Culture of *Chlorella ellipsoidea* in different inexpensive medium and used as food for production of rotifer, *Brachionus calyciflorus*

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Introduction

Rotifers are multicellular animals with body cavities that are partially lined by mesoderm. These organisms are valuable live food for larval fish and crustacean culture. Several characteristics of rotifers, including their nutritional quality, body size and relatively slow motility have contributed to their usefulness as good prey for active larvae (Snell and Carrillo, 1984). In general, rotifers have both nutrient content and a high rate of daily production (Lubzens, 1987). Rotifer transmits adequate supplies of micro and macronutrients, vitamin and even antibodies to the fish larvae (Gatesoupe, 1982). The level of polyunsaturated ω-3 fatty acids in rotifer is believed to affect both survival and growth rate of fish larvae (Koven *et al*., 1990). Rotifer forms an excellent initial food because of its appropriate size (130-320 µm), planktonic nature, rapid production rate, suitability for mass culture under controlled conditions, ability to grow and reproduce in high density cultures and the possibility of artificially manipulating its nutritional qualities along with the euryhaline nature (Dhert *et al*., 2001). Among different genus of rotifer, *Brachionus* has been most widely used as essential food source in raising marine fish, shrimp and crab larvae due to its tolerance to the marine environment (Cheng *et al.,* 2004). Many researches have been done on rotifer culture, enrichment of rotifer culture, development of rotifer culture methods for the purpose of fish larvae feeding for improvement of fish culture industry (Dhert *et al.,* 2001; Hagiwara *et al.,* 2001; Leschenko *et al.,* 2005; Arimoro, 2007; Ludwig *et al.,* 2008). On the other hand, very few studies regarding rotifers culture has been conducted in Bangladesh.

Rotifers have specialized organ systems and a complete digestive tract that includes both a mouth and anus. As rotifers are microscopic animals, their diet must consist of matter small enough to fit through their tiny mouths during filter feeding. Rotifers are primarily omnivorous, but some species have been known to be cannibalistic (Watanabe *et al.,* 1983). The diet of rotifers most commonly consists of dead or decomposing organic materials, as well as unicellular algae and other phytoplankton that are primary producers in aquatic communities (Watanabe *et al.,* 1983). Such feeding habits make some rotifers as primary consumers. Rotifers are in turn prey to carnivorous secondary consumers, including shrimp and crabs. *Brachionus* feeds on microalgae, protozoa, bacteria and dead organic materials (Rezeq and James, 1987) in addition to artificial feeds. Diet is regarded as the most important criterion that could affect growth as well as nutritive quality of rotifers (Nhu, 2004). The lipid content and fatty acid composition of marine microalgae vary among species and culture conditions, and the algae fed to rotifer culture media or larval tanks will alter the lipid and fatty acid composition of the rotifers (Watanabe *et al.,* 1983).

In order to attain stable mass production of rotifers, it is desirable to develop a food source that will support rotifer growth completely by itself. Since large-scale algal production is relatively cheap, both under field and laboratory conditions, various types of algae are routinely being produced for feeding planktonic rotifers (Groeneweg and Schluter, 1981). *Chlorella* is an excellent food for rotifer if supplemented with vitamin B_{12} (Hirayama *et al*., 1989) and some strain of *Chlorella* are known to absorb vitamin B_{12} from culture medium and store this vitamin in their cells (Maruyama and Hirayama, 1993). During the manufacturing process, *Chlorella* cells enriched with vitamin B_{12}, which is essential for rotifers (Hirayama and Funamoto, 1983). In the present study, we cultured *Chlorella ellipsoidea* in different media and used as food in powdered and fresh live form along with Backer's yeast to mass production of rotifers.

Materials and methods

In this study, firstly we cultured *C. ellipsoidea* having three treatments including inorganic expensive medium (T1), pulse bran extract inexpensive medium (T2) and soil extract inexpensive medium (T3). Secondly, we cultured *B. calyciflorus* having three treatments using dried powder *C. ellipsoidea* (T1), fresh live *C. ellipsoidea* (T2) and Baker's yeast (T3) as food.

Inorganic medium was prepared with the inoculation of stock solutions of 8 major (macro) nutrients and 6 minor (micro or trace) nutrients. Ten liter distilled water was taken in a 30 liter plastic bucket and stock solutions were added and mixed well in the bucket and stored in a 15 liter plastic container. Stock solutions were prepared in distilled water using different chemical compounds as major nutrients and trace elements (Stainer *et al.*, 1971).

Pulse bran (Maskalai bran, *Vinga mungo*) was mixed with 20 L tap water in plastic bucket. After one week, 11g urea was added to each bucket. After four weeks, partially decomposed pulse bran mixture was filtered through thin markin cloth and solid materials were discarded. Then after a week the supernatant was siphoned to another bucket and 2g lime (CaO) per liter of medium was mixed to make it clear and pH was adjusted to 7 by adding H_2SO_4. Then, after a week, the clear supernatant was again siphoned to another bucket and this clear solution was ready as algae culture medium.

Soil was collected from bottom of a nursery pond of Field Laboratory Complex of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Textural class of the collected soil was "silty clay loam" which is classified as very fertile soil. After drying for 2 weeks, soil was crushed into powder to facilitate sieving. Soil was sieved through a small mesh sieve usually used to sieve rice powder for making cake. Then 2 kg soil was mixed with 5L tap water in a plastic bucket. Soil-water mixture was kept for 5 days and during this period mixture was stirred for half an hour daily. Then soil-water mixture was kept in this condition for several days till the settling of soil particles at the bottom of the bucket. Then supernatant was sterilized in an autoclave at 121°C and 15 Ib/inch^2 pressure for 20 minutes. The soil extract was treated with commercial urea (5g per liter) and TSP (2.5g per liter) fertilizers.

The seeds of *C. ellipsoidea* were collected from previous and continuous culture maintained in the laboratory of the Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh. Twenty percent seeds of *C. ellipsoidea* were used as inoculums in 200 mL culture medium taken in 1000 mL conical flask. Estimations of cell density of culture of *C. ellipsoidea* collected from twelve conical flasks were done daily by a haematocytometer following the procedure of Rahman (1992).

The seeds of rotifer were collected from different ponds around the Fisheries Faculty Building, Bangladesh Agricultural University, Mymensingh through selective netting with plankton nets (mesh size 55 micrometer and 250 micrometer). The seeds of rotifer were cultured in 4 plastic jars (of 5 liter capacity). Continuous aeration for 24 h by air pumps was arranged. Fresh cultured *Chlorella* was used as food for the rotifer cultured. Samplings were done regularly from each of the plastic jars for preservation (in 5% formalin) and daily analysis under a compound microscope using a special zooplankton counting cell to observe the animal and to find out cultivable rotifer. At the time when the concentration of rotifer was high and the concentration of nauplii, protozoa etc. gradually decreased and finally vanished, then the whole culture was considered as stock culture. *Brachionus calyciflorus* was cultured in nine plastic jars of 3 liter capacity each containing 1 liter of water. Initial density of *B. calyciflorus* was 3 individuals/mL in each jar and it was taken from the stock culture of rotifer. In T1, powdered dried *C. ellipsoidea* was given daily as feed for rotifer at the rate of 0.1 g per liter of water, in T2 fresh cultured *C. ellipsoidea* was given at 40 mL at the concentration of 2.5×10^6 cells/mL and in T3 baker's yeast was given daily at the rate of 0.15 g per liter. Before using in the experiments, the *C. ellipsoidea* powder and the baker's yeast were suspended in small amounts of water and homogenized by hand mixing. Determination of *B. calyciflorus* densities were done daily by using a special zooplankton counting cell under a compound microscope.

Values are expressed as means ± standard error of the mean (SEM). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess statistically significant differences among the different sampling days and different treatments. Statistical significance was set at $p<0.01$. Statistical analyses were performed using SPSS Version 14.0 for Windows (SPSS Inc., Chicago, IL).

Results and discussion

The environmental factors, such as light intensity (lux), water temperature (°C), air temperature (°C), pH, dissolved oxygen (mg/L) were within the productive ranges and showed no abrupt changes during the experimental period in all the treatments. Within limit productive ranges of such water quality parameters have also been observed by a number of authors (Chowdhury *et al*., 2008; Rahman *et al.*, 2012; Talukdar *et al.*, 2012; Siddika *et al.*, 2012; Nupur *et al.*, 2013) for the proper growth and production of phytoplankton and zooplankton in natural condition in the aquaculture ponds of Bangladesh Agricultural University area which are the good agreements of the present study.

Cell densities ($\times 10^6$ cells/mL) of *C. ellipsoidea* cultured in different media for a period of 13 days has been presented in Fig.1. The ranges of cell density of *C. ellipsoidea* were 2.345 to 10.685 ($\times 10^6$ cells/ mL), 3.496 to 12.596 ($\times 10^6$ cells/mL) and 2.765 to 11.698 ($\times 10^6$ cells/mL) in T1, T2 and T3, respectively. Maximum cell densities of *C. ellipsoidea* was recorded in inexpensive pulse bran extract medium (T2) during the culture period. Almost similar cell density (3.36 to 9.37×10^6 cells/mL) of *Chlorella* was recorded after cultured in bean seed powder medium (Karmaker *et al*., 2001).

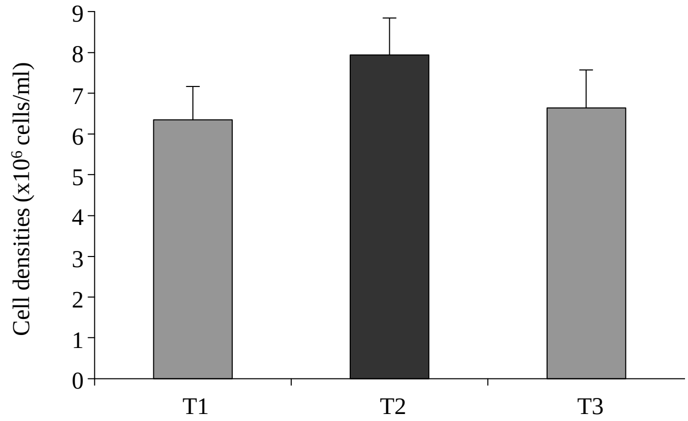

Figure 1: Mean (± SEM) cell densities (× 10^6 cells/mL) of *Chlorella ellipsoidea* in three media during culture period of 13 days.

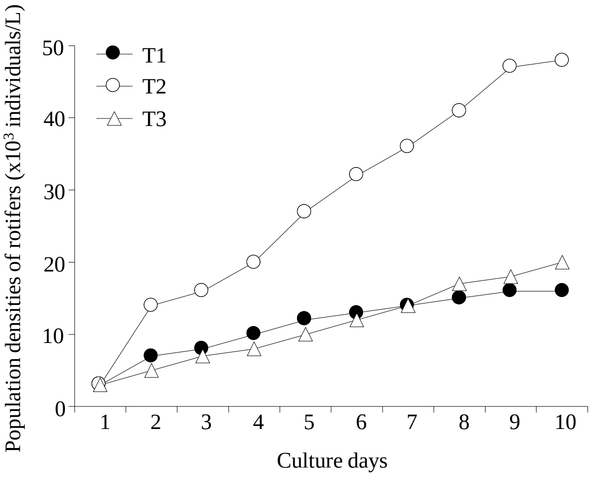

Figure 2: Daily fluctuations of population densities (× 10^6 cells/mL) of *Brachionus calyciflorus* in three treatments during the culture periods.

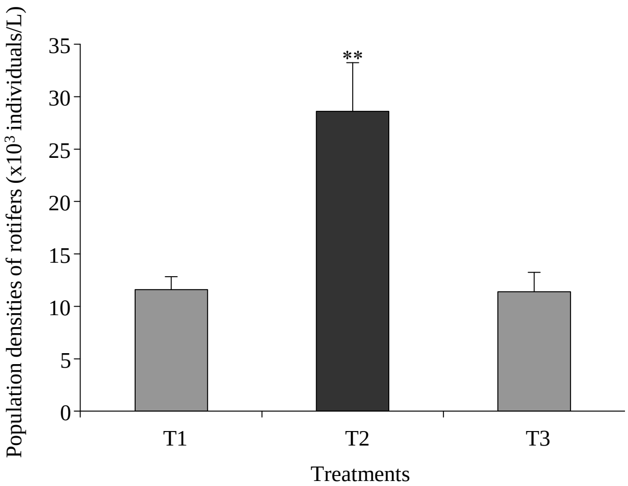

Figure 3: Mean (± SEM) population densities (× 10^6 cells/mL) of *Brachionus calyciflorus* in three treatments during the culture periods. ** indicated significant difference at *p*<0.01.

On the other hand, James *et al.* (1998) observed that the range of cell density was 20 × 10^6 to 80 × 10^6 cells/mL, which was much higher than that of the present experiment. These variations of production of *Chlorella* might be due to culture periods, quality of culture medium and environmental conditions.

Population densities (×10^3 individuals/L) of *B. calyciflorus* cultured in different treatment for a period of 10 days have been presented in Figs. 2 and 3. The ranges of density of *B. calyciflorus* were 3 to 16 (×10^3 individuals/L), 3 to 48 (×10^3 individuals/L) and 3 to 20 (×10^3 individuals/L) in T1, T2 and T3, respectively. Mean population densities were significantly highest (p<0.01) in T2 (28.6 ± 4.64 (×10^3) individuals/L) compared to T1 (11.6 ± 1.24 (×10^3) individuals/L) and T3 (11.4 ± 1.82 (×10^3) individuals/L).

Live *Chlorella* is one of the most widely used foods for culturing planktonic rotifers (Pourriot and Rougier, 1997). The mean value of *B. calyciflorus* density fed on fresh cultured *C. ellipsoidea* under T2 of the present experiment was 28.6 ± 4.64 (×10^3) individuals/L, which is more or less similar to Awaiss *et al*. (1992), who reported that the production of *B. calyciflorus* fed on live *Chlorella* was on average 31.5 ± 3.5 ×10^3 individuals/L. The mean population density of *B. calyciflorus* fed on powdered dried *C. ellipsoidea* under T1 was 11.6 ± 1.24 ×10^3 individuals/L, which strongly agrees with Lucia *et al.* (2001) who found the population density of *B. calyciflorus* cultured feeding with heat-killed *Chlorella* ranged from 6±1 to 26± 6 ×10^3 individuals/L. Hirayama and Nakamura (1976) found 400 individuals/mL during mass culture of *B. plicatilis* feeding with dry powder of *Chlorella* cultured for 41 days, which is higher than the present study, might be due to longer days of culture period. The mean value of *B. calyciflorus* fed on baker's yeast under T3 of the present experiment was 11.4±1.82 ×10^3 individuals/L. Rahman *et al*. (1993) found that the mean values of *B. calyciflorus* fed on baker's yeast was 24.17 ± 5.40 × 10^3 individuals/L which is much higher than that of the present study. It is reflected that the mean population density of *B. calyciflorus* was higher under T2 where rotifer was fed on fresh cultured live *C. ellipsoidea* and the mean population density of *B. calyciflorus* under T3 where rotifer that fed with baker's yeast was lower than those of T1 and T2. This indicates that fresh cultured live *C. ellipsoidea* is the best food for the rotifer, *B. calyciflorus* and dried powder *C. ellipsoidea* is also better than baker's yeast as food for the rotifers. Although a diet of baker's yeast alone was not comparable to that of *C. ellipsoidea*, it can be effectively used at low concentration to supplement algal requirements in rotifer culture system (Sarma *et al.*, 1997). It could be possible that dried and preserved *C. ellipsoidea* will be used when there is no live *C. ellipsoidea* to maintain rotifer culture.

In conclusion, culture experiment of rotifers, *B. calyciflorus* was done fed by powdered dried *C. ellipsoidea*, fresh cultured live *C. ellipsoidea* and baker's yeast. The environmental factors during *C. ellipsoidea* culture and rotifers, *B. calyciflorus* culture were found to vary within suitable ranges. The mean population densities of *B. calyciflorus* under T2 were significantly higher than T1 and T3. The results of present study revealed that fresh cultured live *C. ellipsoidea* was the best food for *B. calyciflorus* production and dried powdered *C. ellipsoidea* was better than baker's yeast as food for the rotifers.

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