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±1°C in 100mL transparent jars, each with 50mL of the test medium, with an initial density of 0.5 indiv/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 (2 800 indiv/mL) for *A. fissa* fed *Chlorella* that was cultured on liquid fertilizers, while *B. rubens* and *M. macrocopa* had peak abundances of 480 and 12 indiv/mL, respectively under similar conditions. Rev. Biol. Trop. 60 (3): 955-965. Epub 2012 September 01.

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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, 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 (300 ind/mL) in a short time (<10 days) (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 20-30 cm 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 (1300µm) 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 NaHCO₃, 60mg CaSO₄, 60mg MgSO₄ 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 1250ppm, B 332ppm, Co 17ppm, 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 (NaHCO₃ 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 1.0x10⁶cells/mL of C. vulgaris or its equivalent dry weight of 0.5x10⁶cells/mL of S. acutus (Mayeli et al. 2004). Experiments were carried out at 23±1°C 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):

\[
\text{Gross reproductive rate} = \sum_{x=0}^{\infty} l_x \cdot m_x
\]

\[
\text{Net reproductive rate} = \sum_{x=0}^{\infty} l_x \cdot m_x \cdot e^{-rx}
\]

\[
\text{Generation time:} \quad T = \frac{\sum_{x=0}^{\infty} l_x \cdot m_x \cdot x}{R_0}
\]

\[
\text{Rate of population increase, Euler equation (solved iteratively)} = \sum_{x=w}^{n} e^{rx} \cdot l_x \cdot m_x = 1
\]

where, \(l_x\) is the probability of an individual to survive to an age class, \(m_x\) is the age specific fecundity, \(R_0\) 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:

\[ r = \frac{(\ln N_f - \ln N_i)}{t} \]

where \( N_i \) is the initial population density, \( N_f \) 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 & Rohlff 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
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,
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...
from 4.5-5.1 µm while that of *S. acutus* from 8.8 to 8.9 µm. Most zooplankton can easily filter algae in the size range of 5-25 µm (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

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**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.
Fig. 4. Demographic variables (mean life span, 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.
remains to be seen whether an exclusive diet 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 Chiropistoma 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, Cladóceros, crecimiento poblacional, algas, dietas, fertilizantes.

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