Growth and development time of subtropical Cladocera *Diaphanosoma birgei* Korinek, 1981 fed with different microalgal diets

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Abstract

The aim of this work was to investigate the growth performance of *Diaphanosoma birgei* fed with two Chlorophyceae algae, *Ankistrodesmus gracilis* and *Haematococcus pluvialis* using monoalgal diets and simpler mixed diets. *D. birgei* was daily fed on four treatments: 1) 100% *Ankistrodesmus gracilis* (Ag); 2) 100% *Haematoccocus pluvialis* (Hp); 3) 25% *A. gracilis* + 75% *H. pluvialis* (Ag-25+Hp-75) and 4) 75% *A. gracilis* + 25% *H. pluvialis* (Ag-75+Hp-25). The fecundity curve of *D. birgei* showed that the mixed feed Ag-25+Hp-75 and temperature 24±2°C triggered fast fecundity at approximately two days. The fecundity was low when based only on *H. pluvialis* (Hp), albeit with greater longevity (19 days) and a higher number of broods (8). *D. birgei* fed on Ag and Ag-75+Hp-25 diets in this experiment sustained higher growth rate and higher lipid content in these treatments. The present study showed that *A. gracilis* diet and mixed microalgae diets tested were able to support the egg production and development of *D. birgei*.

Keywords: Ankistrodesmus gracilis, *Haematococcus pluvialis*, monoalgal diets, mixed diets, growth rates.

Crescimento e tempo de desenvolvimento de uma espécie de Cladocera subtropical *Diaphanosoma birgei* Korinek, 1981 alimentada com dietas de microalgas.

Resumo

O objetivo deste estudo foi avaliar o crescimento de uma espécie de Cladocera *Diaphanosoma birgei* alimentada com dois tipos de microalgas Chlorophyceae, *Ankistrodesmus gracilis* e *Haematococcus pluvialis* cultivadas em monocultura e cultura mista. Quatro dietas foram utilizadas: 1) 100% *Ankistrodesmus gracilis* (Ag); 2) 100% *Haematoccocus pluvialis* (Hp); 3) cultura mista com 25% *A. gracilis* + 75% *H. pluvialis* (Ag-25+Hp-75) e 4) cultura mista com 75% *A. gracilis* + 25% *H. pluvialis* (Ag-75+Hp-25). A curva de fecundidade de *D. birgei* mostrou que a dieta mista Ag-25+Hp-75 e temperatura de 24±2°C promoveu rápida fecundidade em aproximadamente dois dias. A fecundidade foi baixa quando *D. birgei* foi alimentada somente com *H. pluvialis* (Hp), porém foi observada maior longevidade (19 dias) e consequentemente, maior número de descendentes (8). As dietas Ag and Ag-75+Hp-25 apresentaram efeito direto na taxa de crescimento de *D. birgei* com elevados teores de lipídios nestes tratamentos. O presente estudo mostrou que as dietas contendo somente *A. gracilis* e as dietas mistas de microalgas foram capazes de manter níveis adequados na produção de ovos e no desenvolvimento de *D. birgei*.

Palavras-chave: Ankistrodesmus gracilis, *Haematococcus pluvialis*, dietas de monocultura de algas, dietas mistas, taxas de crescimento.

1. Introduction

Several studies have shown that cladoceran have a higher nutritional value than other live food sources for many fish larvae, because their broad size range, high digestibility and high food value allow better grow and development than other live diets (Sipaúba-Tavares and Bachion, 2002; Lemke and Benke, 2003; Sarma et al., 2005; Ismail, et al., 2011).

The cladoceran *Diaphanosoma birgei* is common species in southeast of Brazil and have promising characteristics for aquaculture due resistant to the handling involved in the system and is usually ingested by fish larvae (Pagano et al., 2000; Sipaúba-Tavares et al., 2006). *Diaphanosoma* the largest genus ctenopod cladoceran becomes more abundant and speciose in the pelagic lakes and ponds as one moves from the temperate zone to the tropics (Han et al., 2011). *Diaphanosoma* species also contract with most of, but not all, other pelagic cladoceran taxa in their size preference for food items. They exclusively selected for small particles (Pagano, 2008). *Diaphanosoma* is a tropical limnological genus with high species diversity (20 species) (Sarma et al., 2005).

Some environmental factors influence the growth, reproduction and survival of zooplankton species; the most important are quality and availability of food, temperature and water quality of the culture medium. Freshwater microalgae have been widely used as food, because they can supply energy and nutrients essential for the growth and development of freshwater organisms.

There is a wide range of microalgae available for aquaculture mostly chosen for their specific nutritional qualities because they may be easily grown in mass culture. Some of the most widely used in commercial freshwater are *Haematococcus pluvialis*, unicellular green microalgae, widely used as an additional pigment in aquaculture and a supplement item in the food industry (Sarada et al., 2006) and *Ankistrodesmus gracilis* which is highly appreciated by water organisms owing to its size, shape, thickness of cell walls and easy prey (Hardy and Castro, 2000; Sipaúba-Tavares and Braga, 1999).

However monospecific diets may cause nutritional deficiencies because of the inadequate content of one or more essential nutrients. To reduce this risk, several authors have suggested the use of mixed diets, because their combine nutrient contents are more likely to meet the nutritional requirements of the target species (Cai et al., 2007; Puello-Cruz et al., 2009). In case of cladoceran the cultures are maintained on a monospecific diets (Sipaúba-Tavares and Bachion, 2002; Nandini and Sarma, 2003; Han et al., 2011) and mixture of two or more microalgae (Pagano et al., 2000; Pagano, 2008).

Cladocerans have fast maturation, rapid growth and proliferation, with subsequent more energy allocated to reproduction than to merely somatic growth in early matured animals (Ismail et al., 2011). Growth rate is a critical parameter for most animals since it affects age at first reproduction, adult body size and other aspects of their life history (McFeeters and Frost, 2011).

The availability of food and food type influenced cladoceran reproduction and modified the rate of development, longevity and fecundity. In addition, the development time, size and reproductive output of adult cladoceran varies according to diet or food quality (Matias-Peralta et al., 2012). In aquaculture, the species of algae selected to feed cladoceran should be based on ease of culture and availability, and by matching light and temperature tolerances of algae with the optimum culture conditions for the species of cladoceran being cultured.

The aim of this work was to investigate the growth performance of *Diaphanosoma birgei* fed with two Chlorophyceae algae, *Ankistrodesmus gracilis* and *Haematococcus pluvialis* using monoalgal diets and simpler mixed diets.

2. Material and Methods

2.1. Source and isolation of adult cladoceran

A clone of *Diaphanosoma birgei* isolated from fishponds in the aquaculture farm (21°14'S and 48°17'W), were taken a 58-µm mesh plankton net, and live organisms were brought to the laboratory. They were kept with 2-L glass bottles with water from fishponds. The water was autoclaved and then prepared with feat moss extract in the proportion of 0.44 mg.L^{-1} and maintained at 24±2°C during several weeks for adaptation and they were fed daily with *Ankistrodesmus gracilis* (Sipaúba-Tavares and Bachion, 2002). The remaining adult *D. birgei* were used to start the stock culture.

2.2 Food supply

The algae strain of *Ankistrodesmus gracilis* used in this study was obtained from culture collection no. 005CH, originally from Broa Reservoir (22°15'S and 47°19'W), Brazil and the the algae strain of *Haematococcus pluvialis* was obtained from culture collection CMEA 227 C1 from Rio de Janeiro (22°53'S and 43°17'W), Brazil. The mean cell sizes were respectively 23.8 mm and 52 mm (cysts size). The algae were batch-cultured at 23±2°C, with a light regime of 94.7 mmol m^{-2} s^{-1}, and with fertilizer NPK medium (Sipaúba-Tavares, 1995) to *A. gracilis* and WC medium (Guillard and Lorenzen, 1972) to *H. pluvialis*. Vitamin complex B was added to the culture at a rate of 2-4 mg.L^{-1}, according the cladoceran number. Algal growth was monitored by cell counts in a Neubauer chamber. Algae were harvested at the exponential growth phase, around concentration of 7-9 x 10^6 cells.mL^{-1} to *A. gracilis* and 3-4 × 10^6 cells.mL^{-1} to *H. pluvialis* cysts (with astaxanthin). To evaluate *D. birgei* growth and development rates, the cultures were initiated with 15 females, and four diets were tested daily over a 19-day period. The four diets were as follows: 1) 100% *Ankistrodesmus gracilis* (Ag); 2) 100% *Haematoccocus pluvialis* (Hp); 3) 25% *A. gracilis* + 75% *H. pluvialis* (Ag-25+Hp-75) and 4) 75% *A. gracilis* + 25% *H. pluvialis* (Ag-75+Hp-25).

2.3. Life history

D. birgei were maintained in the laboratory stock culture and 15 ovigerous females (approximately 10 eggs per female) were isolated and transferred to a beaker containing 7-L volume of autoclaved water. Each day the total number of individuals of each diet (Ag; Hp; Ag-25+Hp-75 andAg-75+Hp-25) was counted, and food added (a suspension of 10^5 cells.ml^{-1} of diet). Water and food suspension were totally renewed every two days. The cultures were kept under constant light (30 µmol.m^{-2} s^{-1}) at 24±2°C. Population growth was monitored daily by counting the number of organisms, using a Wild-Leitz M-5 Wild Heerbrugg MDG-17 stereomicroscope. The potential growth of *D. birgei* populations was compared by determining their intrinsic rate of natural increase under defined laboratory conditions, according to the following equation (Odum, 1985):

$$r = \ln N_t - \ln N_0/t$$

where:

N_t = population size at time t
N_0 = population size at time zero.

About 150 organisms at each developmental stage (neonate, primipara and adult) were measured to monitor total length. Animals of different length classes were isolated from stock culture and measured. They were afterwards transferred to aluminum pans, previously dried at 60°C for 24 hours and weighed on Mettler high-precision balance (accuracy ± 0.1 mg). Pans with animals were dried at 60°C for 48 hours and cooled in a desiccator; after cooling they were weighed once more and the dry weight (DW) of the animals was calculated by subtracting the weight of the empty pans from that of the dried animals.

2.4. Growth

Neonates less than 15 hours old were obtained, all from the same female was isolated and transferred individually and maintained in 50-mL beakers under controlled conditions as described above. Duration of embryonic (hours) and post-embryonic (days) development stages, body length, total number offspring per female, mean fecundity (number eggs per female) and longevity (days) were monitored for each organism until natural death occurred. Until the first reproduction organisms were observed at short intervals, many times a day. The experiment was conducted in five replicates.

2.5. Analytical methods

Water samples were monitored twice a week. Dissolved oxygen and temperature were determined with an oxygen meter Orion mod. 810. The pH and conductivity were measured with a Corning PS-17 pH meter and Corning PS-15 conductivity meter. Total phosphorus, orthophosphate, nitrite, nitrate and ammonia in the culture were quantified spectrophotometrically according to techniques describe by Golterman et al. (1978) and Koroleff (1976). Chlorophyll-*a* was extracted with 90% alcohol and quantified at 663 and 750 nm (Nusch, 1980). At the end of the experiment the animals were isolated from stock culture, frozen and lyophilised to analyse total lipids according AOAC (1990).

2.6. Statistical analysis

One-way ANOVA was applied for water parameters and some *D. birgei* characters in order to verify the difference between diets and their interactions, with significance level ($p<0.05$) (Fowler et al., 1998).

3. Results

D. birgei showed exponential growth after the 9th day until 16th day with $28x10^2$ individuals.L^{-1} in Ag diet, 18×10^2 individuals.L^{-1} in Ag-25+Hp-75 diet, and 29×10^2 individuals.L^{-1} in Ag-75+Hp-25 diet. The population growth in Hp diet was very low the higher density was 2×10^2 individuals.L^{-1} in 17th day. Consequently, the intrinsic rate (r) of Hp diet (0.11 days^{-1}) was lower than from the other diets (Table 1; Figure 1).

Animals were born with a mean length between 349 µm (Hp) and 440 µm (Ag-75+Hp-25) and reached maturity with a mean length between 618 mm (Ag-25+Hp-75) and 801 µm (Ag). The length of primipara females was similar when diets were taken into account, with the exception of Ag-75+Hp-25 (Table 1).

A higher number of offsprings per female was observed in the Hp diet (8.4) as a consequence of a greater mean longevity (15.4 days) when compared with that of other diets (7-days in Ag-25+Hp-75 and 9-days in Ag). However, the number of neonates per female was significantly ($p<0.05$) lower than that of neonates fed Ag and mixed microalgae diets (Table 1).

During their lifespan *D. birgei* produced three broods with Ag-25+Hp-75 diet; four broods with Ag diet; five broods with Ag-75+Hp-25 diet and eight broods with Hp diet, at approximately 10.2 hours intervals at the start of the experiment and, as from the third brood, at approximately

Table 1. Life history parameters of *Diaphanosoma birgei* (Cladocera) maintained on diets of 100% *Ankistrodesmus gracilis* (Ag); 100% *Haematoccocus pluvialis* (Hp); 25% *A. gracilis* + 75% *H. pluvialis* (Ag-25+Hp-75) and 75% *A. gracilis* + 25% *H. pluvialis* (Ag-75+Hp-25).

Life cycle characteristics	Diets			
	Ag	Hp	Ag-25+Hp-75	Ag-75+Hp-25
Intrinsic rate of increase (days)	0.20	0.11	0.19	0.21
Mean embryonic development time (hours)	47	33	33	33
Mean post-embryonic development time (days)	1.5	1.5	1.5	1.5
Mean fecundity (number of eggs per female)	15.7	5.35	14.2	11.8
Mean number of offspring per female	5.0	8.4	4.25	4.8
Longevity (days)	9.0	15.4	7.6	8.6
Mean length of neonate (µm)	403 (±61)^{b}	349 (±76)^{c}	414 (±77)^{ab}	440 (±44)^{a}
Mean length of primipara (µm)	439 (±74)^{a}	434 (±83)^{a}	491 (±99)^{ab}	517 (±59)^{b}
Mean length of adult (µm)	801 (±110)^{b}	690 (±158)^{a}	618 (±125)^{c}	739 (±65)^{b}
Dry weight of neonate (µg)	0.05	0.04	0.07	0.09
Dry weight of primipara (µg)	0.17	0.09	0.10	0.13
Dry weight of adult (µg)	0.26	0.35	1.2	0.38
Lipid (% dry weight)	7.02	5.58	5.28	7.31

Means values followed by the same letter do not differ between diets

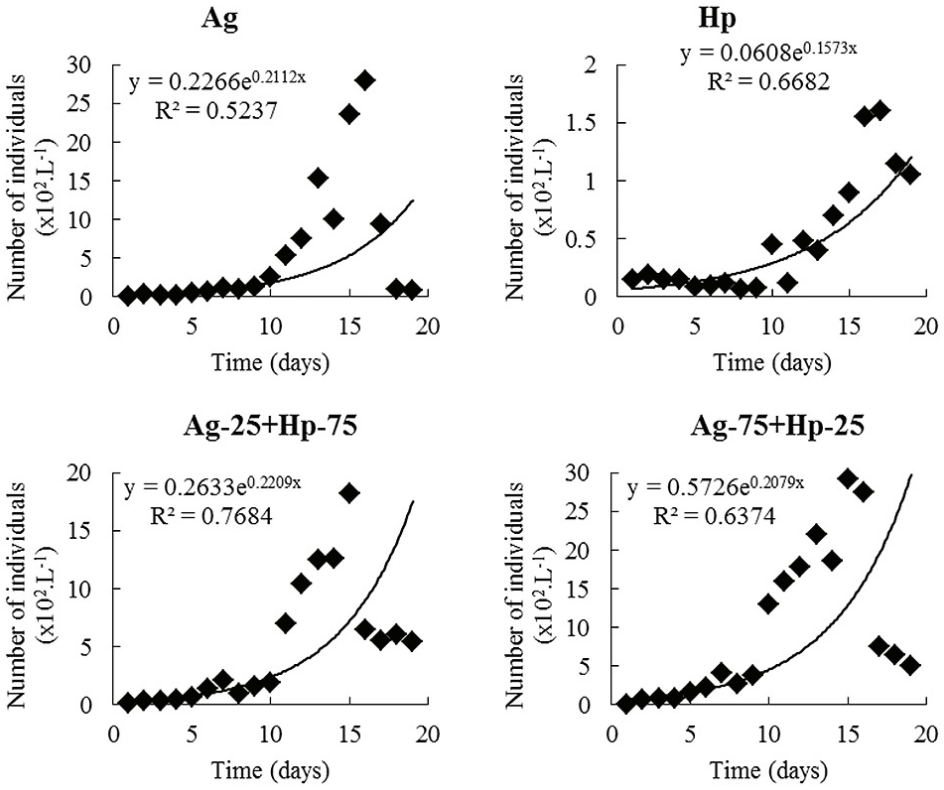

Figure 1. Population growth curve of *Diaphanosoma birgei* maintained on diets of 100% *Ankistrodesmus gracilis* (Ag); 100% *Haematoccocus pluvialis* (Hp); 25% *A. gracilis* + 75% *H. pluvialis* (Ag-25+Hp-75) and 75% *A. gracilis* + 25% *H. pluvialis* (Ag-75+Hp-25) in the laboratory.

26.2 hours intervals (Figure 2). Since Hp diet had a higher lifespan (19-days), the number of broods was higher. Embryonic development time was somewhat higher with Ag diet (47 hours) than the others (33 hours), although the post-embryonic development time was similar in the four diet treatments (1.5-days) (Table 1).

Figure 3 shows the relationship between fecundity and longevity. The highest fecundity produced 23 neonates per female (Ag-75+Hp-25) reached when 5-day old. The dry weights of neonate were higher on Ag-75+Hp-25 diet, primipara females had higher weights on Ag diet and adult females were higher on Ag-25+Hp-75 diet (Table 1). In relation to length, it was higher in the Ag-75+Hp-25 diet for neonate and primipara females and in the Ag diet, it was higher for adult females (Table 1).

There was a difference in lipids among diets for *D. birgei*. Lipid levels in Ag and Ag-75+Hp-25 diet were similar, with lowest percentages in Hp and Ag-25+Hp-75 diets (Table 1).

Water conditions were as a rule similar among diets, except for total phosphorus and chlorophyll-*a* which were significantly ($p<0.05$) higher in Ag diet than in the other diets. Ammonia among the nitrogenated compounds had the highest rate, ranging between 168 $mg.L^{-1}$ (Ag-75+Hp-25) and 188 $mg.L^{-1}$ (Hp). High ammonia concentration directly affecting conductivity values varying between 84 $mS.cm^{-1}$ (Ag) to 90 $mS.cm^{-1}$ (Ag-75+Hp-25). Nitrate and nitrite concentrations were similar in the Ag and Ag-75+Hp-25 diets. Frequent aeration of the water culture favoured dissolved oxygen concentrations above 4 $mg.L^{-1}$. The diet with Ag displayed the highest chlorophyll-*a* concentration. The pH in diets treatments were acid (Table 2).

4. Discussion

Data obtained in the present study clearly showed that *A. gracilis* and *H. pluvialis* affected various production parameters differently. Ultimately, productions goals must be considered, the microalgae fed should be easy to culture, provide efficient population growth and egg production, and thrive in similar temperature and food range as the cladoceran species being cultured.

The relationship between the longevity and fecundity curves provided cumulative information over time reproduction performance associated with a population, and thereby influenced the population dynamics (Dorazio and Lehman, 1983). In the current study, the fecundity curve of *D. birgei* showed that the mixed feed Ag-25+Hp-75 triggered fast fecundity at approximately two days. Further, fecundity was low when based only on *H. pluvialis* (Hp),

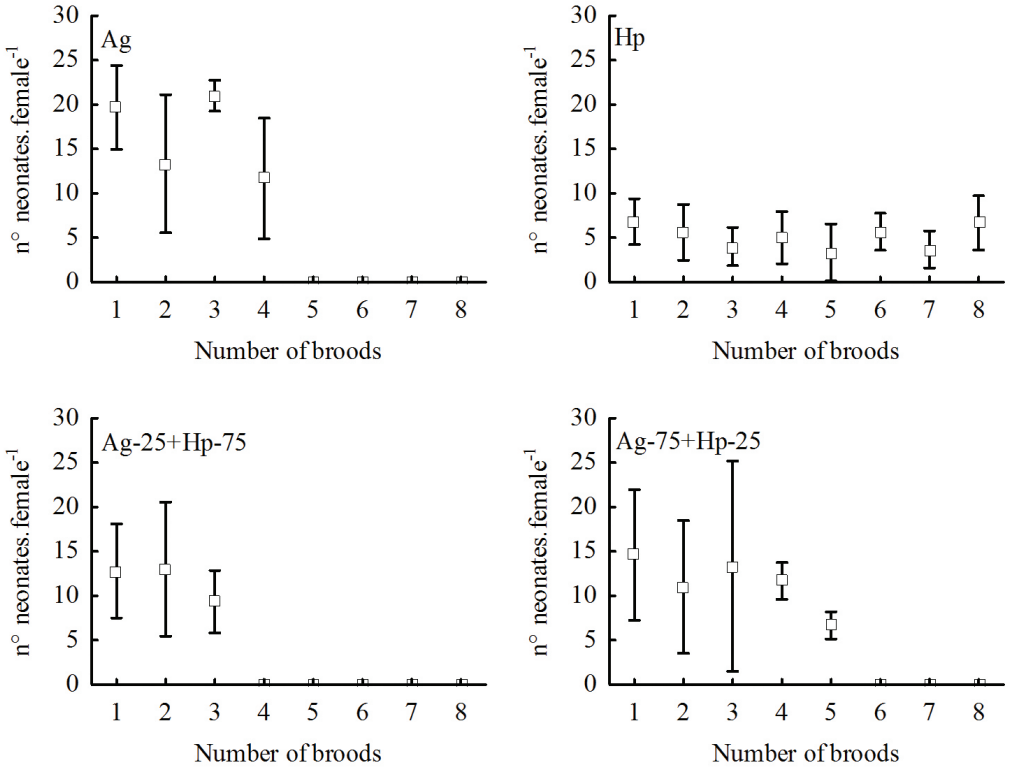

Figure 2. Mean fecundity (number of neonates per female) of *Diaphanosoma birgei* maintained on diets of 100% *Ankistrodesmus gracilis* (Ag); 100% *Haematoccocus pluvialis* (Hp); 25% *A. gracilis* + 75% *H. pluvialis* (Ag-25+Hp-75) and 75% *A. gracilis* + 25% *H. pluvialis* (Ag-75+Hp-25). Mean values (square) and standard deviation (whiskers).

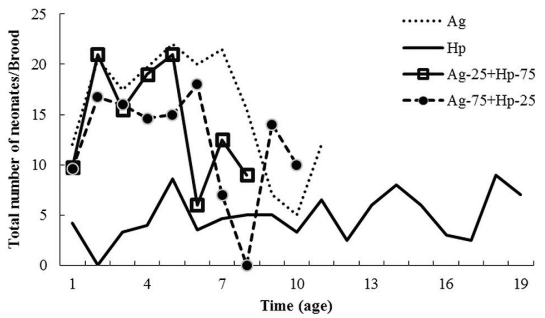

Figure 3. Relationship between fecundity and longevity of *Diaphanosoma birgei* maintained on diets of 100% *Ankistrodesmus gracilis* (Ag); 100% *Haematoccocus pluvialis* (Hp); 25% *A. gracilis* + 75% *H. pluvialis* (Ag-25+Hp-75) and 75% *A. gracilis* + 25% *H. pluvialis* (Ag-75+Hp-25).

albeit with greater longevity (19 days) and a higher number of broods (8). The above fact suggests the importance of mixing food for regulating the reproduction success of *D. birgei*.

The mean number of offspring per female in the current study was low. When the cladocerans were cultured under optimal temperature (25-30°C) and food was given without any restrictions, tropical taxa appear to have lower number of eggs than their temperate counterparts of comparable length. Growth rate increases with temperature increase up to a certain level above which it declines (Sarma et al., 2005).

The lifespan of *D. birgei* ranged between 5 and 13 days depending on the test conditions which included temperature and food concentration (Hardy and Duncan, 1994; Sipaúba-Tavares and Bachion, 2002). In the current study the average lifespan between 7 and 9-days was obtained with Ag and Ag + Hp diets, or rather, within the range reported for this species. In contrast to the mean lifespan of many temperate taxa of *Diaphanosoma* which was usually longer and varied between 2 and 4 weeks (Lemke and Benke, 2003), the lifespan of subtropical species was relatively shorter, or rather, 2 weeks (Sipaúba-Tavares and Bachion, 2002).

Population growth curves of *D. birgei* in microalgae diets had a larger lag phase (9 days). Sudden decrease throughout the growing period of *D. birgei* may not be linked only to food resources and temperature since these parameters are controlled in laboratory experiments. Some cladocerans, such as *Daphnia*, released soluble products and their metabolism could also act in a density-dependent way which, in certain circumstances, augmented the effects of food limitation (Burns, 2000).

Table 2. Estimate parameters of *Diaphanosoma birgei* water cultured during the experimental period for the following diets: 100% *Ankistrodesmus gracilis* (Ag); 100% *Haematoccocus pluvialis* (Hp); 25% *A. gracilis* + 75% *H. pluvialis* (Ag-25+Hp-75) and 75% *A. gracilis* + 25% *H. pluvialis* (Ag-75+Hp-25).

Parameters	Diets			
	Ag	Hp	Ag-25+Hp-75	Ag-75+Hp-25
pH	6.3 (±0.6)[a]	6.3 (±0.5)[a]	6.2 (±0.6)[a]	6.2 (±0.6)[a]
Conductivity (μS.cm^{-1})	84 (±38)[a]	86 (±38)[a]	86 (±37)[a]	90 (±45)[a]
Dissolved Oxygen (mg.L^{-1})	4.0 (±1.7)[a]	6.4 (±2.4)[a]	5.3 (±2.1)[a]	4.9 (±1.6)[a]
Ammonia (μg.L^{-1})	173 (±8.3)[a]	188 (±11)[a]	185 (±20)[a]	168 (±15)[a]
Nitrate (μg.L^{-1})	6.0 (±2.7)[a]	4.5 (±2.1)[a]	5.8 (±2.5)[a]	6.2 (±2.5)[a]
Nitrite (μg.L^{-1})	6.0 (±2.9)[a]	6.5 (±2.2)[a]	6.7 (±2.2)[a]	6.2 (±2.0)[a]
Total Phosphorous (μg.L^{-1})	41 (±13)[a]	29 (±10)[a]	32 (±7.5)[a]	31 (±12)[a]
Orthophosphate (μg.L^{-1})	7.6 (±1.7)[a]	6.7 (±2.1)[a]	7.0 (±1.9)[a]	6.7 (±2.2)[a]
Chlorophyll-*a* (mg.L^{-1})	52 (±25)[a]	14 (±8.0)[b]	21 (±8.1)[b]	32 (±11)[ab]

Means values followed by the same letter do not differ between diets

Under optimal food and temperature conditions for a given volume, smaller tropical taxa were more abundant and one might speculate that numerically they reached greater numbers per unit volume. For example, *D. birgei* 5-6 ind.mL^{-1} (Sipaúba-Tavares and Bachion, 2002) and *D. brachyurum* 1-13 ind.mL^{-1} (Nandini et al., 2007) were comparable with results produced in the current study.

Diaphanosoma generally reached a peak density in about 2 weeks (Lemke and Benke, 2003; Han et al., 2011), a trend also observed in the present study. When raised exclusively on *H. pluvialis*, *D. birgei* showed a lower growth than if they were cultured on mixed food which actually improved growth rates.

Cladocerans' *r* rates varied between 0.01 and 1.5 ind.day^{-1} depending on species, temperature, quality and food amount. *Diaphanosoma brachyurum* had an *r* rate of 0.32 ind. day^{-1} at 25°C (Lemke and Benke, 2003); *D. dubium* had an *r* rate of 0.29 ind.day^{-1} at 29°C (Han et al., 2011) and the *r* rates of *D. birgei* in the current study were similar among the three diets (Ag; Ag-25+Hp-75; Ag-75+Hp-25) at temperature 24°C. In fact, they agreed with published ranges of other cladocerans species reared at similar temperatures and with various food conditions (Lemke and Benke, 2003; Ismail et al., 2011).

Life history traits of cladocerans are known to change according to resource quality or quantity (Acharya et al., 2005) and this view is further supported by this experiment. The longer duration of *D. birgei* in Hp diet revealed that animals which produced more offspring and grew faster during their peak reproduction period died earlier (Ag-25+Hp-75 diet), whereas animals that grew slowly and had a lower reproduction output survived longer (Hp diet).

Acharya et al. (2005) reported similar findings. *Bosmina,* which reproduced more and earlier, had a lower survival rate than those reproducing later and slower. Some researchers have reported that animals which grow faster and put more energy in reproduction tended to have a lower life expectancy (Lemke and Benke, 2003; Sarma et al., 2005; Ismail et al., 2011). Their argument emphasised the advantage of assessing life history over the lifespan of the individual.

This study revealed that the impact of food quality on most life history parameters of *D. birgei* had a greater influence on population increase. The use of freshwater cladoceran as an alternative live food for freshwater aquaculture actually reduced dependence on *Artemia* sp. and artificial food. In fact, the latter failed to support high production especially during the early stages of the fish lifespan. The current study therefore demonstrated that *D. birgei* maintained high growth on a mixed diet and Ag diet, which is an excellent feature to serve as a live food for warm water fish larvae.

It is also possible that *D. birgei* fed on Ag and Ag-75+Hp-25 diets in this experiment sustained higher growth rate because of a higher lipid content in these diets. It was observed that the effects of food quality differentiated the population and this fact might be related to the maintenance of lipid reserves. Results showed that food quality affected the life history parameters of *D. birgei* and that dietary constraints could be an important factor in the determination of population success.

Macedo and Pinto-Coelho (2001) observed that lipid level of *Moina micrura* varied between 11.4% and 19.9% due to the feeding effects of different algal diets. Algal diets, rich in fatty acids, generally supported better survival and growth in many cladoceran species (Nandini et al., 2007).

The post-embryonic development time in current study (1.5 days) at 24°C was lower than that reported for *D. dubium* reared at 23°C (3.3-3.0 days; Han et al., 2011) and half the time reported by Rietzel (1998) for subtropical *D. birgei* reared at 25°C (3.2 days). The embryonic development time for *D. birgei* treated with Ag diet in this study fall within the ranges for other cladocerans species reared at similar temperatures (Rietzel, 1998; Güntzel et al., 2003; Fonseca and Rocha, 2004).

In the present study the body length of *D. birgei* was smaller than that reported for *Daphniopsis australis* (Ismail et al., 2011) and corroborated the fact that individuals in warm waters were often smaller than those in cold ones.

During *D. birgei* population growth, the food level remained high, with chlorophyll-*a* concentration around 14-52 mg.L^{-1}. Not only temperature, food and the intrinsic conditions of animals affected the performance and the development of cladocerans, but morphological changes in response to abiotic factors which also affect survival and reproduction of cladocerans should be taken into account and required greater attention.

Mixed microalgae diets have been suggested for filter-feeders, because monoalgal culture may not meet their nutritional requirements. However, this is not a general rule and it was not the case in this study, because the production obtained with one monospecific culture (Ag) was the same or better results of the mixed microalgae diets. The present study showed that *A. gracilis* diet and mixed microalgae diets tested were able to support the egg production and development of *D. birgei*. These treatments led significant improvement in growth, mean fecundity, lipid contents and intrinsic rate of increase. The life history of *D. birgei* varied according to different food supply. In addition, this study illustrated that mixed microalgae diets and *A. gracilis* were suitable and effective for growth and development of *D. birgei*.

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