

Testing mineral food quality for tropical cladocerans from a shallow Brazilian lake

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ABSTRACT: Testing mineral food quality for tropical cladocerans from a shallow Brazilian lake. Bioassays were carried out to evaluate individual growth and reproduction of Brazilian tropical cladocerans with addition of nitrogen (N) and phosphorus (P) to natural seston. Experiments were made seasonally, except those on the synergic effect of both N and P addition to seston. Cohorts originated from cultivated females were submitted to the following treatments: 1) lake seston, 2) lake seston + P, 3) lake seston + N, and 4) *Scenedesmus spinosus* (1mg C L⁻¹). In the experiments on synergism the last treatment was replaced by lake seston + NP. The sestonic C:P molar ratios varied from low (43-73), medium (119) to high (412-460), and the C:N molar ratios were always low, according to threshold ratios estimated for temperate lakes. P addition to seston did not enhance growth and reproduction of cladocerans when sestonic C:P ratios were low, medium, and high. However, significant higher growth rate of *Daphnia gessneri* was found in the seston + NP compared to seston alone in one experiment on synergism. C, N, P body content of cladocerans was similar to that of temperate counterparts. Energy limitation related to carbon content or food quality seems to be most important in controlling cladoceran populations in this lake than food mineral content.

Key words: C:N ratio, C:P ratio, Mineral limitation, Nitrogen, Phosphorus, Seston, Zooplankton

RESUMO: Teste da qualidade mineral do alimento para cladóceros tropicais de um lago brasileiro raso. Foram realizados experimentos para avaliar o crescimento individual e a reprodução de cladóceros tropicais com a adição de nitrogênio (N) e fósforo (P) ao séston natural. Os experimentos foram realizados sazonalmente, exceto aqueles sobre o efeito sinérgico da adição conjunta de N e P ao séston. Coortes originadas de fêmeas cultivadas foram submetidas aos seguintes tratamentos: 1) séston do lago, 2) séston do lago + P, 3) séston do lago + N e 4) *Scenedesmus spinosus* (1 mg C L⁻¹). Nos experimentos de sinérgismo, o último tratamento foi substituído por séston do lago + NP. As razões molares C:P do séston variaram de baixas (43-73), a médias (119) e elevadas (412-460), sendo as razões molares C:N sempre baixas, de acordo com razões estimadas para lagos temperados. A adição de P ao séston não aumentou o crescimento e a reprodução dos cladóceros quando as razões C:P eram baixas, médias ou elevadas. No entanto, as taxas de crescimento de *Daphnia gessneri* foram significativamente maiores no tratamento séston + NP comparado ao séston natural em um dos experimentos de sinérgismo. O conteúdo corporal de C, N e P dos cladóceros foi similar ao encontrado para cladóceros de região temperada. A limitação por energia relacionada ao conteúdo de carbono ou à qualidade do alimento parece ser mais importante no controle das populações de cladóceros neste lago comparado ao conteúdo mineral do alimento.

Palavras-chave: Fósforo, limitação mineral, nitrogênio, razão C:N, razão C:P, séston, zooplâncton

Introduction

Growth of freshwater zooplankton is determined by several factors, particularly food quantity and quality. Several authors

consider phosphorus (P) and nitrogen (N) fundamental components of food quality and sestonic C:N:P ratios the most important indicators of food quality (Hessen, 1992;

Sommer, 1992; Sterner & Hessen, 1994; Sterner et al., 1992; Urabe and Watanabe, 1992).

Phosphorus has been the focus of considerably recent studies (Gulati & DeMott, 1997; Sterner & Elser, 2002) and as a constituent of phospholipids, related with energy storage (ATP) and the synthesis of nucleic acids plays an important role in growth and reproduction.

However, the role of nitrogen in determining food quality has received less attention in nutritional studies of freshwater zooplankton (Acharya et al., 2004). Nitrogen is an important component of aminoacids and the growth of many animals can be mainly constrained by dietary protein (White, 1993). It is possible that N-limitation of food is more important in tropical lakes and P-limitation in temperate ones (Lewis, 1996; Talling & Lemoalle, 1998).

Herbivores, such as *Daphnia*, are supposed to maintain relatively constant C:P and C:N stoichiometric relationships even when the elements vary in their diets (Hessen, 1990; Sterner & Elser, 2002). However, DeMott et al. (2004) found strong evidence that P content in *Daphnia* in the environment varies according to food P levels, expressed as C:P ratio, contradicting the homeostasis hypothesis.

C:N and C:P ratios of zooplankton are lower than those of phytoplankton (Andersen & Hessen, 1991; Hessen, 1990), suggesting that the animals can concentrate N or P in relation to C in their tissues through feeding and metabolic processes for investing in growth and reproduction (Sterner, 1990). If food C:N or C:P ratios are low, the necessary amount of N and P are easily obtained by the animals, in contrast with conditions of high ratios, when N or P should be concentrated, by reducing C incorporation in the animal tissues. Therefore, zooplankton net production is supposed to be limited by N or P above C:N and C:P thresholds (Urabe & Watanabe, 1992).

The general aims of this paper were to test responses of cladocerans from a shallow tropical lake: 1) to additions of N, P and C to seston; 2) to different conditions of C:N and C:P ratios. To achieve these objectives, the following analyses and experiments were performed: a) elemental analyses of cultivated alga, seston, and cladocerans; b) in vitro experiments for evaluating growth and reproduction of

cladocerans from the lake fed cultivated edible alga, natural seston and seston plus N, P and C (*S. spinosus*) in the four seasons of one year; c) in vitro experiments to test the synergic effect of nutrients by adding both N and P to natural seston; d) in vitro experiments to test responses of cladocerans in conditions of low, medium and high sestonic C:P ratios and low C:N ratios, defined according to threshold ratios estimated for temperate lakes. The aim was to find out whether stoichiometric theory holds for those tropical cladocerans, as well as to estimate threshold ratios based on those usually recorded in the lake.

Material and methods

Study Site

Lake Monte Alegre (21° 11'S, 47° 43'W) is located in southeastern Brazil, and resulted from damming the Laureano Creek, which belongs to River Pardo basin, in 1942. The climate is tropical, marked by a cool-dry season (May to September) and a warm-wet one (October to April). It is a small, eutrophic, shallow (area = 7 ha, maximum depth = 5 m), warm discontinuous polymictic reservoir.

The dam is not manipulated, the winds are weak, the outlet is superficial, the retention time is not so short in relation to lake dimensions (~ 45 days), favoring a tendency for stratification (Arcifa et al., 1990). Stratification is more stable during the warm season, when dissolved oxygen concentrations can drop to zero in the deepest layers of the lake. NH_4^+ and NO_3^- ranged from 0.02 to 1.18 mg L⁻¹ and 0.01 to 0.99 mg L⁻¹, respectively, and PO_4^{3-} from 8 to 67 mg L⁻¹ at the upper layers of the lake during 2001-2002.

Phytoplankton densities varied from 8 to 14 x 10³ ind ml⁻¹ in 1998-1999, being dominated by Chlorophyceae, followed by Cyanophyceae (Fileto, 2001), rarely occurring episodes of water blooms. From 1985 to 2002, eight planktonic cladoceran species have been recorded in the lake in four annual studies (Arcifa et al., in prep.): *Bosmina tubicen*, *Ceriodaphnia cornuta*, *C. richardi*, *Daphnia ambigua*, *D. gessneri*, *Diaphanosoma birgei*, *Moina micrura*, and *M. minuta*. From 1998 to 2001, the most frequent and sometimes the most abundant were *D. gessneri* and *D. ambigua*, but lately peaks of *Ceriodaphnia richardi* are common.

Seston samplings and phytoplankton analysis

Seston was sampled in the deepest region of the lake (5 m) by pumping water from surface to 3.5 m, with a bilge pump (Model 34600-0000, Jabsco ITT Ind., Costa Mesa, USA), delivering 30 L min⁻¹. The water was filtered through a 140 µm net for excluding large zooplankton.

Phytoplanktonic algae were enumerated following Utermöhl's method (1958), the biovolume estimated by geometric forms, and their carbon content assumed to be 10% of the biovolume (Vollenweider, 1974). Species composition, size fractions (< 20 µm and > 20 µm) and morphological characteristics of algae were analyzed and the results presented as density and biomass.

Alga and cladoceran cultures

The chlorophycean *Scenedesmus spinosus* R. Chodat (length 14 µm) was cultivated in MBL medium (Stemberger, 1981) in an environmental chamber (model 347 CDG, FANEM, SP, Brazil) at constant temperature of 23°C and a 12: 12 h photoperiod cycle and constant aeration.

Zooplankton was collected in the lake with a 60 µm mesh net by vertical hauls. Cladocerans were maintained in 500 ml bottles with lake water filtered in glass fiber filters (0.45 µm, Sartorius AG37070, Germany) plus the green alga *S. spinosus* (1 mg C L⁻¹), attached to a plankton wheel, rotating 1 minute every 5 minutes. Several generations were cultivated before the experiments and neonates from the 3rd

generation were used according to von Elert (2002).

Elemental analyses

Seston (initial and final of the experiments) and cultivated alga, concentrated by centrifugation (2000 rpm, 5 min.), were placed in previously weighed tin capsules, which were then dried at 60°C for 24 h. Non egg-bearing adult females from the lake and cultures were separated under a stereomicroscope and placed on glass slides and dried at 60°C for 24 h, being transferred to previously weighed tin capsules. Particulate C and N were evaluated on an elemental analyzer (Carlo-Erba CHN 1110). The analyses of particulate P were made according to Murphy & Riley (1962) with alga and seston samples filtered in glass fiber filters, and the processing of the animals following the same procedures for C/N analyses. C, N, and P content of the seston and the animals in the enrichment treatments were evaluated whenever the quantity of samples of animals and seston were sufficient.

Growth experiments

Six growth experiments were performed and are summarized in Table 1, using the most abundant cladoceran species in the lake during the period of each experiment.

Around 20 individuals, born within 24 h, were placed in 500 ml bottles, on a plankton wheel, rotating 1 minute every 5 minutes. Experiments I to IV, carried out in 2002, tested N and P additions to seston. Treatments, with three replicates each,

Table 1. Summary of the experiments.

Experiment	Date	Cladoceran/Max. length (mm)	Treatments
I	19-24/01/2002	<i>C. richardi</i> (0.80) and <i>D. ambigua</i> (0.90)	1) lake seston;
II	07-11/06/2002	<i>D. ambigua</i> and <i>D. gessneri</i> (1.16)	2) lake seston + P; 3) lake seston + N;
III	03-06/09/2002	<i>C. richardi</i>	4) <i>S. spinosus</i>
IV	03-07/12/2002	<i>C. richardi</i> and <i>D. gessneri</i>	
V	08-12/02/2003	<i>C. richardi</i> and <i>D. gessneri</i>	1) lake seston; 2) lake seston + P; 3) lake seston + N;
VI	21-25/01/2004	<i>C. richardi</i>	4) lake seston + NP

were: 1) lake seston, 2) lake seston + 25 mg PO₄-P L⁻¹ 3) lake seston + 260 mg NO₃⁻ L⁻¹, and 4) the chlorophycean *Scenedesmus spinosus* (1 mg C L⁻¹). Experiments V and VI, made in 2003 and 2005, tested the synergic effect of N and P additions to seston. Treatments, with three replicates each, were: 1) lake seston, 2) lake seston + 25 mg PO₄-P L⁻¹ 3) lake seston + 260 mg NO₃⁻ L⁻¹, and 4) lake seston + 25 mg PO₄-P L⁻¹ + 260 mg NO₃⁻ L⁻¹. Experiments were undertaken at the temperature of 24°C and 12: 12 h photoperiod cycle.

Before starting the experiments, ca. 10 neonates were placed on small, prepared pieces of aluminum foil, dried at 60°C for 24 h to determine the initial body weight. In the subsequent days (2, 3, or 5), depending on the duration of juvenile period of each species, ca. 5-10 animals were taken from each bottle, placed on glass slides, measured, dried at 60°C for 24 h, and weighed for biomass evaluation. The biomass was evaluated by weighing the animals on a microbalance (Mettler Toledo UMT-2) to the nearest 0.1 mg. The exponential growth rate was calculated by the equation:

$$g = [\ln(W_t) - \ln(W_0)]/t,$$

where: W₀ and W_t are the average weight of the animals in time 0 and after t days (3 – 5 days depending on the juvenile period of each species tested), respectively.

Only the juvenile growth was monitored but experiments were extended to evaluate the fecundity (eggs/total female) and clutch size.

The results were analyzed by General Linear Model (Estimate Model) and the significant differences by ANOVA (Systat Program) and post hoc Tukey test (Systat, version 9, 1999, SPSS, Chicago, IL, USA).

Results

Elemental analyses

The relative elemental composition of the green alga *S. spinosus* was 45.50 % ± 1.30 and 0.59 % ± 0.00 of carbon, nitrogen and phosphores, respectively. The elemental composition of lake seston (initial and final) and of seston enriched with N, P and NP are presented in Table II. N and P

Table II: Carbon, nitrogen and phosphorus concentrations and C:N and C:P ratios (molar) of seston offered to cladocerans during the experiments I to VI. Data are means ± SD for three replicates.

Experiments		Seston				
		C (mg L ⁻¹)	N (mg L ⁻¹)	P (mg L ⁻¹)	C:N	C:P
I	Initial seston	0.75 ± 0.05	0.20 ± 0.02	22.00 ± 3.40	4.25 ± 0.60	57.00 ± 13.50
	Final seston	0.65 ± 0.06	0.17 ± 0.03	30.50 ± 0.93	4.30 ± 3.50	46.50 ± 8.75
	Seston + N	0.60 ± 0.30	0.20 ± 0.07	-	4.00 ± 3.00	-
	Seston + P	0.39 ± 0.00	-	50.00 ± 20.55	-	24.95 ± 2.60
II	Initial seston	0.50 ± 0.05	0.11 ± 0.00	20.70 ± 2.60	5.85 ± 0.55	73.00 ± 0.15
	Final seston	0.55 ± 0.00	0.11 ± 0.00	22.70 ± 0.90	5.80 ± 0.30	66.00 ± 2.70
	Seston + N	0.75 ± 0.00	0.20 ± 0.00	-	4.12 ± 0.00	-
	Seston + P	0.70 ± 0.06	0.18 ± 0.00	44.00 ± 1.60	4.70 ± 0.40	44.00 ± 4.10
III	Initial seston	0.60 ± 0.00	0.11 ± 0.00	23.60 ± 2.70	6.35 ± 0.45	64.10 ± 1.00
	Final seston	0.47 ± 0.00	0.09 ± 0.00	26.95 ± 5.80	6.35 ± 0.15	41.45 ± 2.25
	Seston + N	0.44 ± 0.15	0.14 ± 0.00	-	3.60 ± 1.15	-
	Seston + P	0.60 ± 0.01	-	43.00 ± 1.30	-	37.40 ± 0.20*
IV	Initial seston	0.46 ± 0.00	0.09 ± 0.00	28.00 ± 2.30	5.75 ± 0.10	43.00 ± 3.85
	Final seston	0.50 ± 0.10	0.11 ± 0.00	23.00 ± 0.83	5.75 ± 1.30	63.00 ± 17.40
	Seston + N	0.50 ± 0.17	0.22 ± 0.00	-	2.70 ± 0.90	-
	Seston + P	0.50 ± 0.00	0.16 ± 0.00	66.30 ± 3.95	3.60 ± 0.03	20.80 ± 1.70*

Table II: Cont.

Experiments		Seston				
		C (mg L ⁻¹)	N (mg L ⁻¹)	P (mg L ⁻¹)	C:N	C:P
V	Initial seston	0.60 ± 0.03	0.12 ± 0.01	13.10 ± 0.40	5.80 ± 0.03	119.30 ± 0.04
	Seston + N	0.39 ± 0.02	0.13 ± 0.00	-	3.55 ± 0.00*	-
	Seston + P	0.90 ± 0.02	0.20 ± 0.01	17.80 ± 2.80	5.00 ± 0.28	125.20 ± 22.0
	Seston + NP	0.99 ± 0.02	0.25 ± 0.01	25.40 ± 2.10	4.60 ± 0.18	98.55 ± 0.65*
VI	Initial seston	1.70 ± 0.05	0.20 ± 0.01	11.40 ± 0.00	9.50 ± 0.70	412.00 ± 10.70
	Final seston	0.40 ± 0.10	0.045 ± 0.00	2.25 ± 0.00	10.35 ± 0.07	460.00 ± 19.80
	Seston + N	1.70 ± 0.05	0.35 ± 0.00	-	6.00 ± 0.30*	-
	Seston + P	1.70 ± 0.05	-	30.00 ± 0.00	-	160.00 ± 4.25*
	Seston + NP	0.40 ± 0.01	0.27 ± 0.05	29.90 ± 0.00	1.60 ± 0.20*	156.00 ± 4.00

additions significantly increased their concentrations in the seston, except N in experiment I (Tab. II). Sestonic C:N molar ratios were low during the experiments I to V and a little higher in experiment VI. C:P ratios were low in experiments I to IV, medium in experiment V, and high in experiment VI. C:P ratios were significantly reduced in experiments III ($P = 0.020$), IV ($P = 0.023$), V ($P = 0.12$) and VI ($P = 0.023$); C:N ratios in experiments V ($P = 0.026$) and VI ($P = 0.029$ and 0.020 with N and NP additions, respectively). Only the initial seston was analyzed during the experiment V as the final samples were damaged in the elemental analyzer.

Mean body carbon concentrations of cladocerans varied from 40.85 to 54.60%, with no significant differences between individuals from the lake and the laboratory in all experiments (Tab. III). N varied from 6.35 to 15.10% and P from 0.60 to 1.46%. N additions to seston significantly increased the relative content of *Ceriodaphnia richardi* only in experiment IV ($P = 0.020$).

Phytoplankton

Nanoplankton predominated in density (above 75%) and microplankton in biomass, except in experiment II in autumn (Tabs IV, V).

In the experiments I to IV the average algal carbon was lower in autumn (0.10 mg C L^{-1}), increasing in winter, spring and summer (0.35 , 0.45 , and 0.20 mg C L^{-1} , respectively). Nanoplankton contributed 70% (autumn), 54% (winter), 31% (spring), and 40% (summer) to the algal carbon. In

experiment V, average algal carbon was low (0.11 mg C L^{-1}) compared to experiment VI (0.70 mg C L^{-1}). Nanoplankton contribution was 10.2% and 17% to the algal carbon in experiments V and VI (summer), respectively.

Experiments

In the experiments with low C:P molar ratios (I to IV) N or P addition to seston did not enhance cladoceran biomass and growth rates (Figs 1, 2 a and b). However, significantly reduced growth rates of *D. ambigua* were found in experiment I, in summer, with N and P additions ($g = 0.01$ and 0.05 , respectively) compared to seston alone (0.23) in $t = 2$ days ($P = 0.018$ and $P = 0.015$, respectively).

No positive effect was observed on cladoceran growth rates in the seston + N or P treatments with intermediate C:P ratio (experiment V) (Fig. 3 a). However, significant higher growth rate of *D. gessneri* was found in the seston + NP compared to seston + P in $t = 4$ days ($P = 0.046$).

P addition to seston did not improve cladoceran growth in the experiment with high C:P molar ratio (VI) (Fig. 3 b), but N addition to seston negatively affected the initial growth, when loss of weight was observed and shown by the negative value of the exponential growth rate ($g = -0.13$ in $t = 2$ days) followed by the recovery of the initial weight in $t = 4$ days ($g = 0.00$).

Reproduction was not improved with N and P additions in the experiments with low C:P (Fig. 4 a and b). Negative effect on *C. richardi* reproduction occurred with P

Table III: Carbon, nitrogen and phosphorus relative content of cladocerans from the lake, laboratory cultures and seston treatment in the experiments I to VI.

Experiments	C	N	P	C	N	P	
	C. richardi			D. gessneri			
I	Cultures	48.55 ± 7.00	11.20 ± 4.00	1.00 ± 0.08	45.60 ± 2.20	10.30 ± 0.25	1.07 ± 0.15
	Lake	46.50 ± 2.10	12.00 ± 1.00	1.10 ± 0.03	47.25 ± 1.10	6.35 ± 1.10	1.06 ± 0.10
	Seston	52.50 ± 6.90	12.40 ± 1.60	1.10 ± 0.15	50.55 ± 9.25	10.30 ± 0.40	1.15 ± 0.07
	Seston + N	45.75 ± 6.00	10.40 ± 0.40	-	50.40 ± 1.30	12.35 ± 1.25	-
	Seston + P	-	-	1.20 ± 0.20	-	-	1.40 ± 0.02
	D. ambigua			D. gessneri			
II	Cultures	50.50 ± 2.10	10.40 ± 3.40	0.97 ± 0.04	49.10 ± 2.70	10.75 ± 3.90	1.00 ± 0.06
	Lake	40.85 ± 1.65	10.15 ± 0.35	1.00 ± 0.10	42.90 ± 1.40	6.60 ± 0.60	1.25 ± 0.20
	Seston	51.50 ± 9.20	9.55 ± 0.07	1.10 ± 0.14	54.15 ± 3.00	9.50 ± 0.70	1.40 ± 0.06
	Seston + N	50.40 ± 1.30	12.20 ± 1.65	-	54.90 ± 10.00	10.00 ± 0.20	-
	Seston + P	-	-	1.30 ± 0.20	-	-	1.30 ± 0.20
	C. richardi			D. gessneri			
III	Cultures	49.90 ± 2.30	8.55 ± 3.20	1.12 ± 0.20			
	Lake	46.30 ± 1.85	9.15 ± 3.00	1.00 ± 0.10			
	Seston	54.60 ± 3.90	8.85 ± 0.60	1.10 ± 0.15			
	Seston + N	51.20 ± 14.00	9.85 ± 0.90	-			
	Seston + P	-	-	1.40 ± 0.01			
	C. richardi			D. gessneri			
IV	Cultures	52.50 ± 3.25	7.10 ± 0.20	1.30 ± 0.25	48.00 ± 1.60	7.45 ± 0.50	1.00 ± 0.07
	Lake	48.45 ± 4.80	10.24 ± 3.55	1.15 ± 0.08	47.55 ± 3.80	10.75 ± 4.20	1.10 ± 0.20
	Seston	53.50 ± 4.95	9.10 ± 1.50	0.99 ± 0.01	48.15 ± 8.70	10.45 ± 0.15	1.46 ± 0.15
	Seston + N	49.95 ± 1.35	15.10 ± 1.20*	-	55.00 ± 5.65	10.90 ± 0.90	-
	Seston + P	-	-	1.20 ± 0.20	-	-	1.30 ± 0.10
	C. richardi			D. gessneri			
V	Cultures	49.10 ± 6.20	11.75 ± 3.25	0.99 ± 0.01	49.00 ± 2.75	9.80 ± 0.40	1.10 ± 0.10
	Lake	46.60 ± 1.95	10.40 ± 0.20	0.80 ± 0.04	46.50 ± 0.70	9.60 ± 0.05	0.75 ± 0.03
	Seston	52.45 ± 6.90	12.00 ± 1.10	1.10 ± 0.15	50.55 ± 9.25	11.00 ± 0.70	1.10 ± 0.03
	Seston + N	51.40 ± 0.80	10.70 ± 0.85	-	51.65 ± 0.50	12.20 ± 0.02	-
	Seston + P	-	-	1.18 ± 0.25	-	-	0.95 ± 0.02
	Seston +NP	-	-	0.98 ± 0.03			1.10 ± 0.03
	C. richardi			D. gessneri			
VI	Cultures	49.60 ± 0.05	9.10 ± 0.20	1.06 ± 0.00			
	Lake	49.70 ± 0.10	9.00 ± 0.15	1.00 ± 0.01			
	Seston	49.90 ± 0.10	11.60 ± 0.07	-			
	Seston + N	46.50 ± 0.15	7.00 ± 0.05	-			
	Seston + P	-	-	0.80 ± 0.01			
	Seston +NP	-	-	0.60 ± 0.00			

Table IV: Relative density and biomass (in brackets) of seston fractions (nano- and microplankton) regarding algae features during the experiments I to IV. Others = not ornamented, rounded, ovoid and filiform algae, (-) = absent.

Morphology/Exp.	I		II		III		IV	
	≤ 20 μm	> 20 μm						
% of each fraction	80.0 (37.0)	20.0 (63.0)	76.0 (73.0)	24.0 (27.0)	88.0 (53.0)	12.0 (74.0)	87.0 (31.4)	13.0 (66.6)
Colonial/mucilaginous	42.0 (34.4)	12.0 (10.6)	13.0 (3.5)	10.0 (39.5)	2.6 (0.8)	-	20.0 (27.0)	2.0 (3.35)
Spiny	4.0 (10.6)	16.0 (21.6)	3.0 (39.0)	3.0 (37.6)	-	62.0 (93.0)	1.0 (2.1)	4.6 (12.5)
Coenobium	-	3.0 (34.0)	-	-	-	-	2.6 (9.1)	7.0 (56.0)
Filamentous	-	10.0 (26.0)	-	2.0 (5.9)	-	-	-	58.0 (20.6)
Others	64.0 (54.6)	60.0 (5.6)	84.0 (57.5)	86.0 (16.6)	97.6 (99.2)	48.0 (7.0)	76.6 (61.6)	28.6 (5.35)

Table V: Relative density and biomass (in brackets) of seston fractions (nano- and microplankton) regarding algae features during the experiments V and VI. Others = not ornamented, rounded, ovoid and filiform algae, (-) = absent.

Morphology/Experiment	V		VI	
	≤ 20 μm	> 20 μm	≤ 20 μm	> 20 μm
% of each fraction	80.0 (10.2)	20.0 (89.8)	85.0 (17.0)	15.0 (63.0)
Colonial/mucilaginous	32.0 (6.0)	16.0 (0.2)	0.2 (45.2)	12.5 (1.0)
Spiny	-	19.0 (11.0)	0.5 (4.2)	0.5 (4.2)
Coenobium	3.0 (0.5)	0.5 (33.0)	0.3 (0.2)	-
Filamentous	-	1.4 (36.4)	-	28.6 (64.0)
Others	65.0 (93.5)	64.6 (17.4)	90.0 (50.4)	60.6 (30.8)

addition to seston as the cladoceran did not reproduce in the seston + P treatment in experiment III, in winter (Fig. 5 a), coinciding with the reduction of the growth rate from 0.41 in $t = 2$ days to 0.24 in $t = 3$ days (Fig. 2 a), suggesting a toxic effect of P addition.

The average fecundity of *D. gessneri* was significantly lower in the seston + P compared to seston alone in the experiment with intermediate C:P ratio (V) ($P = 0.041$) (Fig. 6 a) and is probably reflecting the lower growth rate found in this treatment

(Fig. 3 a).

There was no difference in reproductive parameters among treatments in the experiment with high C:P ratio (VI) (Fig. 6 b).

Cladoceran growth was significantly higher in experiment I, in summer ($P = 0.000$) (Fig. 1 a), and in experiment III, in winter ($P = 0.021$) when fed the green alga *S. spinosus* (Fig. 2 a). Reproduction of *C. richardi* and *D. ambigua* in experiment I, in summer, was also significantly enhanced in the green alga treatment ($P = 0.000$) (Fig. 4 a).

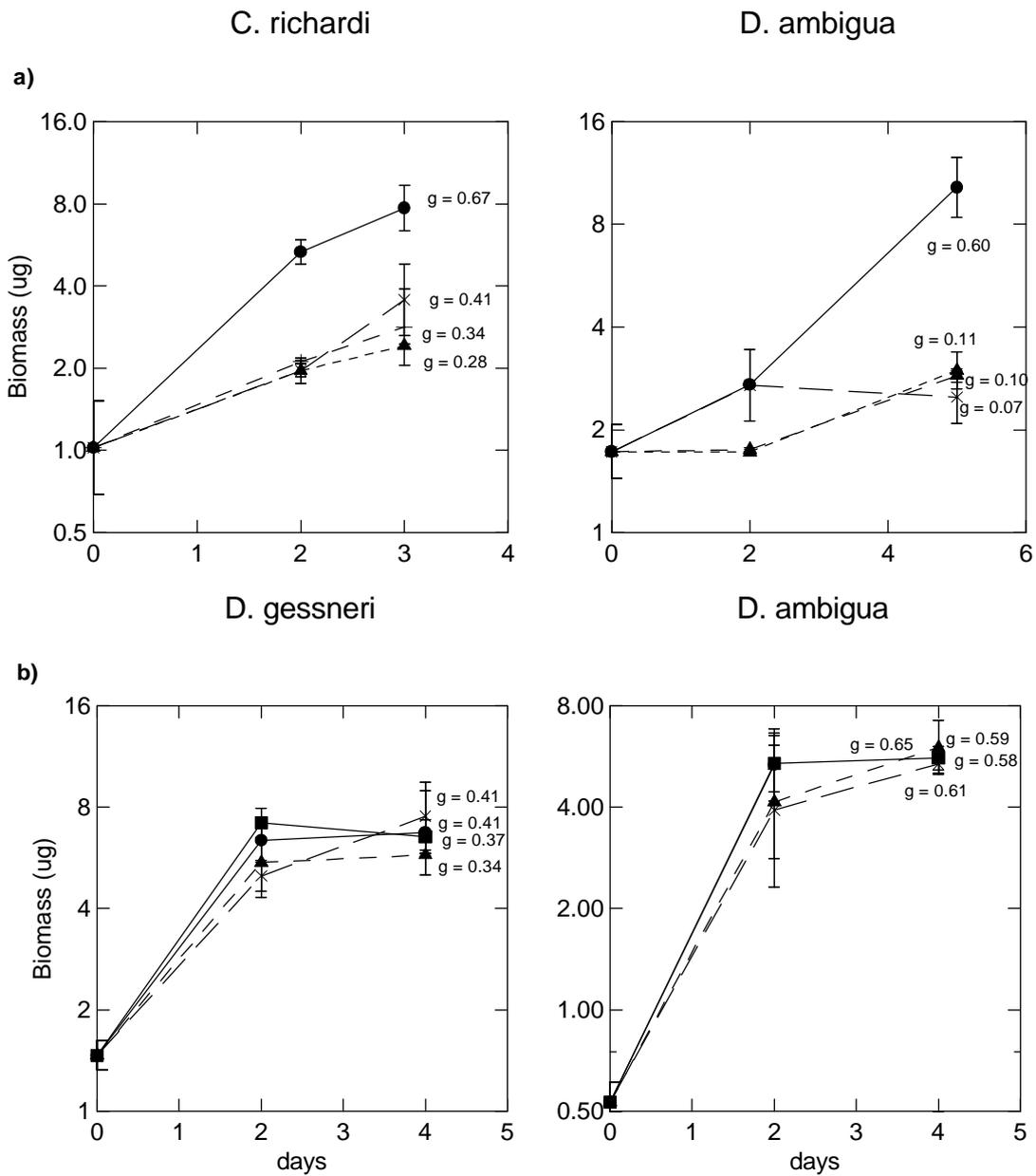


Figure 1: Biomass growth of the cladocerans *C. richardi* and *D. ambigua* (a) and *D. gessneri* and *D. ambigua* (b) in experiments I (summer) and II (autumn). Juvenile growth rates (g) for the last days are given for all species. (-) Chlorophycean, (x) Seston, (▲) Seston + N, (■) Seston + P.

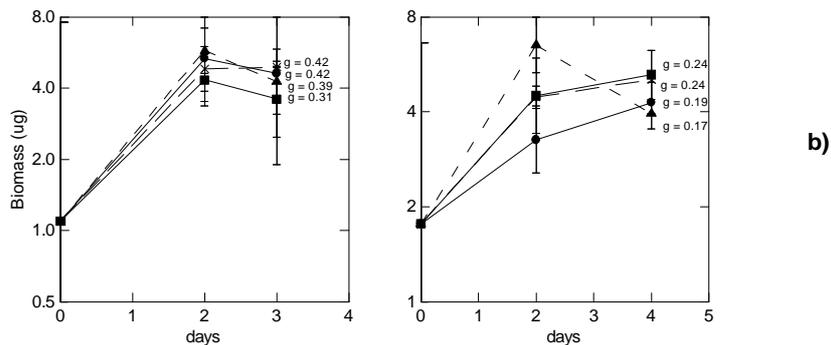
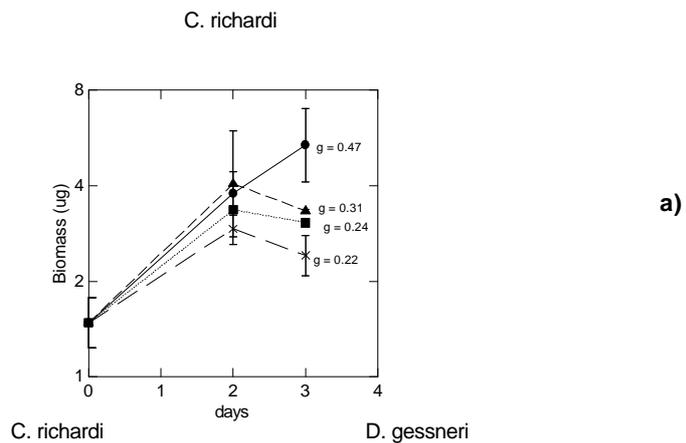


Figure 2: Biomass growth of *C. richardi* (a) and *C. richardi* and *D. gessneri* (b) in experiments III (winter) and IV (spring). Juvenile growth rates (g) for the last days are given for all species. (·) Chlorophycean, (x) Seston, (▲) Seston + N, (■) Seston + P.

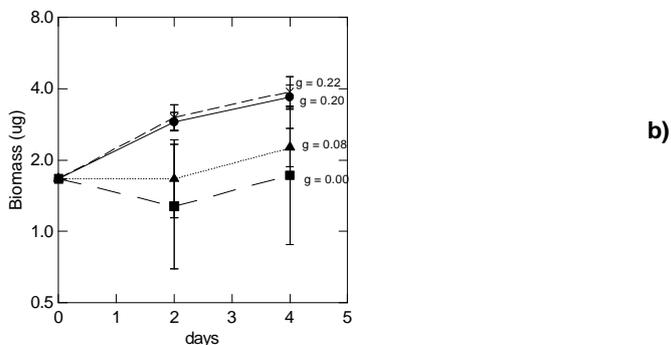
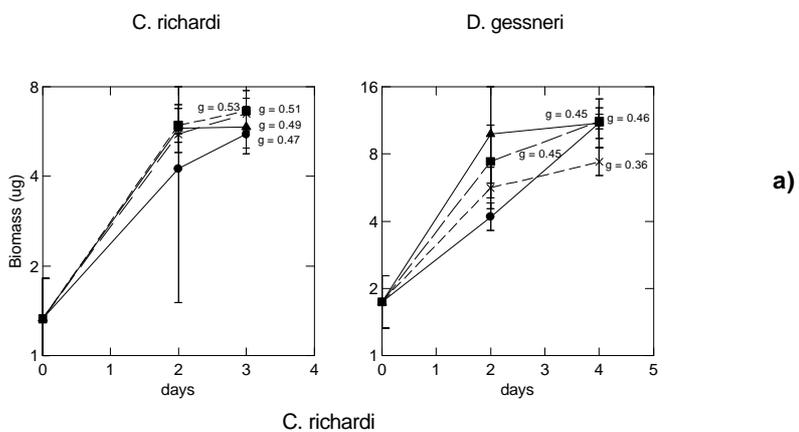


Figure 3: Biomass growth of *C. richardi* and *D. gessneri* (a) and *C. richardi* (b) in experiments V and VI (summer). Juvenile growth rates (g) for the last days are given for all species. (·) Seston, (x) Seston + P, (▲) Seston + N, (■) Seston + NP

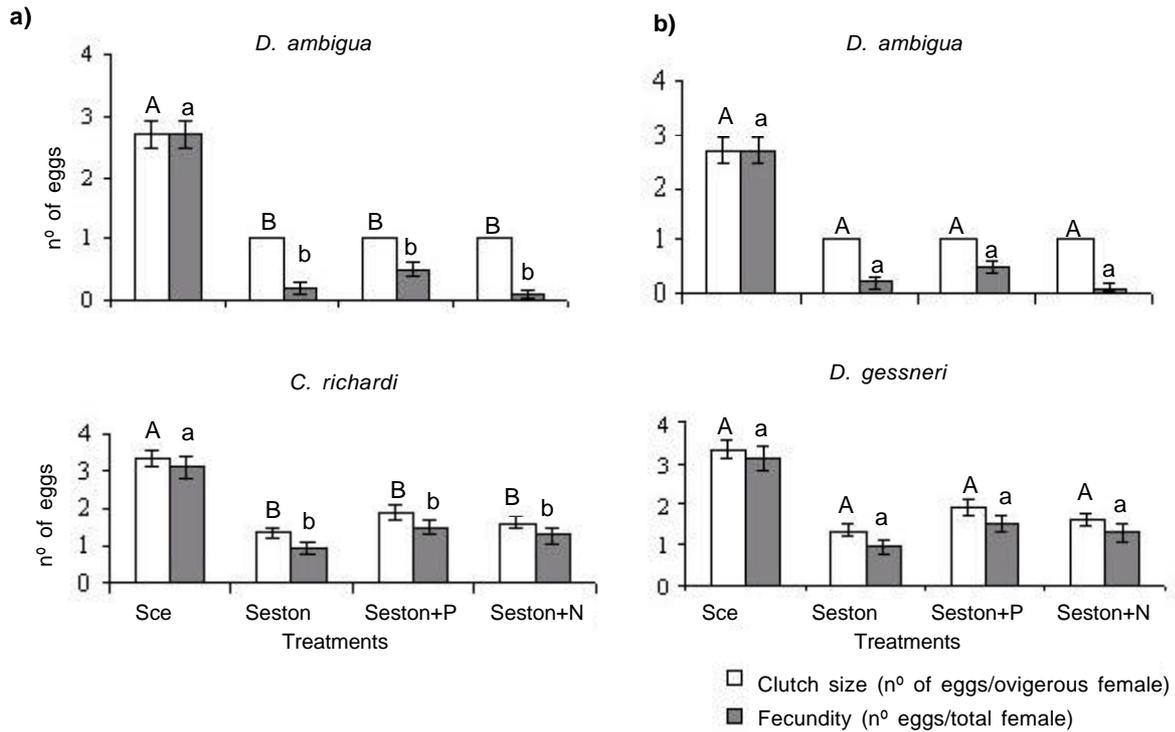


Figure 4: Clutch size and fecundity of cladocerans in experiments I and II (summer and autumn). Different letters above error bars (small letter for fecundity and capital for clutch size) indicate significant differences (ANOVA, $P < 0.05$).

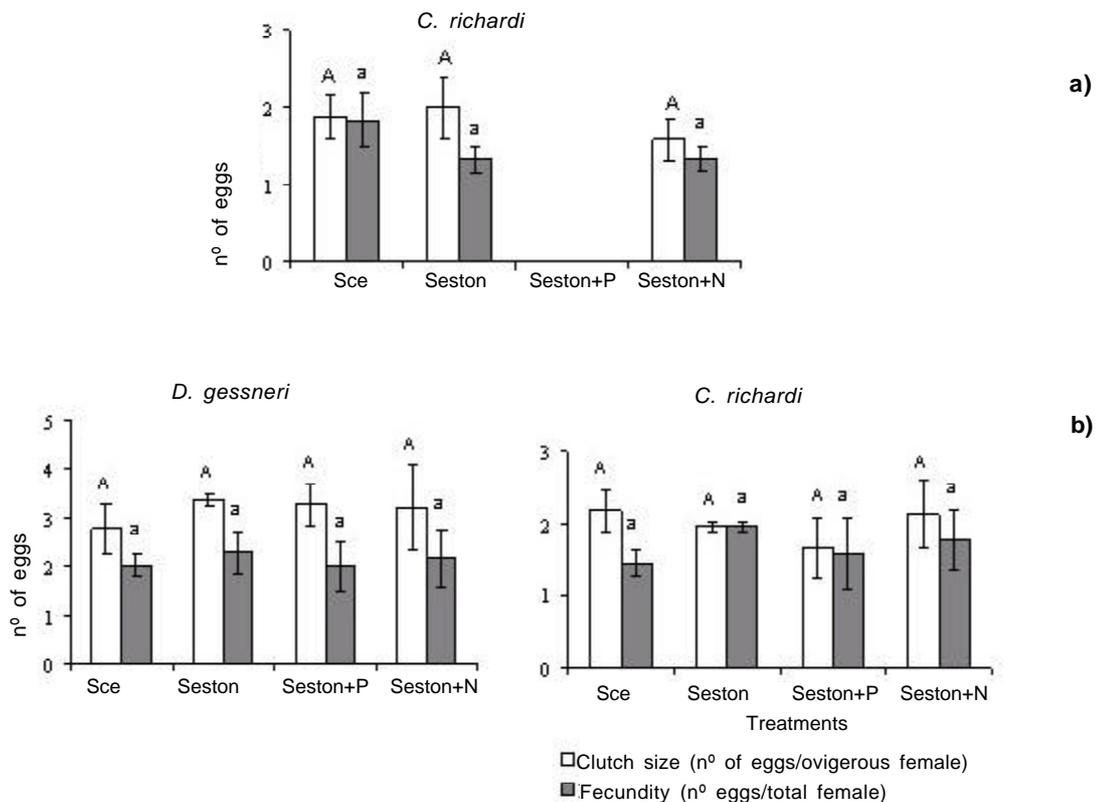


Figure 5: Clutch size and fecundity of cladocerans in experiments III and IV (winter and spring). Different letters above error bars (small letter for fecundity and capital for clutch size) indicate significant differences (ANOVA, $P < 0.05$).

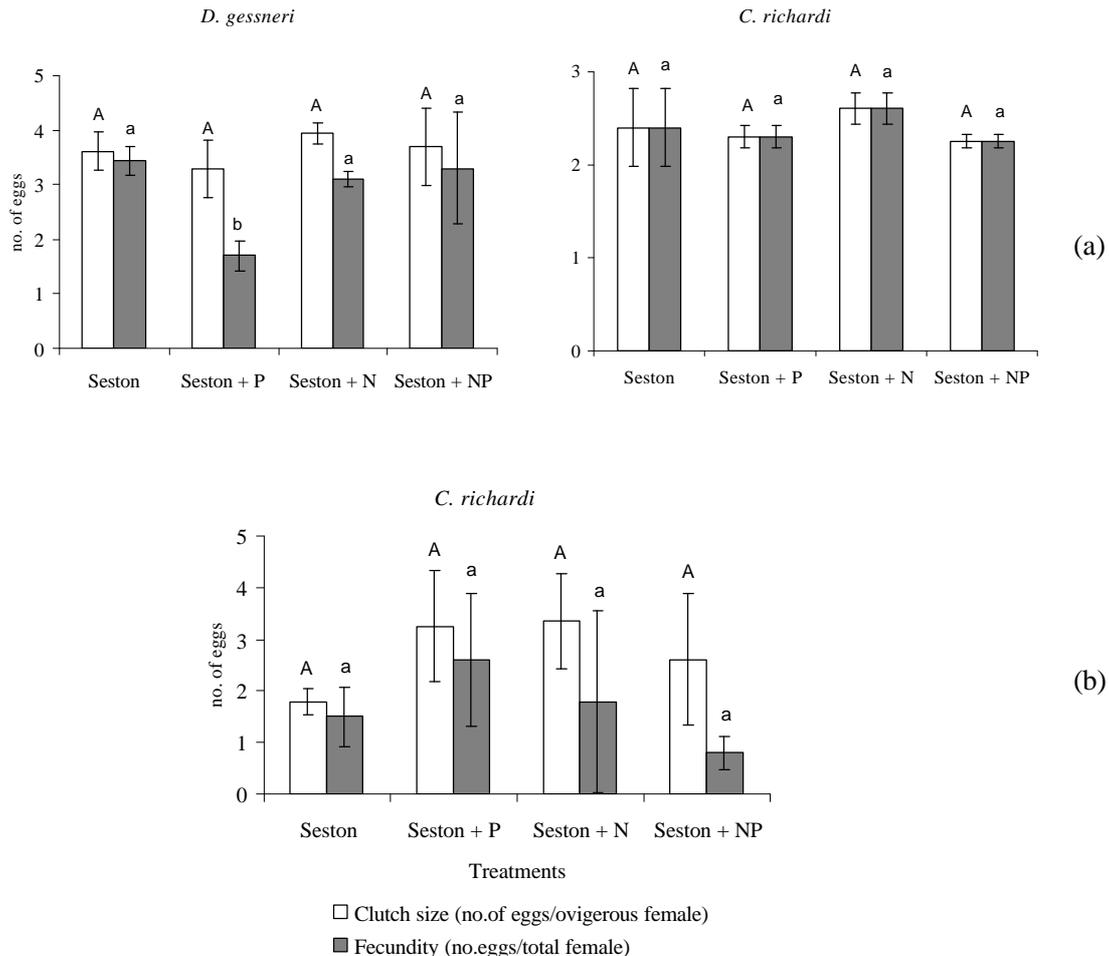


Figure 6: Clutch size and fecundity of cladocerans in experiments V and VI (summer). Different letters above error bars (small letter for fecundity and capital for clutch size) indicate significant differences (ANOVA, $P < 0.05$).

Discussion

Detailed experiments, designed specifically to evaluate the influence of seston mineral content (N and P) and the implications for life strategies of the organisms, have been focused for tropical cladocerans (Ferrão-Filho et al., 2003b; Ferrão-Filho & Arcifa, 2006; Ferrão-Filho et al., 2007). C, N, and P content of food and of consumers in the present experiments have also been analyzed to evaluate the applicability of the strict homeostasis hypothesis to tropical cladocerans.

Limiting C:N and C:P molar ratios estimated for temperate lakes range from 15 to 30 and from 138 to 500, respectively (DeMott & Gulati, 1999; Olsen et al., 1986; Sterner, 1993; Sterner & Hessen, 1994; Urabe & Watanabe, 1992) 300 being the most accepted lower limit for C:P ratio. In the

tropics, limiting values have not yet being established and thus, assuming the temperate values seston was not N-and P-limited during our experiments, except by P in experiment VI.

N and P content of the seston increased with nutrient enrichment even when limitation was not detected, as in experiments I to V. The extra nutrient incorporated by algae had little positive effect on cladocerans in most experiments. However, negative effects have been found such as the decrease of *D. ambigua* growth rates by N and P additions to seston in experiment I, as well as a zero growth rate of *C. richardi* with N addition in experiment VI. N and P addition to the assumed P-limited seston in experiment VI did not influence cladoceran growth; the effect of both nutrient additions was found only in experiment V on synergism, when *Daphnia*

gessneri experienced higher growth rates in the seston + NP compared to seston + P. This suggests a synergic effect or a co-limitation of both nutrients. Reproduction was not affected by N and P enrichment in most experiments. Negative effects (probably toxicity) on cladoceran reproduction with N and P additions to seston occurred on some occasions, as the lack of reproduction and the reduction of growth rates of *C. richardi* in experiment III with P additions to seston.

Literature is controversial on the effects of high sestonic C:P ratios on cladoceran growth. Negative influence of high C:P ratios on *Daphnia* growth has been reported (DeMott, 1998; DeMott & Pape, 2005; Elser et al., 2001; Sommer, 1992; Sterner, 1993; Urabe et al., 1997; Ferrão-Filho et al., 2007). On the other hand, some studies have reported low correlation between *Daphnia* growth and sestonic P content (DeMott & Tessier, 2002; Müller-Navarra, 1995). Negative effect of P addition to seston has been observed on population growth of *Ceriodaphnia dubia* (Matveev & Balseiro, 1990) the same way N or P addition sometimes caused adverse effects in our study.

Ferrão-Filho & Arcifa (2006) did not find any increment on cladoceran growth and reproduction in experiments with P addition to seston from Lake Monte Alegre with very high C:P molar ratios (936-1043), in summer. Only in autumn, when C:P ratios varied from 401 to 505, *Daphnia gessneri* growth rate was significantly increased with P enrichment. The authors pointed out that seston C:P ratio was not a good predictor of cladoceran P limitation and that residual limitation indicates other factors, and emphasized the need for more studies on food limitation in tropical cladocerans.

The decrease of P content in herbivores might indicate a P-limited growth in the field or a tendency for stress caused by the diet (DeMott et al., 2004). In our study, N and P content has not significantly differed in animals from the laboratory and the lake during the experimental period, indicating that cladocerans were not stressed by diet or animal P content is not a good indicator of food quality in the lake.

Relative contents of C, N, and P in cladocerans from Lake Monte Alegre fall in the same range for temperate counterparts. The C content in temperate *Daphnia* is, on average, 44% (Hessen 1990), and N varies

from 8 to 10% in cladocerans (Andersen & Hessen, 1991). P content, especially in *Daphnia*, ranges from 0.60 to 1.50% (Andersen & Hessen, 1991), 1.33% being found in average for nine *Daphnia* species (Brett et al., 2000). Variation in N content among taxa could be attributed to distinct pool of proteins (Elser et al., 1996) and differences in P content among species could be related to variation in RNA content (Mckee & Knowles, 1987).

The increase in N and P content of one cladoceran, *Ceriodaphnia richardi*, with addition of nutrients in two experiments in our study has not enhanced growth rates. This agrees with DeMott et al. (2004) who found that P addition to seston increased *Daphnia* P content without any effect on growth rate.

P content of zooplankton species was unrelated to P variation of the diet in several studies, what has been predicted by the strict homeostasis model (reviewed by Sterner & Hessen, 1994). Other papers, however, report decreases of P content in cladocerans fed P-deficient diets either in laboratory experiments (DeMott, 1998; DeMott, 2003; DeMott & Pape, 2005; Plath & Boersma, 2001) or in the field (DeMott et al., 2001, 2004; DeMott et al., 2004). Ferrão-Filho et al. (2007) tested three cladoceran species from Lake Monte Alegre and found that two of them (*M. micrura* and *C. richardi*) showed higher homeostasis coefficients to P compared to the other one, *D. ambigua*, meaning that those two species showed little variation in P content, even when fed P-limited diets. Homeostasis seems to be a characteristic of the cladocerans of Lake Monte Alegre tested in the experiments, except *C. richardi*.

Energy can be suggested as a factor influencing cladoceran growth in the experiments as shown by better performance of cladocerans in the green alga. Reproduction has been also improved in the alga treatment in summer. The importance of sestonic energy limitation for cladoceran individual growth and reproduction has already been reported in Lake Monte Alegre by Ferrão-Filho et al. (2003; 2005). Fileto et al. (2004) found better performance of cladocerans from the lake in the nanoplankton composed of rounded and non-spiny algae and those free of gelatinous sheaths. Thus, algae size and form as well as other characteristics and the contribution of detritus and bacteria are

relevant aspects to be taken into consideration in studies of food influence on growth and reproduction.

Food limitation by carbon in the seasons during the experiments I to IV is unlikely, considering that sestonic carbon concentrations exceeded threshold values commonly reported for cladocerans (0.005 - 0.31 mg C L⁻¹) (conversion of data reviewed by Sarma et al., 2005, using 0.5 dry weight = carbon concentration).

In spring and summer (experiments IV and I, respectively), algal carbon was predominantly composed of microplankton, as previously observed in the lake (Ferrão-Filho et al., 2005). The lower growth rates of *Daphnia gessneri* in spring and *D. ambigua* in summer in the seston treatment could be related to the dominance of microplankton, mostly composed of an inedible coenobitic alga (*Coelastrum reticulatum* - 40 µm) and of a filamentous one (*Cylindrospermopsis raciborskii* - 120 µm). In experiment VI (summer), algal carbon was also dominated by microplankton in agreement with observations made in the summer of 1999 (Ferrão-Filho et al., 2005). The zero growth rate of *C. richardi* in experiment VI was unrelated to the presence of filamentous algae as the filaments did not affect cladoceran growth in the two treatments without N addition. It seems to be related, however, to N enrichment of seston in addition to the high C:P ratio found.

The occurrence of high C:P ratios were rare in Lake Monte Alegre, including the period the experiments were made. Characteristics such as shallowness and low thermal stability with short periods of stratification (Arcifa et al., 1990) can lead to partial or total mixing of the water column and, consequently, nutrient replacement for phytoplankton.

Maybe the hypothesis of mineral limitation is plausible for explaining the phytoplankton influence on zooplankton development in temperate region, where most lakes are oligotrophic or ultra-oligotrophic (Sarma et al., 2005). In the tropics, mesotrophic, eutrophic, and even hypereutrophic lowland lakes are more common (Harper, 1992), where nutrient limitation is probably rare.

For Boersma & Kreutzer (2002) mineral limitation is not as important for organism growth when food is scarce as energy is for maintaining individual metabolism. It is

possible that in eutrophic tropical lakes other food characteristics such as quantity and quality in terms of size, form, and digestibility (Fileto et al., 2004; Ferrão-Filho & Arcifa, 2006) can have a major role in regulating planktonic populations than mineral deficiency.

In summary, in this study cladoceran growth and reproduction were not influenced by P addition to seston with low (~ 43-73) and medium (~119) C:P molar ratios, which could be expected based on the estimated threshold C:P ratio (300) for temperate cladocerans. However, the lack of cladocerans' responses to P addition to seston with high C:P ratios (~ 412-460) is in disagreement with stoichiometric theory prediction. Besides, lack of cladocerans' responses to P enrichment of seston with very high C:P ratios (up to 1043) (Ferrão-Filho & Arcifa, 2006) lead to hypothesize that only extremely high C:P ratios would be limiting or that stoichiometric theory does not hold for cladocerans of this tropical eutrophic lake.

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