely, even severely phosphorus-limited *Scenedesmus* are probably not so digestion resistant that this plays a major role in this model system (Boersma and Kreutzer 2002), but, in systems that are dominated by algae that are difficult to digest, the digestibility of the food is probably the primary determinant of food quality, followed by phosphorus content of the food as long as this is below the threshold, and only then the biochemical content.

Thus far, studies on the effect of different quality factors in the food have almost exclusively studied somatic growth (e.g., Urabe et al. 1997; Elser et al. 2001). Only recently, Urabe and Sterner (2001) included reproductive traits. They suggested that the suite of elements or biochemicals necessary for somatic growth of young individuals differs from the suite needed by adult individuals for reproduction. They argued that N and P content relative to C in body tissues are higher for smaller *Daphnia* individuals (DeMott et al. 1998), whereas the opposite is the case for eggs (Sterner and Schulz 1998), because yolk is the energy source for embryonic development. This difference in the C:nutrient ratio between eggs and postembryonic individuals should imply that young individuals require more N and P relative to C for their growth, whereas less N and P relative to C is required for the development of eggs. More generally, this would imply that nutrients are more likely to limit somatic growth, whereas other factors should be more important for reproduction. Our results tend to support this. The increase in somatic growth rates after EPA addition was less pronounced than the increase in the number of viable offspring (somatic growth rates at the highest two P additions only increased by 23% and 36%, whereas the number of produced juveniles increased by 97% and 45%), thus suggesting a higher importance of EPA for reproduction. At the same time, adding phosphorus increased the somatic mass by $119 \mu\text{g}$ (difference in weight at maturity between the lowest and highest addition), whereas the reproductive output (difference in egg number times average egg weight) increased by $40 \mu\text{g}$. Even

though we observed that most of the variance for both somatic growth and reproduction was explained by the phosphorus content of the algae, this is most likely at least partly explained by the higher number of treatments. We also observed that the variance explained by the fatty acid treatments was more than double for reproduction as for somatic growth (3% vs. 7%), thus suggesting a higher importance of the biochemical limitation for reproduction.

In their study on the effect of different nutritional limitations on the size of the offspring produced, Urabe and Sterner (2001) observed that daphniids fed P-limited algae produced offspring that were smaller than those fed P-sufficient algae. In our study, we found a similar tendency, although here this result is only marginally significant. In contrast, Boersma and Kreutzer (2002) observed no significant effects in offspring weight between offspring born from mothers grown on P-limited and -sufficient algae. The stronger effect observed by Urabe and Sterner might have been a result of the fact that they did not directly measure weight of the eggs but established the size of the eggs. If the quality of the eggs changes with food quality for the mother (DeMott et al. 1998) this would not be the most accurate of measures, especially given that Boersma and Kreutzer observed that the amount of visible lipid droplets is actually larger in offspring born from P-limited mothers. Nevertheless, we conclude that the food quality of the mother affects the quality of the offspring, and these quality differences had obvious effects when we studied the fate of the different neonates under different conditions (Fig. 6). Mothers that had been supplied with EPA produced neonates that grew faster than neonates from the controls. Moreover, when control juveniles were fed EPA-enriched food, they increased growth to similar levels as the EPA-enriched neonates. On the other hand, there was only a small effect of the EPA enrichment on neonates from mothers fed sufficient P. These results suggest that internal EPA pools in animals are quickly refilled, even though P-sufficient algae contain no or only traces of EPA, and are most likely explained by the observations of von Elert (2002), who found that animals fed algae lacking EPA still had an internal pool of this fatty acid, thus suggesting synthesis from other fatty acids—for example, linolenic acid.

We observed that mothers with access to EPA made a larger and earlier investment in reproduction. At the same time, they had a lower survival. One could speculate that this lower survival is a cost of reproduction, which is a generally accepted phenomenon when comparing a range of different species (e.g., Bell 1984). Species that put more energy in reproduction tend to have a lower life expectancy. However, in many of the studies that have investigated within species variation, correlations tended to be nonsignificant or even positive (see Roff 1992). This is normally explained by correlations between female quality in general and reproduction and survival. With clonal organisms, these correlations are not possible obviously, but Bell (1984) also observed these positive correlations in daphniids and rotifers. Moreover, the observation that daphniids kept under limiting conditions have a higher life expectancy has been made several times (Ingle et al. 1937; Martínez-Jerónimo et al. 1994; Rose et al. 2000), but none of those authors linked this caus-

ally to lower reproductive rates. Lynch and Ennis (1983) did make this link, but they found no negative correlation between longevity and reproduction, stating that their results were inconsistent with the “cost of reproduction hypothesis” (Williams 1966) or with the “rate of living” hypothesis. In contrast, we found this negative correlation, suggesting that the animals that reproduce more at an earlier age trade this off against a decreased survival. It remains to be seen, however, whether this tradeoff is the result of an evolutionary link between fast growth and high potential mortality later in life as a result of predation or is the result of constraints within the animal that allow it to spend its energy only once.

In summary, we observed that different aspects of food quality not only changed in importance depending on the severity of the limitations present but also that they might play a role in different phases of an animal's life. We conclude that phosphorus limitation overrides biochemical limitations if the C:P ratio of the food is >350 and that only when phosphorus is present in ample supply will other factors such as the content of fatty acids become important (see also Boersma 2000). Ontogenetic differences in food preferences are probably not very important in daphniids, and one would expect such changes in limiting factors to be more important in for example copepods, where food spectra and prey size can change considerable during life (e.g., Hart and Santer 1994; Villar-Argaiz et al. 2002). Nevertheless, our results suggest that, even in daphniids such shifts in importance of different aspects of food quality could occur. Hence, the step that is needed next in the research on the effects of essential components of the food are more detailed investigations where which factor is limiting production of the zooplankton rather than a continued discussion on the importance of single factors.

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