Freshwater Biology (2003) **48**, 759–767

Effects of essential fatty acids and N and P-limited algae on the growth rate of tropical cladocerans

ALOYSIO S. FERRÃO-FILHO*, CLÁUDIA FILETO†, NORBERTO P. LOPES‡ AND MARLENE S. ARCIFA†

*Instituto de Biofísica Carlos Chagas Filho, CCS, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Ilha do Fundão, RJ 21949–900, Brazil

†Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14040–901, Brazil

‡Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14040–901, Brazil

SUMMARY

1. In this study, the effects of nutrient (N and P) deficiency and the importance of essential polyunsaturated fatty acids (PUFA) [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] to tropical cladocerans, growth and reproduction were determined in a growth bioassay.
2. The animals were fed N/P-sufficient, N-deficient and P-deficient algae, and also N and P-deficient algae supplemented with fish oil emulsions rich in EPA and DHA.
3. Cladocerans showed different responses to nutrient-deficient algae and also to supplements of fish oil emulsions. *Moina micrura* was most sensitive to P-deficient alga and, surprisingly, grew better and produced more eggs in N-deficient alga than in N/P sufficient alga. *Ceriodaphnia cornuta* was less sensitive, growing well in both N and P-deficient algae. This species, however, had a lower clutch size in N-deficient alga. On the other hand, *Daphnia gessneri* was the most sensitive to mineral limitation, showing decreased growth and clutch size in both nutrient-deficient algae.
4. The PUFA supplements to nutrient-deficient algae increased growth rates only for *M. micrura* and *C. cornuta*, suggesting that these fatty acids are important food requirements for these species.

Keywords: docosahexaenoic acid, eicosapentaenoic acid, mineral limitation, polyunsaturated fatty acids, tropical zooplankton

Introduction

Discussion about the biochemical versus mineral limitation of zooplankton has increased lately, with some supporting biochemical (Müller-Navarra, 1995a,b; Tung & Dam, 1999; Müller-Navarra *et al*., 2000), and some supporting mineral limitation (Sterner, 1993; Sterner & Schulz, 1998; Urabe, Clasen & Sterner, 1997; Elser, Hayakawa & Urabe, 2001). Some authors also consider both factors to be limiting in different situations (Sundbom & Vrede, 1997; Boersma, 2000). Biochemical limitation often refers to essential polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA; 20 : 5ω3) and docosahexaenoic acid (DHA; 22 : 6ω3), while mineral limitation refers to elements such as nitrogen and phosphorus. There is also evidence that proteins can be limiting to *Daphnia* fed on lake seston (Goulden *et al*., 1998).

Elements such as N and P are essential nutrients by definition and must be obtained from the diet. Nitrogen is important in the amino acid and protein

Correspondence: Aloysio S. Ferrão-Filho, Instituto de Biofísica Carlos Chagas Filho, CCS, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Ilha do Fundão, RJ 21949–900, Brazil. E-mail: aloysio@biof.ufrj.br

synthesis. Phosphorus is important as a component of phospholipids, in energy storage metabolism (i.e. ATP) and in nucleic acid synthesis, being therefore directly involved in growth and reproduction. Studies have suggested that high levels of P in cladocerans are associated with high content of RNA (Hessen, 1990; Main, Dobberfuhl & Elser, 1997). Thus, fast-growing species may have a high demand for P and are likely to be more affected by P-deficient algae than slow-growing species (Main *et al*., 1997; Sterner & Schulz, 1998).

Essential fatty acids play an important role in cellular metabolism, as a precursor of eicosanoids (i.e. prostaglandins) in animals, and also as part of cell membranes, regulating membrane fluidity, and acting as an antifreeze in organisms that live in low temperatures (Brett & Müller-Navarra, 1997). Eicosanoids play an important role in the physiology of invertebrates, regulating egg production, egg-laying, spawning and hatching, among other functions (Stanley-Samuelson, 1994a,b).

Some studies on the effect of PUFA additions to monoalgal diets have shown dietary dependence of cladocerans, especially temperate *Daphnia* species, on essential fatty acids (DeMott & Müller-Navarra, 1997; Sundbom & Vrede, 1997), but few studies have shown the same responses in tropical cladocerans (Ferrão-Filho, Azevedo & DeMott, 2000). As tropical cladocerans are not subject to freezing temperatures, as are cladocerans in the temperate lakes, the functional role of PUFA as an antifreeze (Brett & Müller-Navarra, 1997) may not be as important for tropical cladocerans as it is for temperate ones. Thus, for tropical cladocerans, essential fatty acids must be more important in regulating functions related to reproduction.

It is also known that elemental limitation, especially P-limitation, can also influence lipid contents and the fatty acid composition of algae (Müller-Navarra, 1995b; Kilham *et al*., 1997; Lurling & Van Donk, 1997). In general, under P-limitation, algae tend to decrease essential PUFA and increase saturated fatty acids' content (Morris, 1981; Piorreck & Pohl, 1984; Harrison, Thompson & Calderwood, 1990). Thus, it has been thought that growth limitation of cladocerans by P-deficient algae might be an indirect effect of altered algal biochemical composition (Müller-Navarra, 1995b; Sundbom & Vrede, 1997; Weers & Gulati, 1997).

The aim of the current work was to test the hypothesis that nutrient-limited algae can limit growth and reproduction of cladocerans and that PUFA are important for tropical cladoceran nutrition. We used a supplementation approach to test the effects of additions of fish oil emulsion to nutrient-limited algae on the growth rate and reproduction of cladocerans from Lake Monte Alegre, Brazil. These cladocerans showed different degrees of food limitation in experiments carried out in two seasons in Lake Monte Alegre using lake seston (Ferrão-Filho, Arcifa & Fileto, in press) and we wanted to test specifically the hypothesis that PUFA limitation is an indirect effect of mineral limitation.

Methods

Animals and algae

Ceriodaphnia cornuta Sars, *Daphnia gessneri* Herbst and *Moina micrura* Kurz were isolated from Lake Monte Alegre (Ribeirão Preto, SP, Brazil) and cultivated in the laboratory as monoclonal cultures for several generations prior to the experiments. They were maintained in 500-mL bottles with filtered lake water and fed *Scenedesmus spinosus*, R. Chodat, at a concentration of 0.5 mgC L^{-1}. For the feeding of cladocerans, *S. spinosus* was cultured in 500 mL of MBL medium (Stemberger, 1981) with vitamins, in an environmental chamber (model 347 CDG FANEN; São Paulo, Brazil), at 23 °C and with a 12/12 h photoperiod. For the experiment, *S. spinosus* was cultured in 1.5 L of MBL medium in three conditions (two replicates each): (1) N/P-sufficient, with original MBL medium (600 μM N; 30 μM P); (2) N-deficient, with N sources reduced 20 times (30 μM N; 30 μM P); and (3) P-deficient, with P source reduced 50 times (600 μM N; 0.6 μM P). These cultures were inoculated in fresh medium using the same amount of stock culture (in original MBL) and maintained at a room temperature of 23 °C and under fluorescent light and a 12/12 h photoperiod. Algae for the experiment were harvested between 12 and 18 days of cultivation.

Chemical analyses

Samples for algal C, N and P analyses were taken at the beginning and at the end of the experiment, filtering 30–100 mL onto preignited glass-fiber filters (Sartorius AG 37070, Goettingen, Germany). At the end of the experiment, the remaining cultures (*c*.1 L)

were harvested and freeze-dried for fatty acid analysis. Particulate organic carbon was analysed with dichromate-sulphuric acid digestion and by reading absorbance at 440 nm (Strickland & Parsons, 1972), nitrogen (Mackareth, Heron & Talling, 1978), and particulate phosphorus (Fassbender, 1973) with persulphate digestion and colorimetric methods. For the fatty acids analysis, we used a modified method from Kreeger *et al*. (1997). Lyophilised cells (10 mg) were extracted with 1.0 mL chloroform : methanol (2 : 1 v/v) assisted by sonication (5 min), following centrifugation for an additional 5 min (4000 *g*). The supernatant was transferred to another clean tube to which KCl solution (0.9%) was added as 20% of the volume and the tube was shaken vigorously. The extract was then centrifuged for 2 min (2500 r.p.m.). The organic phase was removed to another clean tube, and dried under N_2. To the dried extract was added 0.5 mL of BF3/methanol, and the tube was sealed and put in 80–90 °C water bath for 15 min. After that, 0.5 mL of distilled water and 0.6 mL of chloroform were added to the tube, which was shaken for 30 s. The chloroform phase (lipid fraction) was then removed and analysed by Gas Chromatography (GC). Gas chromatography was performed on a GC Hewlett Packard (Wilmington, DE, U.S.A.) Series II GC using column HP-1 (30 m × 0.25 mm, 0.25 μm), FID detector at 300 °C, manual injector at 250 °C and hydrogen as the carrier gas (linear velocity 39 cm s^{-1}). Column oven temperature conditions were as follows: initial temperature of 100 °C was held for 1 min and then increased 5 °C min^{-1} to 190 °C, then held constant for 6 min and finally increased 8 °C min^{-1} to 250 °C, where it was held constant for 5 min. A quantity of 2 μL of the samples were injected on the column, at a split rate of 1 : 70.

Growth bioassay

A growth bioassay was performed with the three cladocerans from Lake Monte Alegre from 4 to 8 October 2000. Initially ($t = 0$), 10 animals less than 24 h old (20 for *C. cornuta*) were placed on small preweighed aluminium foil to determine initial weight. These animals were dried at 60 °C overnight and then stored in a dessicator with silica gel prior to weighing. About 25–30 animals of each species were placed in 500-mL stoppered bottles for the experiment. Bottles were placed in a plankton wheel with a rotation speed of *c.* 1 r.p.m., at a room temperature of 23 °C. Experimental treatments were: (1) N/P-sufficient *S. spinosus*; (2) N-deficient (–N); (3) P-deficient (–P); (4) –N + PUFA and (5) –P + PUFA. The algal concentration was 0.5 mg C L^{-1} in each treatment and resources were replaced every other day during the experiment. There were three replicate bottles per treatment. Commercial fish oil capsules (Flor & Erva Homeopathy & Manipulation Pharmacy, Ribeirão Preto, SP, Brazil) were used for the PUFA addition treatment, with 18.1% EPA and 12.1% DHA content. Fish oil emulsions were prepared with 500 mg capsules, mixing the oil in 500 mL of distilled water with a shaker for 15 min. Oil droplets of 1–3 μm diameter were formed through this process. Using an estimate of 80% carbon content for fish oil emulsions (DeMott & Müller-Navarra, 1997), 0.3 mg C L^{-1} of fish oil was added in the N and P-deficient algae treatments. Animals for final weighing were collected after 2 days for *M. micrura*, 3 days for *C. cornuta* and 4 days for *D. gessneri*. Different days were chosen for each species because of different ages at maturity. Juvenile growth rates were calculated according to the formula:

$$g = [ln(M_t) - ln(M_o)]/t$$

where M_o and M_t are mean individual mass initially and after t days. At the end of the experiment, the number of eggs present in the brood chamber was counted. Two-way ANOVA was used to test for the main effects of species (three levels), treatment (five levels) and the interaction between these two factors. Differences between treatments for each species were tested using multiple comparison Tukey tests.

Results

Scenedesmus spinosus showed different elemental composition in each culture condition (Table 1). C : N and C : P ratios of N/P-sufficient alga were lower than nutrient-deficient alga. However, N-deficient alga had both C : N and C : P ratios higher than N/P-sufficient algae, showing also some P-deficiency. On the other hand, P-deficient alga had C : N ratios lower than N-deficient alga and C : P ratios much higher than other algae, showing extreme P-deficiency. P-deficient alga also showed some N-deficiency relative to N/P-sufficient alga.

Table 1 Elemental composition of *Scenedesmus spinosus* in the three culture conditions. Data are means ± SE for two replicate cultures in each date

Date	Culture condition	C : N	C : P
Initial (10/04/00)	N/P-sufficient	8.5 ± 0.5	217.2 ± 15.2
	N-deficient	14.9 ± 0.6	596.3 ± 44.0
	P-deficient	12.4 ± 1.0	1215.9 ± 53.7
Final (10/08/00)	N/P-sufficient	6.4 ± 0.7	340.7 ± 35.1
	N-deficient	32.8 ± 5.5	443.7 ± 110.4
	P-deficient	14.6 ± 0.9	2792.2 ± 314.2

Fatty acid composition also varied among different culture conditions (Table 2). N/P-sufficient algae had much higher contents of saturated fatty acids, such as palmitic acid (16 : 0) and stearic acid (18 : 0), than nutrient-deficient algae and lower contents of PUFA such as linoleic (18 : 2ω6) and linolenic acids (18 : 3ω3). Oleic acid (18 : 1ω9), a precursor of linoleic acid, remained relatively constant in the different culture conditions, so the increase in linoleic acid in nutrient-deficient algae was probably through the conversion of palmitic acid, which is also a precursor of linoleic acid. No EPA (20 : 5ω3) or DHA (22 : 6ω3) were found in *S. spinosus*.

The three cladocerans responded differently to treatments in the growth bioassay (Fig. 1). ANOVA revealed significant differences among cladoceran species, treatments and a significant interaction between species and treatment (Table 3). *M. micrura* had the highest growth rate, even with nutrient-deficient algae (Fig. 1). *C. cornuta* had moderate growth rates while *D. gessneri* had the lowest growth rates even in the N/P-sufficient algae. The nutrient deficiency affected most cladocerans, decreasing growth rates relative to nutrient sufficient algae (Fig. 1). However, there was no significant difference between nutrient-sufficient and deficient algae for *C. cornuta*. Also, no significant difference was found between N/P-sufficient and N-deficient algae for *M. micrura*. The latter species, however, had a significant decrease in growth rate in P-deficient alga. For *D. gessneri*, both nutrient-deficient algae decreased growth rate relative to N/P-sufficient alga.

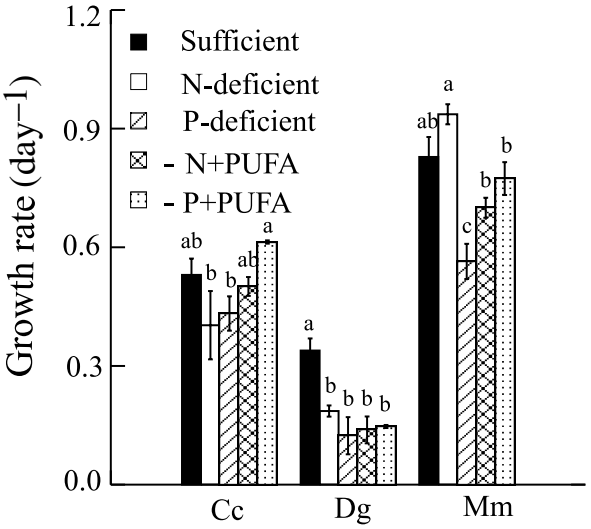

Fig. 1 Growth rates of cladocerans in nutrient-limited alga (*Scenedesmus spinosus*) and with supplements of fish oil rich in PUFA. Algal concentration in all treatments was 0.5 mg C L^{-1} and fish oil emulsions were 0.3 mg C L^{-1}. Data are mean growth rates ±SE. Different letters above bars indicate significant differences (Tukey test, $P < 0.05$).

Nutrient deficiency also affected clutch size (number of eggs/female) (Fig. 2). ANOVA revealed significant differences among cladoceran species, treatments and a significant interaction between species and treatment (Table 4). The clutch size of *M. micrura* was higher than the other cladocerans, even in nutrient-

Table 2 Relative fatty acid composition (as percentage of total FA) of *Scenedesmus spinosus* in the three culture conditions

Fatty acids	N/P-sufficient	P-deficient	N-deficient
14 : 0 (Miristic)	0.3	0.5	0.7
16 : 0 (Palmitic)	40.6	17.1	12.5
16 : 1 (Palmitoleic)	0.5	0.4	0.3
18 : 0 (Estearic)	3.5	1.7	2.0
18 : 1ω9 (Oleic)	14.8	13.7	15.3
18 : 2ω6 (Linoleic)	2.1	7.2	8.2
18 : 3ω3 (Linolenic)	1.3	6.5	9.8
20 : 5ω3 (EPA)	ND	ND	ND
22 : 6ω3 (DHA)	ND	ND	ND

ND = non-detected.

Table 3 Results of the ANOVA for the effects of species, treatment and interaction in the growth rate of cladocerans

Source	Sum of squares	d.f.	Mean squares	*F*	*P*
Species	2.474	2	1.237	455.9	<0.0001
Treatment	0.195	4	0.049	17.9	<0.0001
Species × treatment	0.215	8	0.027	9.9	<0.0001
Error	0.081	30	0.003		

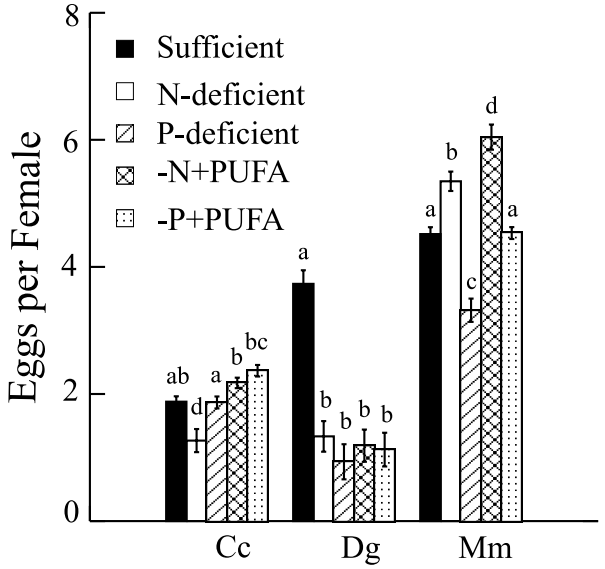

Fig. 2 Clutch size of cladocerans in nutrient-limited alga (*Scenedesmus spinosus*) and with supplements of fish oil rich in PUFA. Same concentrations as in Fig. 1. Data are mean number of eggs ± SE. Different letters above bars indicate significant differences (Tukey test, $P < 0.05$).

Table 4 Results of the ANOVA for the effects of species, treatment and interaction in the number of eggs produced

Source	Sum of squares	d.f.	Mean squares	*F*	*P*
Species	686.8	2	343.4	638.5	<0.0001
Treatment	67.7	4	16.9	31.5	<0.0001
Species × treatment	147.1	8	18.4	34.2	<0.0001
Error	180.2	335	0.538		

deficient algae (Fig. 2), and followed the same pattern of growth rates (Fig. 1). The response of *M. micrura* to N-deficient algae was curious, producing significantly more eggs per female than with N/P-sufficient algae and showing evident increase in lipid reserves (drops) in their tissues. *D. gessneri* had relatively high clutch size in N/P-sufficient algae, but did poorly in all other treatments (Fig. 2). Most animals produced only one or no eggs in nutrient-deficient algae. On the other hand, *C. cornuta* had an intermediate clutch size in all treatments, showing a significant decrease only in N-deficient algae.

The addition of PUFA to nutrient-limited algae had different effects on growth rates and egg production of cladocerans (Figs 1 and 2). For *C. cornuta*, PUFA additions to P-deficient alga significantly increased growth rate and egg production relative to P-deficient and also to N-deficient algae. When PUFA were added to N-deficient alga, however, *C. cornuta* did not significantly improve growth, but significantly increased clutch size. For *D. gessneri*, the addition of PUFA did not result in any significant improvement of growth rate or clutch size. For *M. micrura*, there was a significant increase in growth rate only when PUFA were added to P-deficient alga. Growth rate of *M. micrura* in –N + PUFA was significantly lower than in N-deficient alga, probably because of an experimental artifact, as there was no significant difference between these treatments and N/P-sufficient alga. Also, clutch size was significantly increased when N-deficient alga was supplemented with PUFA.

Discussion

The colimitation in N and P showed in this work has also been reported in other studies (Sterner, 1993; Müller-Navarra, 1995b; Weers & Gulati, 1997; Sundbom & Vrede, 1997; Lürling & Van Donk, 1997) and is probably related to a reduction in protein synthesis (i.e. enzymes) and accumulation of carbohydrate and lipids in nutrient-limited algae (Darley, 1988).

Increases in linoleic and linolenic acids and no EPA and DHA in P-deficient *Scenedesmus* were also found by Müller-Navarra (1995b) and Boersma (2000). Other studies have also shown that *Scenedesmus* have non-detectable or trace amounts of EPA and DHA, while having substantial amounts of other PUFA such as linoleic and linolenic acids (Ahlgren *et al*., 1990; Ahlgren, Gustafsson & Boberg, 1992; De Lange & Van Donk, 1997; Isik *et al*., 1999; Boersma, 2000). Weers & Gulati (1997) also found an increase in linolenic acid in *Chlamydomonas reinhardtii* under nutrient limitation and no EPA and DHA in any nutrient condition.

In our study we did not measure all classes of fatty acids, thus we cannot conclude that there was a general trend to accumulate saturated fatty acids at the expense of PUFA, especially ω3 and ω6 fatty acids (Müller-Navarra, 1995b; Weers & Gulati, 1997). In fact, two important PUFA increased in nutrient-deficient algae. Linoleic and linolenic acids are essential for almost all animals because they are not capable of synthesising either (Stanley-Samuelson, 1994a). Linolenic acid can be further metabolised into EPA and

DHA. Many organisms, such as freshwater fish and terrestrial vertebrates, are able to synthesise EPA and DHA (Watanabe, 1982; Lehninger, Nelson & Cox, 1993), but conversion rates are insufficient to maintain optimal growth in aquatic invertebrates (Jones, Kanazawa & Ono, 1979; Kanazawa, Teshima & Endo, 1979; Waldock & Holland, 1984). It is not known, however, whether cladocerans are able to convert linolenic acid into EPA and DHA. Certain algae, such as diatoms and crytophyceans, are rich in ω3 fatty acids such as EPA and DHA, and therefore are an excellent source of essential fatty acids for freshwater cladocerans. Other algae, such as cyanophyceans and chlorophyceans, are poor sources of these fatty acids (Ahlgren *et al*., 1992; Müller-Navarra, 1995b; Brett & Müller-Navarra, 1997; Lurling & Van Donk, 1997; Isik *et al*., 1999).

As *S. spinosus* did not have EPA and DHA, fish oil additions were the only source of these fatty acids. In the case of *C. cornuta* and *M. micrura*, animals significantly increased growth rate and clutch size when PUFA were added to P-deficient alga, suggesting that the limited growth was probably related to deficiency in fatty acids. Also, growth rates in –P + PUFA were similar to N/P-sufficient algae, suggesting that nutrient deficiency was not the primary cause of reduced growth and was overcome by the addition of essential fatty acids. This was not observed for *D. gessneri*, which suggests that mineral deficiency was more important than limitation of fatty acids for this cladoceran. On the other hand, *M. micrura* had an exceptionally high growth rate and clutch size in the N-deficient alga.

The evident increase in lipid reserves (drops) in *M. micrura* tissues may be related to the increase in linoleic and linolenic acids in N-deficient alga, both considered essential to cladocerans (Weers & Gulati, 1997). This does not explain, however, why *M. micrura* did not grow and produce eggs as well in P-deficient alga, which also had an increase in those fatty acids. Probably, P-deficiency is more costly to *M. micrura* than N-deficiency, as it is a fast-growing species and may have high phosphorus demands (Sterner & Schulz, 1998; Main *et al*., 1997). However, given the extreme P limitation of the algae, it was surprising that the fast-growing *M. micrura* was able to grow and reproduce at rates even higher than the other cladocerans. This suggests that, even though the P-balance of their tissues should have declined in P-deficient diet, they were able to assimilate enough of the scarce element to allow growth and reproduction.

According to A. S. Ferrão-Filho (unpublished data), *M. micrura* and *C. cornuta* have higher P-contents (1.5–2.0%) in their tissues than temperate *Daphnia* species (1.0–1.5%; DeMott, Gulati & Van Donk, 2001; DeMott in press). Thus, one should expect that these small, fast-growing cladocerans should have higher P demands and hence were severely affected by P deficiency, which were not the case in our study. This suggests that, contrary to stoichiometry theory, the P-content of the animals and its diet were not good predictors of cladocerans response to P-deficient food. Other traits must be involved, such as metabolic rate, digestive and absorption capabilities of the species.

Contrary to Müller-Navarra (1995a), Wacker & von Elert (2001) did not find a significant correlation between growth rates of *Daphnia* in seston and EPA or DHA. They found, however, a strong and significant correlation of *Daphnia* growth rates with α-linolenic acid. Also, after supplementing seston with *Stephanodiscus hantzschii*, rich in EPA, Wacker & von Elert (2001) did not observe any change in the growth rates of *Daphnia*, and concluded that these two fatty acids, EPA and α-LA, were not mutually substitutable resources and may have different physiological functions. In their study, seston C : P ratios never exceeded 300, which is considered a threshold for *Daphnia* growth (Urabe & Watanabe, 1992; Sterner, 1993), and thus biochemical constraints were more important than P-limitation. In our study, given the extreme P-limitation of the algal diet, *D. gessneri* was not able to maintain good growth, even when supplemented with fish oil rich in EPA, showing that elemental limitation was more important than fatty acids limitation for this cladoceran.

Von Elert & Wolffrom (2001) also showed that supplementation of lipids as single or mixtures of fatty acids (Linoleic and EPA) to *Synechococcus elongatus*, which is poor in PUFA, did not enhance growth rates of *Daphnia galeata*. They found, however, that feeding the animals first with *Scenedesmus obliquus*, which had no EPA, the low food quality of the cyanobacterium was partially mitigated and animals significantly increased growth, suggesting that the poor growth with *S. elongatus* was because of a deficiency in lipids other than PUFA. These findings, together with our data from tropical cladoceran species, revealed puzzling, as all these cladocerans

are considered closely related species, and suggest that differential mechanisms to cope with food quality can operate in a very small range of genetic variability.

Digestion resistance could be one of the reasons why cladocerans had lower growth rate and clutch size when fed P-limited algae (Van Donk & Hessen, 1993, 1997). The addition of fish oil emulsions, on the other hand, could compensate for the lower assimilation of algal carbon and increases in growth rate and clutch size would be explained by an increase in energy availability. In no case, however, did the addition of PUFA to nutrient-deficient algae increase the growth rate of cladocerans to values higher than the N/P-sufficient algae, which suggests that the addition of extra energy (lipids) had no important effect on growth rates. A treatment with addition of PUFA to N/P-sufficient algae would have allowed stronger inferences about energy limitation because of digestion resistance in P-limited alga. In this case, if the addition of fish oil emulsion increased growth rates to values higher than with the N/P-sufficient alga, we would have evidence of energy limitation rather than limitation by fatty acids. However, as even N/P-sufficient *S. spinosus* did not have any EPA or DHA, we would still have the possibility of PUFA limitation. Adding PUFA-poor and PUFA-rich emulsions to P– (deficient) and P+ (sufficient) *S. obliquus*, Boersma (2000) found increases in the growth rate of *Daphnia magna* when PUFA emulsions were added only to P+ alga, and argued that PUFA served merely as a source of energy and not of essential fatty acids. In our study, however, additions of PUFA-rich emulsions to P-deficient alga significantly increased growth rates and clutch sizes of *M. micrura* and *C. cornuta*. On the other hand, *D. gessneri* did not improve growth and egg production when supplemented with PUFA, so energy limitation can be disregarded here.

N/P-sufficient and N-deficient algae showed also some degree of P-limitation during the growth phase (Table 1) and this could also influence cladoceran performance. However, growth rates were near to maximal rates reported in other studies with the same species (Ferrão-Filho & Azevedo 2003; Ferrão-Filho *et al*., in press) and it is likely that the small degree of P-limitation was not enough to impose a measurable limitation in growth. The C : P ratio of P-deficient algae, however, was very high and comparable with the severely P-limited *Scenedesmus* (LOP) used in the studies of Sterner (1993) and Urabe *et al*. (1997). Differently from the results found in these two studies, in which *Daphnia* was affected only by P-limited algae, our results showed that cladocerans were differentially affected by nutrient-deficient algae. While *M. micrura* had decreased growth and reproduction with P-deficient algae, *C. cornuta* was affected only by N-deficient algae, having decreased clutch sizes. On the other hand, *D. gessneri* was equally affected by both nutrient-deficient algae. This shows that these closely related cladocerans differ in their sensitivity to the nutrient status of the food.

Responses to PUFA additions also differed among cladocerans, suggesting that essential fatty acids are more important for some species than others. The increase in clutch size of *M. micrura* and *C. cornuta* with PUFA supplements also shows that these fatty acids were readily assimilated and allocated to reproduction. DeMott & Müler-Navarra (1997) also found increased clutch sizes in *Daphnia* when PUFA were added to a cyanobacterium (*S. elongatus*) rich in P but poor in essential fatty acids. In another study, Ferrão-Filho *et al*. (2000) also showed increased clutch sizes in the tropical cladoceran *Moinodaphnia macleay* with PUFA supplements to the same cyanobacterium. In experiments with microencapsulated fish oil, rich in EPA & DHA, Sundbom & Vrede (1997) found increased somatic growth and fecundity in *D. galeata* above levels observed for P-rich but not for P-deficient *Scenedesmus*. As suggested by Sterner & Schulz (1998), the results of the above studies show that limitation by fatty acids may be important at low C : P ratios, and that P-limitation is more important than PUFA limitation at high C : P ratios. These authors concluded that P-limitation can shift to fatty acid limitation in nature, when P-rich cyanobacteria dominate. In this study, however, we found evidence that PUFA were important even in very high C : P ratios (>1000).

We conclude that PUFA are important resources for tropical cladocerans, increasing growth and clutch size when supplemented to nutrient-deficient algae. Differences between cladoceran species, regarding sensitivity to elemental and biochemical deficiencies in algae, can be an important mechanism of selectivity in environments dominated by nutrient-limited or PUFA-limited algae.

Acknowledgments

We thank Dr W. R. DeMott for a review of the manuscript and Dr João Atílio Jorge for the use of his laboratory and equipment. We also thank FAPESP for a research grant to MSA (97/10407–6) and for grants to ASFF (98/12999–0) and CF (98/14823–7).

References

Ahlgren G., Gustafsson I.-B. & Boberg M. (1992) Fatty acid and chemical composition of freshwater microalgae. *Journal of Phycology*, **28**, 37–50.

Ahlgren G., Lundsted L., Brett M. & Fosberg C. (1990) Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. *Journal of Plankton Research*, **12**, 809–818.

Boersma M. (2000) The nutritional quality of P-limited algae for *Daphnia*. *Limnology and Oceanography*, **45**, 1157–1161.

Brett M.T. & Müller-Navarra D.C. (1997) The role of highly unsaturated fatty acids in aquatic food web processes. *Freshwater Biology*, **38**, 483–499.

Darley W.M. (1988) *Algal Biology: A Physiological Approach.* Blackwell Scientific Publications, Oxford, London.

De Lange H.J. & Van Donk E. (1997) Effects of UV-B irradiated algae on life history traits of *Daphnia pulex*. *Freshwater Biology*, **38**, 711–720.

DeMott W.R. (in press) Implications of element deficits for zooplankton growth. *Hydrobiologia*, in press.

DeMott W.R. & Müler-Navarra D.C. (1997) The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. *Freshwater Biology*, **38**, 649–664.

DeMott W.R., Gulati R.D. & Van Donk E. (2001) Effects of dietary phosphorus dificiency on the abundance, phosphorus balance, and growth for *Daphnia cucculata* in three hypereutrophic Dutch lakes. *Limnology and Oceanography*, **46**, 1871–1880.

Elser J.J., Hayakawa K. & Urabe J. (2001) Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. *Ecology*, **82**, 898–903.

Fassbender H.W. (1973) Simultane P-Bestimmung in N-Kjeldahl-AusfschluB von Bodenproben. *Die Phosphorsäure*, **30**, 40–53.

Ferrão-Filho A.S. & Azevedo S.M.F.O. (2003) Effects of unicellular and colonial forms of toxic *Microcystis aeruginosa* from laboratory cultures and natural populations on tropical cladocerans. *Aquatic Ecology*, **37**, 23–35.

Ferrão-Filho A.S., Arcifa M.S. & Fileto C. (in press) Resource limitation and food quality for cladocerans in a tropical Brazilian lake. *Hydrobiologia*, in press.

Ferrão-Filho A.S., Azevedo S.M.F.O. & DeMott W.R. (2000) Effects of toxic and non-toxic cyanobacteria on the life history of tropical and temperate cladocerans. *Freshwater Biology*, **43**, 1–19.

Goulden C.E., Moeller R.E., McNair J.N. & Place A.R. (1998) Lipid dietary dependencies in zooplankton. In: *Lipids in Freshwater Ecosystems* (Eds M.T. Arts & B.C. Wainman). Springer-Verlag, New York, pp. 91–108.

Harrison P.J., Thompson P.A. & Calderwood G.S. (1990) Effects of nutrient and light limitation on the biochemical composition of phytoplankton. *Journal of Applied Phycology*, **2**, 45–56.

Hessen D.O. (1990) Carbon, nitrogen and phosphorus status in *Daphnia* at varying food conditions. *Journal of Plankton Research*, **12**, 1239–1249.

Isik O., Sarihan E., Kusvuran E., Gül Ö. & Erbatur O. (1999) Comparison of the fatty composition of freshwater fish larvae *Tilapia zillii*, the rotifer *Brachionus calyciflorus*, and the microalgae *Scenedesmus abundans*, *Monoraphidium minitum* and *Chlorella vulgaris* in the algae-rotifer-fish larvae food chains. *Aquaculture*, **174**, 299–311.

Jones D.A., Kanazawa A. & Ono K. (1979) Studies on the nutritional requirements of the larval stages of *Panaeus japonicus*. *Marine Biology*, **54**, 261–267.

Kanazawa A., Teshima S. & Endo M. (1979) Requirements of prawn, *Panaeus japonicus*, for essential fatty acids. *Memoirs of the Faculty of Fisheries*, Kagoshima University, 28, 27–33.

Kilham S.S., Kreeger D.A., Goulden C.E. & Lynn S.G. (1997) Effects of algal food quality on fecundity and population growth rates of *Daphnia*. *Freshwater Biology*, **38**, 639–647.

Kreeger D.A., Goulden C.E., Kilham S.S., Lynn S.G., Datta S. & Interlandi S.J. (1997) Seasonal changes in the biochemistry of lake seston. *Freshwater Biology*, **38**, 539–554.

Lehninger A.L., Nelson D.L. & Cox M.M. (1993) *Principles of Biochemistry*, 2nd edn. Worth Publishers, New York, NY.

Lurling M. & Van Donk E. (1997) Life history consequences for *Daphnia pulex* feeding on nutrient-limited phytoplankton. *Freshwater Biology*, **38**, 693–709.

Mackareth F.J.H., Heron J. & Talling J.F. (1978) *Water Analysis: Some Revised Methods for Limnologists* (scientific Publication n.36). Freshwater Biological Association, Cumbria and Dorser, England.

Main T.M., Dobberfuhl D.R. & Elser J.J. (1997) N : P stoichiometry and ontogeny of crustacean zooplankton:

a test of the growth rate hypothesis. *Limnology and Oceanography*, **42**, 1474–1478.

Morris I. (1981) Photosynthetic products, physiological state, and phytoplankton growth. *Canadian Bulletin of Fisheries and Aquatic Sciences*, **210**, 83–102.

Müller-Navarra D.C. (1995a) Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. *Archiv für Hydrobiologie*, **132**, 297–307.

Müller-Navarra D.C. (1995b) Biochemical versus mineral limitation in *Daphnia*. *Limnology and Oceanography*, **40**, 1209–1214.

Müller-Navarra D.C., Brett M.T., Liston A.M. & Goldman C.R. (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature*, **401**, 74–77.

Piorreck M. & Pohl P. (1984) Formation of biomass, total proteins, chlorophylls, lipids and fatty acids in green and blue-green algae during one growth phase. *Phytochemistry*, **23**, 217–223.

Stanley-Samuelson D.W. (1994a) Assessing the significance of prostaglandins and other eicosanoids in insect physiology. *Journal of Insect Physiology*, **40**, 3–11.

Stanley-Samuelson D.W. (1994b) The biological significance of prostaglandins and related eicosanoids in invertebrates. *American Zoologist*, **34**, 589–598.

Stemberger R.S. (1981) A general approach to the culture of planktonic rotifers. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 721–724.

Sterner R.W. (1993) *Daphnia* growth on varying quality of *Scenedesmus*: mineral limitation of zooplankton. *Ecology*, **74**, 2351–2360.

Sterner R.W. & Schulz K.L. (1998) Zooplankton nutrition: recent progress and a reality check. *Aquatic Ecology*, **32**, 261–279.

Strickland J.D.H. & Parsons T.R. (1972) *A Practical Handbook of Seawater Analysis*. Bulletin of Fishery Research Bd, Canada, 167, 310 pp.

Sundbom M. & Vrede T. (1997) Effects of fatty acids and phosphorus content of food on the growth, survival and reproduction of *Daphnia*. *Freshwater Biology*, **38**, 665–674.

Tung K.W. & Dam H.G. (1999) Limitation of zooplankton production: beyond stoichiometry. *Oikos*, **84**, 537–542.

Urabe J. & Watanabe T. (1992) Possibility of N or P limitation for planktonic cladocerans: an experimental test. *Limnology and Oceanography*, **87**, 244–251.

Urabe J., Clasen J. & Sterner W. (1997) Phosphorus limitation of *Daphnia* growth: is it real? *Limnology and Oceanography*, **42**, 1436–1443.

Van Donk E. & Hessen D.O. (1993) Grazing resistance in nutrient stressed phytoplankton. *Oecologia*, **93**, 508–511.

Van Donk E. & Hessen D.O. (1997) Altered cell morphology in nutrient-deficient phytoplankton and its impact on grazers. *Limnology and Oceanography*, **42**, 357–364.

Von Elert E. & Wolffrom T. (2001) Supplementation of cyanobacterial food with polyunsaturated fatty acids does not improve growth of *Daphnia*. *Limnology and Oceanography*, **46**, 1552–1558.

Wacker A. & von Elert E. (2001) Polyunsaturated fatty acids: evidence for non-substitutable biochemical resources in *Daphnia galeata*. *Ecology*, **82**, 2507–2520.

Waldock M.J. & Holland D.L. (1984) Fatty acid metabolism in young oysters, *Crassiostera gigas*: polyunsaturated fatty acids. *Lipids*, **19**, 332–336.

Watanabe T. (1982) Lipid nutrition in fish. *Comparative Biochemistry and Physiolgy (B)*, **73B**, 3–15.

Weers P.M.M. & Gulati R.D. (1997) Growth and reproduction of *Daphnia galeata* in response to changes in fatty acids, phosphorus, and nitrogen in *Chlamydomonas reinhardtii*. *Limnology and Oceanography*, **42**, 1584–1589.

(*Manuscript accepted 13 January 2003*)