POPULATION GROWTH AND DEVELOPMENT OF TWO SPECIES OF CLADOCERA, *Moina micrura* AND *Diaphanosoma birgei*, IN LABORATORY

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(With 3 figures)

ABSTRACT

The objective of the present work was to investigate the influence of four diets on population growth, development, total length, dry weight, and nutritional value of two zooplanktonic species, *Moina micrura* and *Diaphanosoma birgei*. The four dietary treatments were: algae alone (A); algae + vitamins (AV); algae + ration (AR); and algae + ration + vitamins (ARV). Growth rate peak for both species occurred faster with AV treatment. In general, AV treatment for *M. micrura* showed better results for intrinsic rate, fecundity, and embryonic and post-embryonic development. On the other hand, longevity and total spawning number were better with AR treatment ($p < 0.05$). Vitamin and ration treatments produced the best results in *D. birgei* species ($p < 0.05$). The highest percentage of protein and lipids for both cladocerans was verified for ration treatments. Carbohydrate was higher for the treatment containing algae alone ($p < 0.05$). Generally, diets containing ration and vitamin showed better results in cladocerans development, with water quality adequate for culture systems. Ration and vitamin diets may also be used in high-density cultures in the laboratory.

Key words: zooplankton, population growth, biochemical composition, algae.

RESUMO

Influência da dieta alimentar (alga + suplemento) no crescimento e desenvolvimento populacional de duas espécies de Cladocera, *Moina micrura* e *Diaphanosoma birgei,* em laboratório

O objetivo do presente trabalho foi testar a influência de quatro dietas alimentares sobre o crescimento populacional, desenvolvimento, comprimento total, peso seco e valor nutricional de duas espécies zooplanctônicas, *Moina micrura* and *Diaphanosoma birgei*, com os seguintes tratamentos alimentares: somente alga (A), alga + vitaminas (AV), alga + ração (AR) e alga + ração + vitaminas (ARV). O pico de crescimento para as duas espécies estudadas ocorreu mais rápido no tratamento AV. Em geral, o tratamento AV para *M. micrura* mostrou melhores resultados para taxa intrínseca, fecundidade, desenvolvimento embrionário e pós-embrionário. Já a longevidade e número total de desovas apresentaram melhores resultados no tratamento AR ($p < 0{,}05$). Para *D. birgei,* os melhores resultados foram obtidos nos tratamentos contendo ração e vitamina ($p < 0{,}05$). A maior porcentagem de proteínas e lipídeos para os dois cladóceros ocorreu nos tratamentos contendo ração, já o carboidrato foi maior no tratamento contendo somente alga ($p < 0{,}05$). Em geral, as dietas contendo ração e vitamina apresentaram os melhores resultados para o desenvolvimento dos cladóceros, com qualidade de água adequada para cultivo, podendo ser utilizadas em culturas com altas concentrações em laboratório.

Palavras-chave: zooplâncton, crescimento populacional, composição bioquímica, alga.

INTRODUCTION

In Brazil, few studies have emphasized plankton development and its nutritive value (Rocha & Matsumura-Tundisi, 1990; Hardy & Duncan, 1994; Sipaúba-Tavares *et al.*, 1994; Hardy & Castro, 2000; Macedo & Pinto-Coelho, 2000).

Cladocerans culture offers the possibility of obtaining a large number of individuals quickly under appropriate conditions of temperature, food, and water quality, due to parthenogenetic reproduction of these organisms.

Zooplankton reproduction and growth rate increases result in higher availability and, sometimes, better food quality for subsequent trophic levels.

Currently, a large variety of live organisms is used in larviculture, mainly due to their superior nutritional value as compared to that of formulated diets. Natural diets include different species of phytoplankton, zooplankton, and invertebrate larvae. However, some species have been selected as food sources in larviculture based on the following criteria (Watanabe & Kiron, 1994):

- Physical qualities such as purity, availability, and acceptance;
- Nutritional indicators, such as digestibility and organism nutrients/energy;
- Easily obtainable;
- Easy reproduction;
- Economically feasible.

Moina micrura and *Diaphanosoma birgei* species were chosen because of their virtual year-round presence in fishponds. In addition, they are resistant to the handling involved in the culture system and are selected as food by fish larvae.

According to Fim (1992) and Sipaúba-Tavares & Braga (1999), since tambaqui and pacu larvae feed on *M. micrura* and *D. birgei* during their first days, these are promising species for possible use as food in larviculture.

For herbivorous zooplanktonic organisms, algae are the main food source; however, food quantity and quality are important factors controlling zooplankton development, growth, and reproduction.

Vitamins have also been added to algae culture used as food by zooplankton and result in significant increase in organism production.

The laboratory study of planktonic organisms will provide input useful for large-scale production. Increases knowledge of their biology and the influence of factors such as temperature, food type on population growth, and zooplankton nutritional value will allow adaptation of culture conditions to ensure plankton culture success.

The objective of this study was to verify the influence of diets (algae + supplement) on the development of two Cladocera species, *Moina micrura* and *Diaphanosoma birgei*. In the laboratory life cycle of both species were studied, focusing on growth, development period, reproduction, and biochemical composition. The influence of water quality on culture was also investigated.

MATERIALS AND METHODS

Zooplankton collection

Moina micrura and *Diaphanosoma birgei* were collected in fishponds (21°15'S; 48°18'W) using 58 µm mesh-size plankton net. Acclimation period in the laboratory lasted 2 months. The specimens were placed in 2 L glass bottles filled with filtered fishpond water (filter GFC, 0.47 µm pore) at 25 ± 1°C and only fed *Ankistrodesmus gracillis* algae. Before beginning the experiment, they were not fed for 24 hours to empty the digestive tract.

Food supply

The algae used, *Ankistrodesmus gracillis,* originated in São Carlos University collection number 005 CH and was isolated from Broa Reservoir (SP, Brazil).

The algae species were batch-cultured in the laboratory at 24°C, D light regime 5,200 lux, and fertilized with NPK (Sipaúba-Tavares, 1995). Stock culture was maintained in 2 L flasks; during this time the flasks were refilled with filtered fresh water and fertilized as required. Mean cell size was 23.8 x 3.3 µm.

To evaluate *M. micrura* and *D. birgei* growth and development rates, the cultures were initiated with 15 females, and 4 diets were tested daily over a 15-day period. The 4 treatments were as follows: 1. algae alone (A), at 3 to 4 x 10^6 cells/mL density; 2. algae + vitamin (AV), B vitamin complex varying from 2 to 4 mg, according to population growth; 3. algae + ration (AR), commonly used to feed fish larvae, varying from 0.05 to 0.1 g; and 4. a diet containing all three ingredients (ARV).

Growth rate

Cladocerans were maintained in laboratory stocking culture and 15 ovigerous females (approximately 4 eggs/female) were selected and isolated. The cultures were kept under constant light (300 lux), in 1,800 mL volume, at 25 ± 1°C temperature for a 15 day-period.

Population growth was monitored daily by counting the number of organisms, using a Wild-Leitz M-5 Wild Heerbrugg MDG-17 stereomicroscope.

About 150 organisms at each developmental stage (neonate, young, and adult) were measured to monitor total length and dry weight.

Duration of embryonic (hours) and post-embryonic (days) developmental stages, total number of offspring/female, mean fecundity (number of neonate/offspring/female), and longevity (days) were monitored for each organism until natural death occurred. The experiments were conducted with six replicates.

Cladocerans' potential growth was determined using intrinsic rate of natural increase, under defined laboratory conditions (Bottrell *et al.,* 1976; Vijverberg, 1989).

Biochemical composition

Protein was analyzed according to Lowry *et al.* (1951) using bovine albumin, and carbohydrates according to Dubois *et al.* The method (1956) using glucose solution and lipids by sulpho-phosphovanillin method was also used, through chemical digestion proposed by Meyer & Walter (1988).

Dry weight was determined with a Mettler precision balance (accuracy ± 0.1 µm) according to the Berberovic & Pinto-Coelho method (1989).

Hydrological data

To evaluate the effect of the diets on the water quality, several limnological parameters were analyzed as shown in Table 1.

Data analysis

Comparison among mean values of different biological treatments was made by the Tukey test (Pimentel-Gomes, 1976). Different dietary treatments were analyzed by entirely randomized design (ERD) due to homogeneity of experimental conditions.

RESULTS

Growth

Moina micrura organisms fed algae + vitamin (AV) reached growth peak faster, on the eighth day, and the average value was 3,915 ind/L. This result was followed by that of organisms fed algae alone (A), algae + ration (AR), and algae + ration + vitamin (ARV), which reached growth peak between the 11th and 14th days, with average values of 3,880, 3,830, and 6,383 ind/L, respectively.

Diaphanosoma birgei growth rate was similar to that of *M. micrura*. The 4,593 ind/L average value was reached on the 10th day with AV treatment, followed by 3,927 ind/L for A, 4,517 ind/L for AR, and 5,608 ind/L for ARV reached between the 12th and 14th days.

TABLE 1
Limnological variables analysed.

Limnological variables	Methods
pH	Corning PS 15 pH meter
Electrical conductivity	Corning PS 17 conductivity meter
Dissolved oxygen	Winkler method Golterman *et al.* (1978)
Total alkalinity and CO_2	Mackereth *et al.* (1978)
Ammonia	Koroleff (1976)
Nitrite and nitrate	Golterman *et al.* (1978)
Total phosphorus and orthophosphate	Golterman *et al.* (1978)
Chlorophyll *a*	Golterman *et al.* (1978)

Average maximum number of organisms per liter was significantly different ($p < 0.05$) for ARV treatment in both species. The other treatments did not show significant differences ($p > 0.05$).

The highest intrinsic rate of natural increase (r) was obtained through AV treatment, with **r** values of 0.69 and 0.57 for *M. micrura* and *D. birgei*, respectively. This treatment also resulted in faster reaching of growth peak (Table 2).

Treatment AV for *M. micrura* was better with respect to embryonic and post-embryonic development, as well as fecundity. Treatment AR and ARV ($p < 0.05$) resulted in longer periods of embryonic and post-embryonic development, totaling 34.46 hours and 2.13 days, respectively (Table 2).

Number of offspring for treatments containing ration (AR and ARV) was higher compared to that for algae (A) and algae + vitamin (AV) treatments, averaging 3.97 and 3.05 offspring/females, respectively (Table 2).

For species *M. micrura*, average longevity was not significantly different among treatments ($p > 0.05$). However, it was slightly higher for diet AR, by a 6-day average (Table 2).

For species *D. birgei*, fecundity was higher for ARV and AV treatments ($p < 0.05$), with averages of 8.66 and 8.15 neonate/offspring/female, respectively. Total number of offspring/female was higher for treatments containing vitamins ($p < 0.05$), AV and ARV, with average values of 10.08 and 7.65 offspring/female, respectively. Embryonic development under AV treatment ($p < 0.05$) lasted 44.91 hours, and post-embryonic development was faster for A treatment ($p < 0.05$), lasting 2.26 days. Treatments containing vitamins (AV and ARV) showed the best longevity results, a 16-day average (Table 2).

For *M. micrura* cladocera, average length varied from 740.87 to 776.77 μm for adult females; 606.33 to 638.06 μm for juveniles; and 486.39 to 502.78 μm for neonates. As for *D. birgei*, which is larger than *M. micrura*, average length varied from 929.56 to 961.57, 608.53 to 653.65, and 438.16 to 475.56 μm for adult females, juveniles, and neonates, respectively (Fig. 1).

M. micrura adult females were larger when fed A, AV, and AR ($p < 0.05$) diets, while juveniles were larger when fed the ARV diet ($p < 0.05$). Neonate average length was not significantly different for any of the diets ($p > 0.05$). *D. birgei* average length at different developmental stages differed only slightly for all diets (Fig. 1).

Average dry weight varied between the two species for different diets. It ranged from 3.29 to 3.74 μg and 5.68 to 6.28 μg for adult females; 2.52 to 2.90 μg and 3.09 to 4.44 μg for juveniles; and 0.18 to 0.22 μg and 0.26 to 0.35 μg for neonates of *M. micrura* and *D. birgei*, respectively (Fig. 2).

Average dry weight of *M. micrura* adult females was higher for the AV and AR diets, and that of juveniles for the AV diet ($p < 0.05$). On the other hand, neonate dry weight was not significantly different for any diet ($p > 0.05$). *D. birgei* neonates and adult females presented higher dry weight under AV treatment ($p < 0.05$). However, juveniles showed better results with AV and AR treatments ($p < 0.05$) (Fig. 2).

Biochemical composition

For cladocerans *M. micrura* and *D. birgei*, the highest protein percentages were found for ARV treatment ($p < 0.05$), 80.23% and 76.40%, respectively. For these two species, protein levels for AV and AR treatments were similar, and the lower percentages were found for treatment A, 56.40% and 58.47%, respectively (Fig. 3).

As for carbohydrate percentages, an opposite trend was found, i.e., the highest values were found for treatment A ($p < 0.05$), reaching values of 34.33% and 21.29% for *M. micrura* and *D. birgei*, respectively. The AR and ARV treatments yielded the lowest carbohydrate percentages, with average values of 13.53% and 11.89%, respectively (Fig. 3).

There was no significant difference with respect to lipids for *D. birgei* among the diets ($p > 0.05$). As for *M. micrura,* ARV yielded the highest value ($p < 0.05$) compared to others, with a 21.5% average (Fig. 3).

Limnological characteristics

Some limnological variables were influenced by the water supply (from a well). However, the values found for the treatments were appropriate for organism culture, mainly due to nitrite and ammonia low concentrations. Nitrogen compounds were directly influenced by the well water, which was contaminated by chemical fertilizer due to agricultural activities in the area (Table 3).

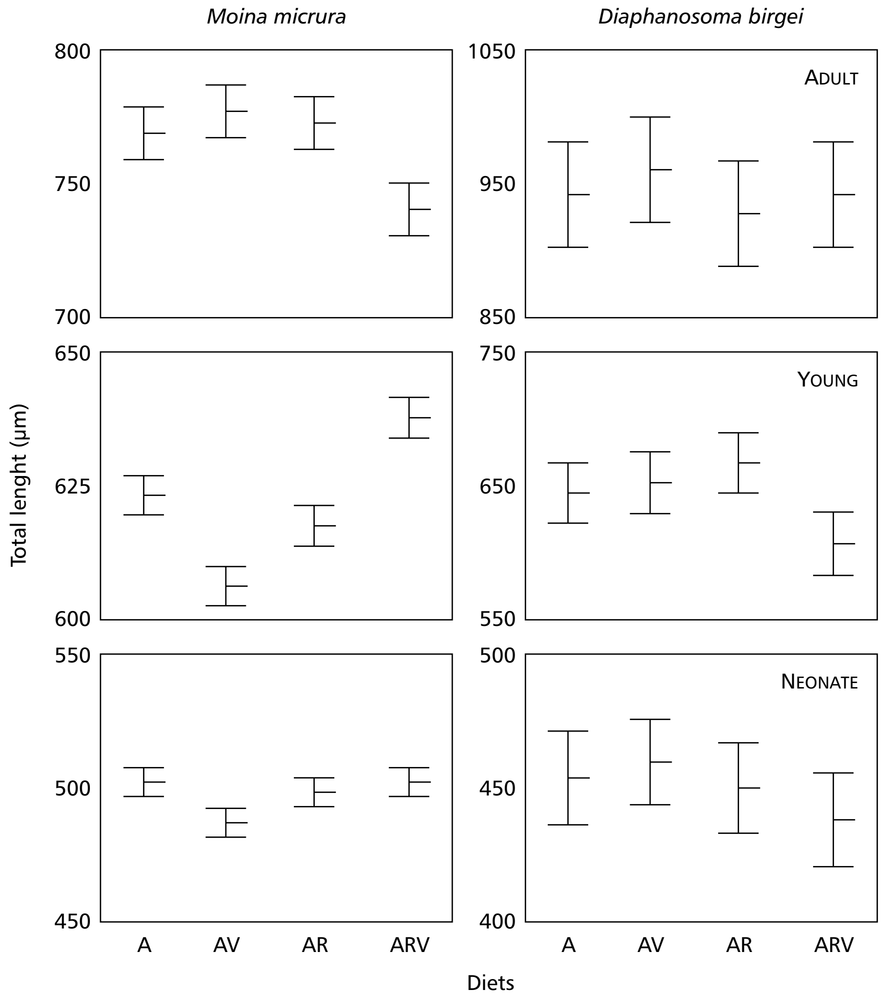

Fig. 1 — Average and standard error of total length (µm) for adults, young, and neonates of *Moina micrura* and *Diaphanosoma birgei*, treated with algae alone (A), algae + vitamin (AV), algae + ration + vitamin (ARV), and algae + ration (AR).

Generally, AV treatment yielded the best results, and ARV, the worst. This was basycally true for phosphorus, conductivity, and inorganic carbon forms. With respect to nitrogen compounds, treatment A displayed the highest nitrate and nitrite concentrations for *M. micrura* culture, with averages of 209.4 and 9.9 µg/L, respectively.

While the treatments with ration (AR and ARV) displayed the highest ammonia value for the two species, for *M. micrura* the values were 15.6 and 10.6 µg/L, and for *D. birgei,* 44.4 and 33.9 µg/L, respectively. For ration treatments (AR and ARV), nitrite levels were five times higher than those for A and AV (Table 3).

The alkaline pH oscillated mildly during the period and conductivity was higher for ARV treatment, probably due to higher nutrient content in this diet, except for nitrite and nitrate in *M. micrura* culture, which was higher in treatment A. Conductivity was higher for treatments containing ration and for *D. birgei* culture as compared to *M. micrura*. Inorganic carbon in the medium was influenced by alkaline pH, with bicarbonate dominating (Table 3).

TABLE 2
Life history characters of the cladocerans, *Moina micrura* e *Diaphanosoma birgei*, treated with algae alone (A), algae + vitamin (AV); algae + ration (AR), and algae + ration + vitamin (ARV).

Characters	***Moina micrura***				***Diaphanosoma birgei***			
	Feeding treatments							
	A	**AV**	**AR**	**ARV**	**A**	**AV**	**AR**	**ARV**
Intrinsic rate (r)	0.50	0.69	0.43	0.43	0.37	0.57	0.42	0.44
Mean fecundity (n. neonate/offspring/female)	5.37 ± 0.51	8.63 ± 0.51	5.78 ± 0.51	7.10 ± 0.51	7.16 ± 0.62	8.15 ± 0.62	6.73 ± 0.67	8.66 ± 0.62
Mean number of offspring/female	2.30 ± 0.99	2.83 ± 0.99	3.97 ± 0.99	3.05 ± 0.92	6.17 ± 2.19	10.08 ± 2.19	5.46 ± 1.09	7.65 ± 2.19
Mean embryonic duration (hours)	27.23 ± 3.75	24.29 ± 4.02	34.46 ± 4.02	29.11 ± 4.02	46.79 ± 2.14	44.91 ± 1.88	44.91 ± 1.87	45.98 ± 1.87
Mean postembryonic duration (days)	1.73 ± 0.25	1.38 ± 0.31	1.50 ± 0.23	2.13 ± 0.25	2.26 ± 0.06	2.72 ± 0.11	2.72 ± 0.10	2.90 ± 0.10
Longevity (days)	5	5	6	5	14	16	13	16

Dissolved oxygen levels were above 67% saturation, due to constant aeration (Table 3).

In general, chlorophyll *a* rates for both cultures were high, mainly in treatments ARV for *M. micrura* and AV, AR, and ARV for *D. birgei*. For both species, treatment A presented the lowest values with averages of 78.6 µg/L and 89.9 µg/L for *M. micrura* and *D. birgei*, respectively (Table 3).

DISCUSSION

In the present work, cladocerans production was 1.3 times higher for the diet containing algae + ration + vitamins compared to the other diets. The addition of vitamins and ration as a support for bacteria production had a positive result on *M. micrura* and *D. birgei* production.

According to Yu *et al.* (1994), bacteria are important sources of vitamin B_{12}, thus some zooplanktonic species could grow rapidly when certain bacteria strains are present in the culture. Most vitamins in aqueous media are often found in very low levels (nanograms) and originate from bacterial excretion, phytoplankton, and autholysis of senescent cells. In the laboratory, it is possible to eliminate all unfavorable conditions existing in nature, e.g., predation, illnesses, large temperature variations, and competition for food and space.

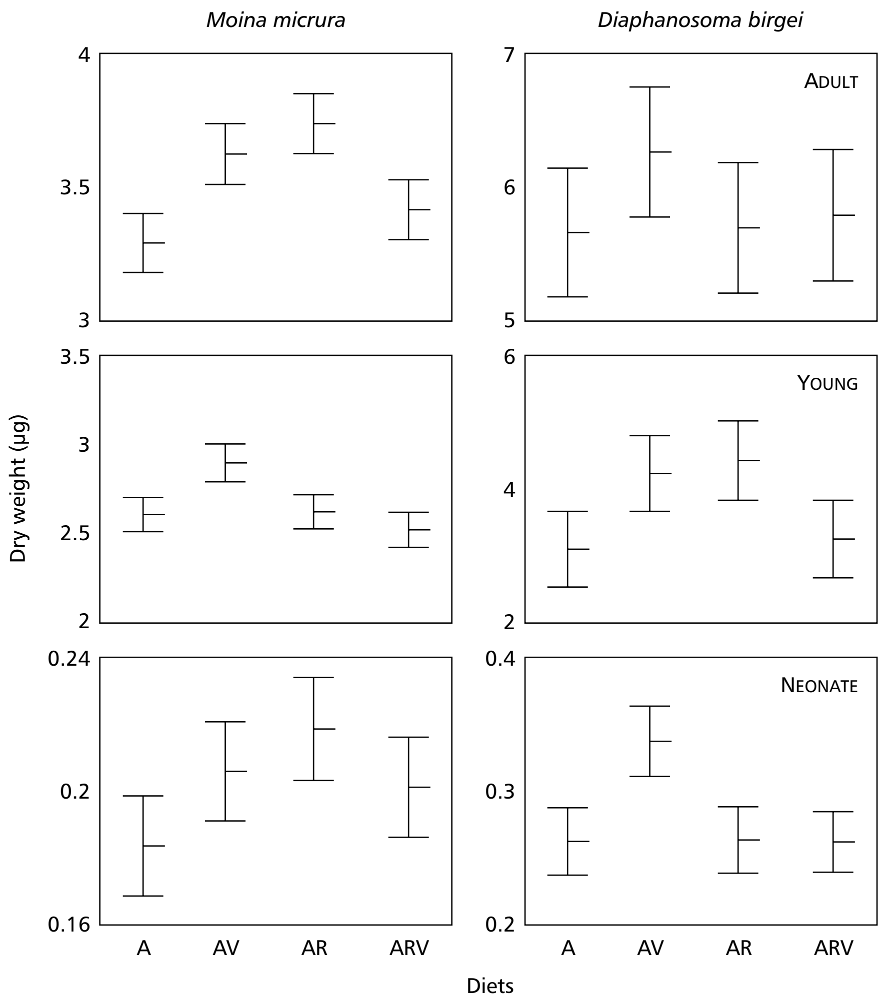

Fig. 2 — Average and standard error of dry weight (µg) for adults, young, and neonates of *Moina micrura* and *Diaphanosoma birgei*, treated with algae alone (A), algae + vitamin (AV), algae + ration + vitamin (ARV), and algae + ration (AR).

TABLE 3
Mean variation of limnological variables of the culture water of zooplanctonic species during the experimental period for these feeding treatments: algae alone (A), algae + vitamin (AV), algae + ration + vitamin (ARV), algae + ration (AR), and control (C) (referring to water only, with no treatment).

Limnological variables	C	*Moina micrura*				*Diaphanosoma birgei*				Units
		A	AV	AR	AVR	A	AV	AR	AVR	
pH	7.5	7.9	7.8	7.8	7.3	7.7	7.8	7.8	7.7	
Conductivity	25	46.0	46.0	76.1	83.3	160.0	157.0	172.0	184.0	µS/cm
Dissolved oxygen	66.0	84.0	80.1	74.2	67.3	80.3	80.5	78.7	79.2	%SAT
Alkalinity	19.0	12.0	12.0	36.0	39.0	16.0	24.0	27.0	37.0	mg/L
Bicarbonate	12.2	14.6	14.6	43.7	47.5	20.2	30.0	33.7	44.6	mg/L
Free CO_2	6.7	1.5	1.17	2.2	3.8	0.8	0.9	1.0	1.1	mg/L
Total CO_2	18.9	16.1	15.8	46.0	51.3	22.0	31.9	35.7	46.9	mg/L
Ammonia	0.1	4.0	6.2	15.6	10.6	15.4	16.8	44.4	33.9	µg/L
Nitrite	1.6	9.9	8.8	4.8	2.7	7.8	8.5	48.2	42.2	µg/L
Nitrate	249.1	209.4	180.6	6.6	7.0	23.1	20.8	20.2	20.1	µg/L
Total phosphorus	*	18.0	18.8	36.6	31.0	15.1	16.2	19.0	19.5	mg/L
Orthophosphate	*	0.8	1.2	1.9	2.0	0.9	1.1	1.2	1.5	mg/L
Chlorophyll *a*	*	78.6	80.8	79.2	130.8	89.8	112.3	127.3	146.2	µg/L

* Below detection limit

For cladocerans, **rm** varies between 0.2 and 0.6. In this study, it varied from 0.43 to 0.69 and 0.37 to 0.57 for *M. micrura* and *D. birgei*, respectively. The highest values were found for AV treatment and also the highest number of neonates per female for *M. micrura*.

Body size is of fundamental importance in zooplanktonic organism studies because of its relationship with rates of physiological processes such as growth, respiration, feeding, and excretion (Jayatunga, 1986).

M. micrura females were smaller for ARV treatment, probably due to high population density. However, during the juvenile stage, ARV treatment resulted in the largest body size, while the opposite was verified for *D. birgei* at the same developmental stage. Jana & Pal (1985) observed that *M. micrura* length was strongly affected by population density. Due to space limitations the same effect might have occurred in this study.

Another important factor in population development studies of zooplanktonic organisms is dry weight, which may vary for same-size animals, depending on their nutritional value, life-cycle stage, and reproductive conditions (Rocha & Matsumura-Tundisi, 1990). In addition, temperature and food concentration influence size, weight, and embryonic and post-embryonic development period of zooplanktonic organisms.

In this study, *D. birgei* embryonic development was not significantly different for any diet. However, *M. micrura* embryonic development was slower for the algae + ration diet, probably due to its higher ammonia and total phosphorus levels.

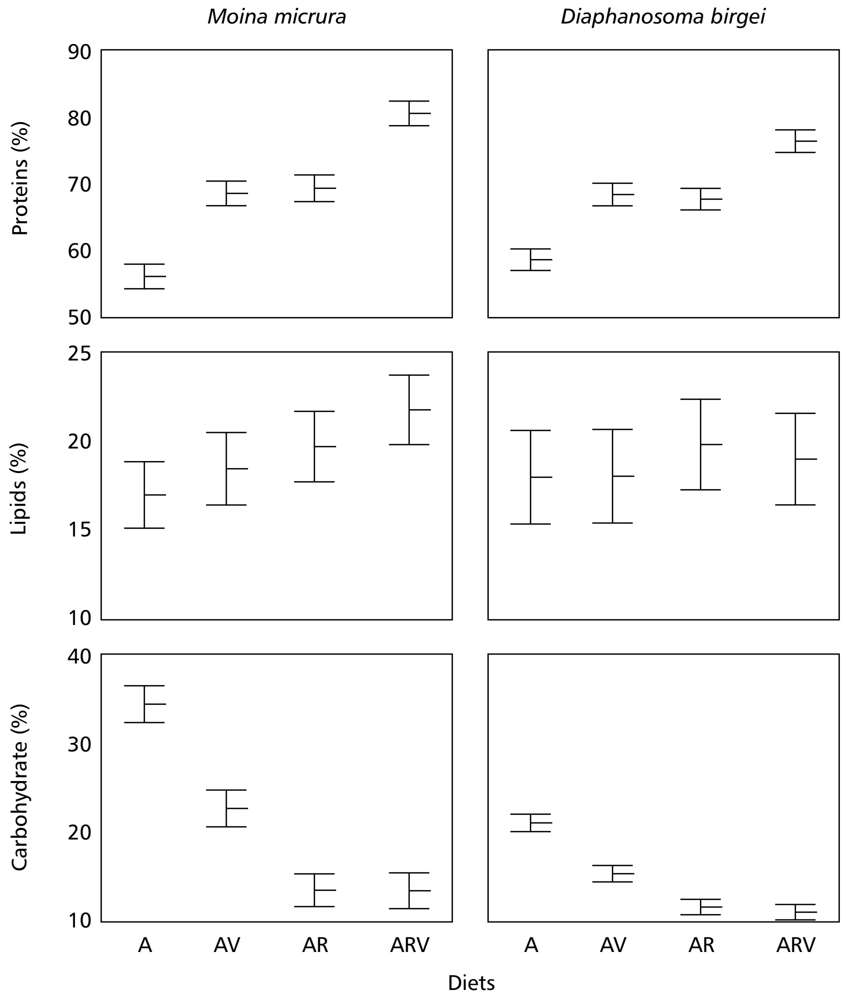

Fig. 3 — Average and standard error of lipid, carbohydrate, and protein percentage (% dry weight) for *Moina micrura* and *Diaphanosoma birgei*, treated with algae alone (A), algae + vitamin (AV), algae + ration + vitamin (ARV), and algae + ration (AR).

Fim (1992), who while studying the biology of *M. micrura* fed *Scenedesmus quadricauda* in the laboratory, reported a 21-hour embryonic developmental period. In this work, the fastest development was verified for AV treatment, with a 24.29-hour average, and the slowest for AR treatment, with 34.46 hours for the same species. Embryonic and post-embryonic development of *M. micrura* was faster than that of *D. birgei* for all studied treatments.

Hardy & Duncan (1994) observed that embryonic and post-embryonic development of *Moina reticulata* was faster when compared to that of *Diaphanosoma sarsi* and *Daphnia gessneri* cultivated in similar conditions. According to these authors, temperature was as important as food quality in determining embryonic and post-embryonic development of these three species of tropical cladocerans.

From the results presented, it is evident that food type influenced number of offspring/female. Offspring number for *M. micrura* fed ration treatments, mainly AR, was higher compared to results of A and AV without ration. As for *D. birgei*, offspring number was higher for treatments containing vitamins (AV and ARV).

Number of eggs per female is directly affected by the food supply. The studied species presented high fecundity, compared to that indicated by values reported by Fim (1992) and Fonseca (1991), due to temperature control and vitamin addition to the treatments.

According to Lynch (1980), larger *Daphnidae* invest less energy in growth and more in reproduction after they reach maturity. They present higher longevity compared to smaller *Daphnidae*.

The longevity of *M. micrura,* which was not significantly different ($p > 0.05$) under any of the treatments, was lower than that of *D. Birgei*; however, they reached maturity much faster under all treatments. In general, treatments containing vitamins (AV and ARV) presented better results in *D. birgei*.

It is not yet completely clear which diet is preferable for zooplankton. Food quality is a crucial factor for these organisms, since they store large lipid reserves from the medium depending on their ability to assimilate nutrients from the algae.

According to Kilham *et al.* (1997), there are intraspecific and interspecific variations in the biochemical composition of live food as well as a large variation in herbivore response to different diets. This complicates the interpretation of feeding studies.

Ration and vitamin additions to the treatments caused higher protein and lipid percentages. However, carbohydrates were higher for the treatment containing algae.

Macedo & Pinto-Coelho (2000) verified that *M. micrura* and *D. laevis* fed *Ankistrodemus gracilis* presented higher lipid levels compared to those fed *Scenedesmus quadricauda*.

Water quality also influenced survival and development of these aquatic organisms. In all treatments, limnological parameters were at acceptable levels except for total phosphorus and orthophosphate, probably reflecting excretion and organic matter decomposition accumulation during culture. Moreover, ration addition may have influenced the behavior of these nutrients in the medium.

High percentages of saturated oxygen were associated with constant aeration; ARV treatment displayed the highest concentrations of chlorophyll *a* due to algal growth caused by nutrients released by the ration via decomposition and presence of vitamins.

Slightly alkaline pH caused bicarbonate dominance for all treatments and high conductivity in the culture systems was a consequence of nutrient availability due to ration presence, since the highest values found were for AR and ARV.

For the majority of aquatic species, adequate development happens when water pH is close to neutral.

Moina micrura and *Diaphanosoma birgei* may be considered promising species for feeding fish larvae and fingerlings in large-scale cultivation. They show high intrinsic rates, short life-span, small size, quick embryonic development, and abundant energy invested in reproduction. In general, diets containing ration and vitamins showed better results in population growth, and protein levels. They can be used as additional components in the diets of planktonic organisms used directly as natural food or indirectly as inoculum in culture ponds to increase system productivity. This diet is economically feasible, considering the low cost of NPK used in the algae culture and the small quantities of vitamin and ration added to improve the growth rates of these zooplanktonic species.

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