Demography of zooplankton (*Anuraeopsis fissa*, *Brachionus rubens* and *Moina macrocopa*) fed *Chlorella vulgaris* and *Scenedesmus acutus* cultured on different media

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Abstract: Generally zooplankton growth is often limited by the quality of their algal diet. A cheaper common practice in aquaculture, is to culture algae with fertilizers; however, the demography of zooplankton when fed these algae has not yet been evaluated. We studied the population growth and life table demography of the rotifers *Anuraeopsis fissa* and *Brachionus rubens*, and the cladoceran *Moina macrocopa*. For this, the algae *Scenedesmus acutus* or *Chlorella vulgaris* were cultured on defined (Bold's basal) medium or the commercial liquid fertilizer (Bayfolan). Experiments were conducted at one algal concentration $1.0x10^6$cells/mL of *C. vulgaris* or its equivalent dry weight of $0.5x10^6$cells/mL of *S. acutus*. The population dynamics were tested at 23±1ºC in 100mL transparent jars, each with 50mL of the test medium, with an initial density of 0.5indiv/mL, for a total of 48 test jars (3 zooplankton 2 algal species x 2 culture media x 4 replicates). For the life table experiments with *M. macrocopa*, we introduced 10 neonates (<24h old) into each test jar containing the specific algal type and concentration. For the rotifer experiments, we set 5mL tubes with one neonate each and 10 replicates for each algal species and culture medium. We found that the average rotifer life span was not influenced by the diet, but for *M. macrocopa* fed *S. acutus* cultured in Bold's medium, the average lifespan was significantly lower than with the other diets. The gross and net reproductive rates of *A. fissa* (ranging from 18-36 offspring per female) were significantly higher for *C. vulgaris* cultured in Bold medium. Regardless of the culture medium, *Chlorella* resulted in significantly higher gross and net reproductive rates for *B. rubens* than *S. acutus* diets. The reproductive rates of *M. macrocopa* were significantly higher in all the tested diets except when fed with *S. acutus* in Bold medium. The population increase rate, derived from growth experiments of *A. fissa* and *B. rubens*, ranged from 0.1-0.25/d and were significantly higher on *C. vulgaris* cultured in liquid fertilizer as compared to the other diets. The growth rates of *M. macrocopa* ranged from 0.1 to 0.38/d, and were highest with diets of *C. vulgaris* cultured in Bold medium and *S. acutus* cultured in fertilizer. Thus, regardless of the culture medium used, the growth rates of the evaluated zooplankton species were higher with *Chlorella* than with *Scenedesmus*. The peak population density was highest (2 800ind/mL) for *A. fissa* fed *Chlorella* that was cultured on liquid fertilizers, while *B. rubens* and *M. macrocopa* had peak abundances of 480 and 12ind/mL, respectively under similar conditions. Rev. Biol. Trop. 60 (3): 955-965. Epub 2012 September 01.

Key words: Rotifera, Cladocera, algae, fertilizer, diet, population growth, zooplancton.

The production of adequate amounts of live food such as zooplankton for fish larvae remains a 'bottleneck' in aquaculture. Many reviews had well documented that the zooplankton requirement declines after the first 4-6 weeks, but during this period, demand is very high (50 000 rotifers per week per larva: Lubzens *et al*. 1989). This way, aquaculture facilities have to establish economical but large scale cultures of freshwater zooplankton.

The live food predominantly used in freshwater aquaculture includes rotifers and cladocerans, this is because of their adequate body size for the gape of young larvae, high growth rates and ease of maintenance. The gape size of oviparous fish can be 10-25% of the total length (Østergaard *et al*. 2005) resulting in the need of small sized but nutritive prey. Rotifers are among the best suited prey during these early stages due to their small size (<100µm) and their ability to reach high densities (300ind/mL) in a short time (<10days) (Sarma 1991).

Brachionids, particularly *Brachionus calyciflorus*, *B. rubens* and *B. plicatilis* (in marine systems) are most frequently used as live prey for fish. *Anuraeopsis fissa* is the smallest brachionid with high growth rates (Sarma *et al*. 1996) and therefore could serve as an important first diet for small fish species of the genus *Chirostoma* (although these measure only 20-30cm as adults, they have a high commercial value in Mexico (Chacón-Torres & Rosas-Monge 1995). *Brachionus rubens* is also useful due to its high growth rates (Azuara-García *et al*. 2006) and wide distribution under tropical conditions. Among cladocerans, *Daphnia* spp. are widely used in temperate countries but in the tropics *M. macrocopa* should be preferred, particularly due to its wide distribution, high maximal densities and growth rates (Sarma *et al*. 2005) and the high preference that fish larvae have for it (Zaret 1980).

The stoichiometric ratio of zooplankton is of paramount importance for using live food in aquaculture. Rotifers contain, as dry weight, 28-63% proteins and 9-28% lipids (Lubzens & Zmora 2003). Cladocerans, particularly *Moina* contain 59-78% proteins and 12-27% lipids (Watanabe *et al*. 1983). Physico-chemical parameters regulate, not-only the growth rates of the plankton but also their nutritional quality. Rotifers, for instance, have higher lipid content at 10°C than at 25°C (Lubzens *et al*. 1995). These factors affect the population growth of zooplankton as well as the fish larvae. The quality of zooplankton as diet for fish larvae can be tested using proximal analyses as well as growth bioassays. Previous studies have indicated that the growth rates of rotifers are significantly influenced by the quality of the diet. Growth rates are lower on yeast than on algae-yeast mixed diets (Peña-Aguado *et al*. 2005). These effects are also evident at the next trophic level. For instance, *B. rubens* cultured on the organic wastes had higher growth rate than *Chlorella*-fed populations; the nutritional quality of the rotifers cultured on this diet was further reflect in bioassays conducted with the predatory rotifer *Asplanchna sieboldi,* which had growth rates ranging from 0.05 to 0.08 per day on *B. rubens* cultured on *C. vulgaris* but 0.14 to 0.22 per day on rotifers fed organic wastes (Sarma *et al*. 2003). In an earlier study, Kibria *et al*. (1999) showed that the somatic growth of the perch (*Perca fluviatilis*) was higher on *Daphnia carinata* cultured in waste water than with *Moina australiensis* reared on the same medium.

Green algae grow well on commercial fertilizers (Chaumont 1993). Our preliminary experiments have shown that the liquid fertilizer of Bayfolan (Bayer product) supports algal growth comparable to the defined algal media such as Bold's basal. This is important in terms of aquacultural requirements since culturing algae with fertilizers would significantly bring down the production costs (Jana & Webster 2003). Several studies show that both *Scenedesmus* and *Chlorella* (Flores-Burgos *et al*. 2003) can be used for zooplankton culture. However, few laboratory studies considered growing algae on commercial liquid fertilizers because of possible nutritional limitations.

In this study, we compared the population growth and life table demography of *A. fissa*, *B. rubens* and *M. macrocopa* fed *Chlorella*

vulgaris or *S. acutus*, grown on Bold´s basal medium or the commercial liquid fertilizer Bayfolan (Bayer).

MATERIALS AND METHODS

Culture assays: *Anuraeopsis fissa* (body length, 70µm) and *M. macrocopa* (1 300µm) were isolated from local waterbodies in Puebla City, and *Brachionus rubens* (120µm) from a small pond in Tepozotlan town, all in Mexico. All the zooplankton species were cultured in moderately hard water (EPA medium) and fed a mixture of *S. acutus* and *C. vulgaris* at 23 ± 2°C. The EPA medium was prepared by dissolving 96mg $NaHCO_3$, 60mg $CaSO_4$, 60mg $MgSO_4$ and 4mg KCl in one liter of distilled water (Weber 1993). The algae were separately cultured, from the first day, on Bold's basal medium (Borowitzka & Borowitzka 1988) or in the commercial liquid fertilizer Bayfolan (Bayer, 0.5mL/L). The fertilizer composition was: N 9.1%, P 6.6%, K 5.0%, S 1 250ppm, B 332ppm, Co17ppm, Zn 664ppm, Cu 332ppm, Mo 42ppm, Ca 207 ppm, Mn 332ppm, Fe 415ppm, Mg 207ppm, Thymine clohydrate 33ppm and Indolacetic acid 25ppm. The cultures were exposed to continuous fluorescent illumination (1700 lux) and aeration. Sodium bicarbonate ($NaHCO_3$ 0.25g/L) was added every third day as a source of carbon. The algae were harvested after 8-10 days, allowed to sediment in a refrigerator for 24h, decanted and the density was estimated using a Neubauer haemocytometer.

Life table studies: All experiments were conducted at one algal concentration $1.0x10^6$cells/mL of *C. vulgaris* or its equivalent dry weight of $0.5x10^6$cells/mL of *S. acutus* (Mayeli *et al*. 2004). Experiments were carried out at 23±1ºC in 100mL transparent jars, each with 50mL of the respective test medium, algal species and density. For the life table experiments with *M. macrocopa*, we introduced 10 neonates (<24h old) into each jar containing the specific algal type and concentration. For the two rotifer species, the experiments were conducted in 5mL tubes with one neonate each. For these experiments, we set up 10 replicates for each algal species and culture medium. The number of individuals in each cohort was counted daily. The neonates and dead individuals of the original cohort when present were counted and eliminated. The surviving individuals of the cohort were transferred to fresh medium containing appropriate concentration of the algae. Experiments were maintained until the last adult of each cohort died.

Jack-knife method was used to derive means and standard errors of the demographic variables of rotifers (Meyer *et al*. 1986). The survivorship and fecundity data were used to calculate variables such as average lifespan (ALS), gross and net reproductive rates, generation time (T), and the rate of population increase per day (r) using the following equations (Krebs 1985):

Gross reproductive rate

$$= \sum_{0}^{\infty} m_x \qquad (1),$$

Net reproductive rate

$$R_o = \sum_{0}^{\infty} l_x \cdot m_x \qquad (2),$$

Generation time:

$$T = \frac{\sum l_x \cdot m_x \cdot x}{R_o} \qquad (3),$$

Rate of population increase, Euler equation (solved iteratively)

$$\sum_{x=w}^{n} e^{-rx} \cdot l_x \cdot m_x = 1 \qquad (4),$$

where, l_x is the probability of an individual to survive to an age class, m_x is the age specific fecundity, R_o is the average number of offspring per female, and r is the population growth rate.

Population growth experiments: Population growth experiments were conducted under similar conditions mentioned above, in 100mL recipients with 50mL of the test medium with the desired algal concentration.

Every jar included 25 individuals of a mixed population of each of the test species. In total there were 48 test jars (three zooplankton taxa x two algal species x two culture media x four replicates). The individuals were counted and transferred to fresh medium with the appropriate algae concentration in a daily basis. The experiments were continued over a three-week period, until the populations began to decline. Population growth rates were calculated using the formula:

$$r = (lnN_t - lnN_o)/t$$

where N_o is the initial population density, N_t is the population density at time t and t is the time in days (Krebs 1985).

Data from demography and population growth experiments were assessed using one way analysis of variance (ANOVA) (Sokal & Rohlf 2000). Post-hoc (Holm-Sidak test) analysis was used for multiple comparisons utilizing the software Statistica ver. 6.

RESULTS

Demography: The survivorship curves of *A. fissa* showed a steady decline in the rate of survival with age, regardless of the diet (Fig. 1). The fecundity was highest on *Chlorella* cultured on Bold's medium. *B. rubens* showed a steep decline in survivorship (Fig. 1) as compared to *A. fissa*. The fecundity was also significantly higher with *Chlorella* as compared *S. acutus*. The survivorship and fecundity of *M. macrocopa* (Fig. 1) were higher with *Chlorella* cultured in Bold's medium and *Scenedesmus* cultured in Bayfolan. These variables were much lower when fed *Scenedesmus* cultured on Bold's medium.

For the rotifers, no significant impact of the tested diets was found on the average lifespan, which was eight days in the case of *A. fissa* and five days in that of *B. rubens*. The average lifespan of *M. macrocopa* was about 14 days for all the tested diets except for *S. acutus* cultured on Bold's medium for which was significantly lower (F-test, $p<0.05$).

The gross and net reproductive rates of *A. fissa* ranged from 18-36 offspring per female and were significantly higher with *C. vulgaris* cultured on Bold's medium as compared to the other diets tested (Fig. 2). Regardless of the culture medium, *Chlorella* resulted in significantly higher (F-test, $p<0.05$) gross and net reproductive rates for *B. rubens* when compared to *S. acutus* diets. The reproductive rates of *M. macrocopa* were significantly higher (F-test, $p<0.05$) on all the test diets except for *S. acutus* on Bold's medium. It varied from 30 to 85 offspring per female. The generation time ranged from 4 to 6 days for rotifers. In *A. fissa* it was significantly longer with *C. vulgaris* diet on Bold's medium when compared to all other tested media. For *B. rubens*, it was not significantly influenced by the diet, while in *M. macrocopa* it was significantly lower only on *S. acutus* cultured in Bold's medium. The population growth rate of *A. fissa* ranged from 0.55 to 0.60 with no significant differences due to diet type. For *B. rubens* it was significantly higher on *Chlorella* (0.75-0.85 per day) than on *S. acutus* (0.25-0.40 per day). The growth rate of *M. macrocopa* ranged between 0.2-0.40 per day and was significantly lower (F-test, $p<0.05$) for *S. acutus* cultured on Bold's medium as compared to the other diets.

Population growth: The rotifer species *A. fissa* and *B. rubens* had higher growth rates on a diet of *C. vulgaris* than on *S. acutus* (Fig. 3). Regardless of the culture medium *S. acutus* did not support higher reproductive output. On the other hand, *C. vulgaris* cultured on the fertilizer was a better diet due to significantly higher growth rates of both the rotifer species as compared to those observed on diets of *C. vulgaris* cultured on Bold´s medium. The cladoceran *M. macrocopa* also grew better on a diet of *C. vulgaris* than on *S. acutus* (Fig. 3). Growth rates were higher for *C. vulgaris* cultured in Bold's medium than for *S. acutus* cultured on the fertilizer.

The population growth rates of both, *A. fissa* and *B. rubens*, ranged from 0.1-0.25/d (Fig. 4). These were significantly higher on

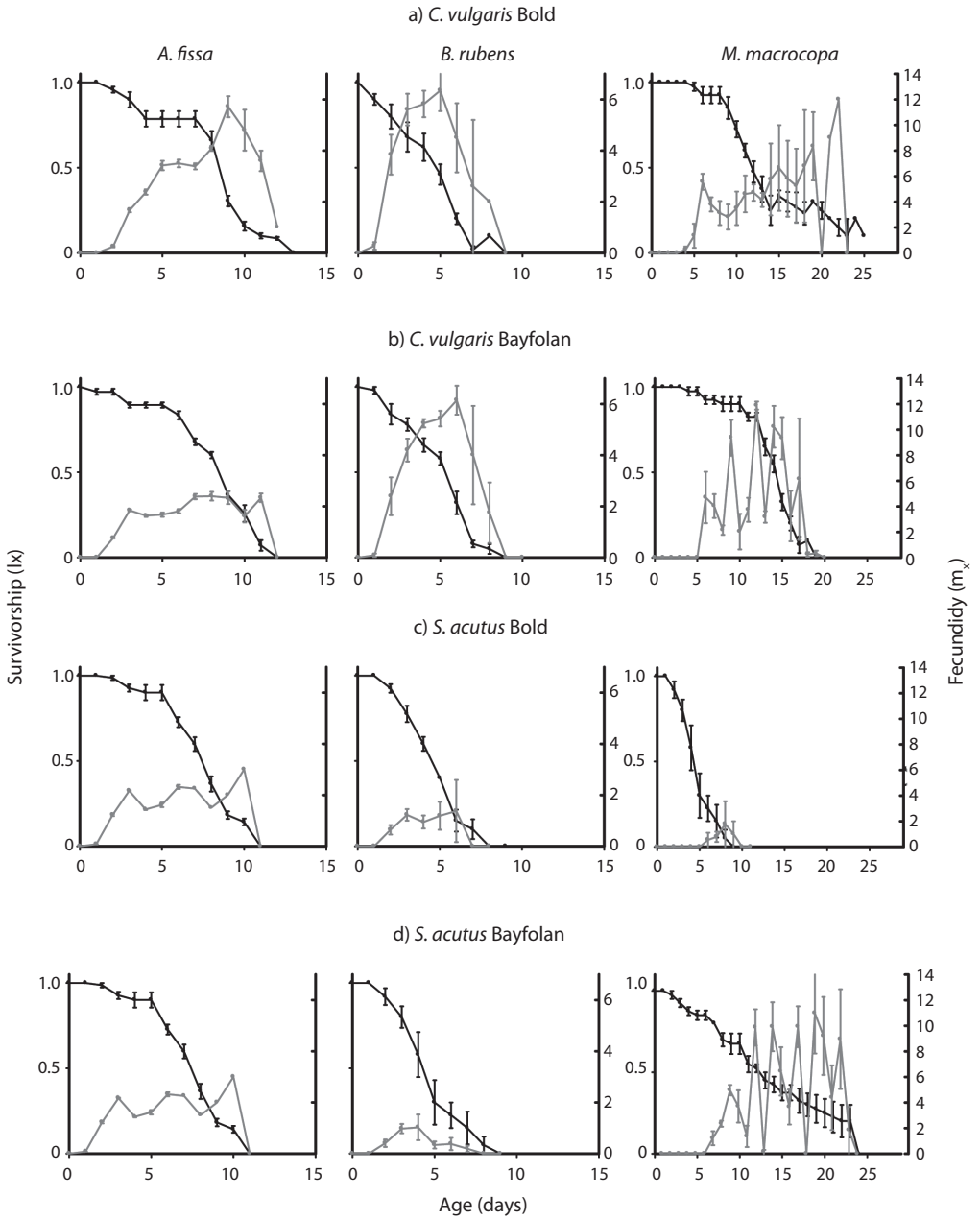

Fig. 1. Survivorship (black) and fecundity (grey) curves of *Brachionus rubens*, *Anuraeopsis fissa* and *Moina macrocopa* fed (a) *Chlorella* grown on Bold´s medium, (b) *Chlorella* on the liquid fertilizer Bayfolan (c) *Scenedesmus* on Bold's medium and (d) *Scenedesmus* on the fertilizer. Shown are the mean ± SE.

C. vulgaris cultured on fertilizer as compared to any of the other diets ($p<0.05$, F-test, post-hoc Tukey's test). The growth rates of *M. macrocopa* ranged from 0.1 to 0.38/d, and were highest on diets of *C. vulgaris* cultured on Bold's medium and *S. acutus* cultured on the fertilizer. Thus, in all the three zooplankton species, regardless of the culture medium,

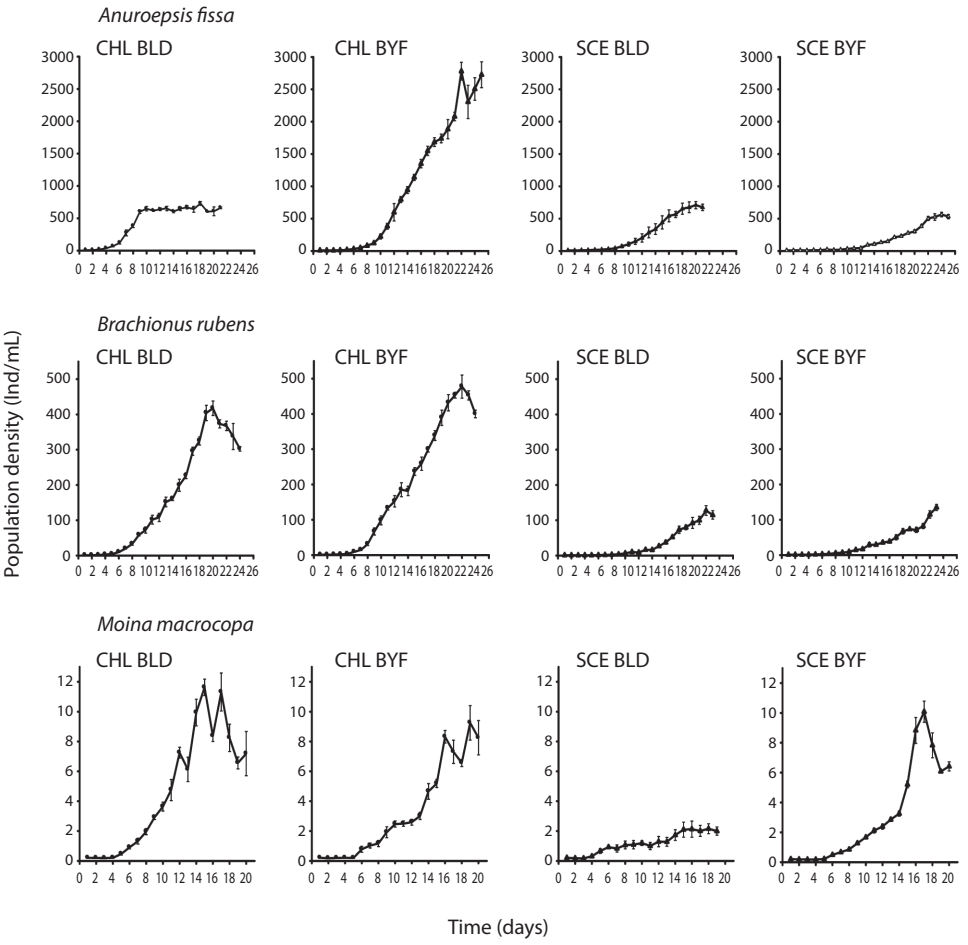

Fig. 2. Population growth curves of *B. rubens*, *A. fissa* and *M. macrocopa* fed (a) *Chlorella* grown on Bold´s medium, (b) *Chlorella* on the liquid fertilizer (c) *Scenedesmus* on Bold's medium and (d) *Scenedesmus* on the fertilizer. Shown are the mean ± SE.

the growth rates were highest on *Chlorella* than on *Scenedesmus*.

The peak population density reached was highest of 2 800indiv/mL in the smallest species tested, *A. fissa*. *Brachionus rubens* reached a density of 480indiv/mL while for *M. macrocopa* was of 12indiv/mL. The rotifer species attained the peak population density between 18 to 21 days, while *M. macrocopa* reached peak densities between 15 to 18 days. There were no significant differences in the day at which peak densities were reached in relation to the diet.

DISCUSSION

Zooplankton bioassays are sensitive enough to test the quality of algal (Nandini *et al*. 2010) or seston (Gulati *et al*. 2001) diets. Our study also shows that all the three species used in this study showed significant differences in the life-history parameters with relation to, not only the differences between algal species but also the medium on which each alga was cultured. We found that, in general *C. vulgaris* was a more suitable diet than *S. acutus*. In our study, the size of *C. vulgaris* ranged

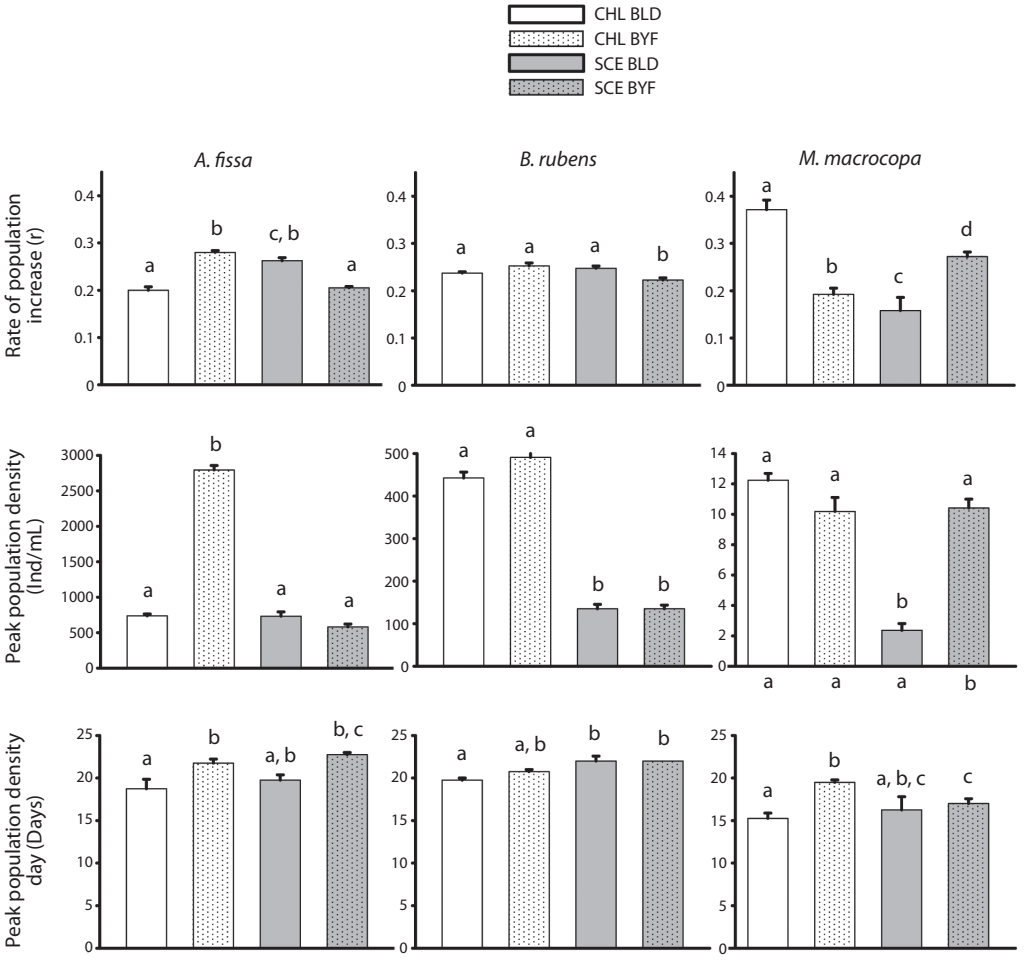

Fig. 3. Day at maximum population density, maximum population density and population growth rate of *B. rubens*, *A. fissa* and *M. macrocopa* fed (a) *Chlorella* grown on Bold´s medium, (b) *Chlorella* on the liquid fertilizer (c) *Scenedesmus* on Bold's medium and (d) *Scenedesmus* on the fertilizer. Shown are the mean ± SE.

from 4.5-5.1µm while that of *S. acutus* from 8.8 to 8.9µm. Most zooplankton can easily filter algae in the size range of 5-25µm (Monakov 2003), therefore the algal size may not have been the reason for poor growth on *S. acutus*. This is most probably due to the fact that the cell wall of the former is about 20 nm (Northcote *et al*. 1958) while that of *Scenedesmus* is 36 nm (Bisalputra & Weier 1963). This may have resulted in *S. acutus* being more difficult to digest by zooplankton than *C. vulgaris*.

The quality of algal diets depends significantly on the culture medium as well as physicochemical parameters such as light and temperature. Fatty acids are more sensitive to changes in the medium than are proteins (Rai *et al*. 1997). Although it has been suggested that in the face of poor food quality organisms would increase their intake (Brett 1993, Hessen 1993), this actually does not occur (Kilham *et al*. 1997). This is the reason why we found significant differences in the growth rates of the

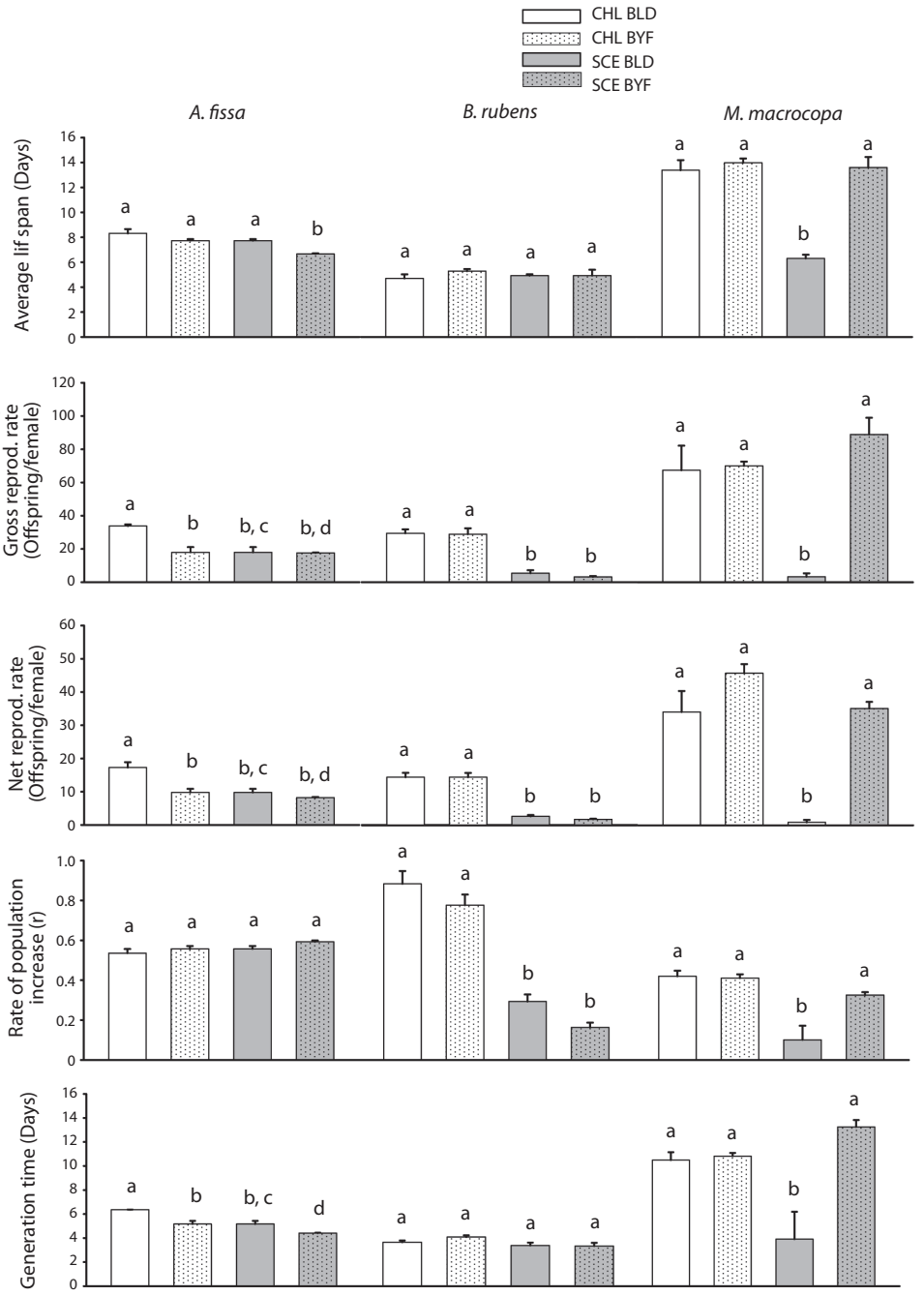

Fig. 4. Demographic variables (mean life spam, gross reproductive rate, net reproductive rate, generation time and rate of population increase) of *B. rubens*, *A. fissa* and *M. macrocopa* fed (a) *Chlorella* grown on Bold´s medium, (b) *Chlorella* on the liquid fertilizer (c) *Scenedesmus* on Bold's medium and (d) *Scenedesmus* on the fertilizer. Shown are the mean ± SE.

zooplankton in relation to the culture medium of the algae. It was evident that the *S. acutus* cultured on Bold's medium resulted in higher growth rates of *M. macrocopa* while *Chlorella* cultured on Bayfolan resulted in higher or similar growth rates of the rotifers as compared to same alga cultured on Bold's medium. This clearly indicates that Bayfolan is adequate for plankton production for aquaculture practices and would help in bringing down the costs of algal production significantly.

That *C. vulgaris* cultured on the commercial fertilizer is adequate for the rotifers is also evident from the peak densities attained; for *A. fissa* this was more than five times that reached on *C. vulgaris* cultured on Bold's medium. In the case of *B. rubens* significant differences were observed only in relation to the algal species but not with culture medium. This clearly indicates that culturing algae in commercial fertilizers will yield high densities of algae of an adequate quality for aquaculture. Several studies indicate that algae and zooplankton grown in diverse culture media are suitable food for zooplankton, mollusks and fish (Ahlgren *et al*. 1990).

Our study reiterates the importance of *M. macrocopa* as a live food for aquaculture. This species is known to have a higher protein content and lower ash content as compared to *Artemia* (Watanabe *et al*. 1983) or *Daphnia* (Kibria *et al*. 1999). It is one of the few cladoceran genera with much shorter age at first reproduction and lifespan which in turn result in higher growth rates. As compared to several cladoceran taxa, *Moina* frequently has growth rates values above 0.6 per day (Sarma *et al*. 2005). We also found that regardless of the algal type or culture medium used, the gross reproductive rate of *Moina* were up to 90. This indicates that a healthy, large scale, *Moina* culture would ensure sufficient prey for the fish larvae. *Moina* is also a preferred prey for commercially important fish species. For example, from first to third week after hatching, larval *Chirostoma riojai* showed high preference for *M. macrocopa* and consume it in large numbers (Morales-Ventura *et al*. 2004). It remains to be seen whether an exclusive diet of *M. macrocopa*, as compared to an artificial but balanced diet, improves the survivorship and growth of larval fish.

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RESUMEN

Generalmente el crecimiento del zooplancton está a menudo limitado por la calidad de su dieta de algas. La demografía del zooplancton durante la alimentación con algas no ha sido estudiada, a pesar de que el cultivo de algas con fertilizantes es una práctica económica común en acuacultura. Se analizó la demografía de *Anuraeopsis fissa* y *Brachionus rubens* (rotíferos) y *Moina macrocopa* (cladóceros), alimentados con las algas verdes *Scenedesmus acutus* o *Chlorella vulgaris* cultivadas en medio Bold o fertilizante líquido comercial (Bayfolan, de Bayer). En los rotíferos no se observaron diferencias significativas en el promedio de vida, sin embargo, este parámetro en *M. macrocopa* con *S. acutus* cultivada en Medio Bold, fue significativamente menor que en otras dietas. Las tasas de reproducción bruta y neta de *A. fissa* fueron significativamente mayores con *C. vulgaris* cultivada en medio Bold, que con el fertilizante; estas tasas en *B. rubens*, independientemente del medio de cultivo, resultaron significativamente mayores con *Chlorella* que *S. acutus*. La tasa de reproducción de *M. macrocopa* fue significativamente mayor en todas, a excepción de *S. acutus* en Bold. En el crecimiento poblacional con *A. fissa* y *B. rubens* la tasa de crecimiento poblacional varió de 0.1 hasta 0.25/d, significativamente mayores en *C. vulgaris* cultivadas con fertilizante, en comparación con las otras dietas; en *M. macrocopa* la tasa de crecimiento varió desde 0.1 hasta 0.38/d, las más altas fueron: con *C. vulgaris* cultivadas en medio Bold y *S. acutus* cultivadas con fertilizante. Así, en todas las especies, la tasa de crecimiento fue más alta con *Chlorella* que con *Scenedesmus*.

Palabras clave: Rotífera, Cladócera, crecimiento poblacional, algas, dietas, fertilizantes.

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