The Bangladesh Veterinarian (2010) 27(2) : 91 – 98

Culture of *Brachionus plicatilis* feeding with powdered dried *Chlorella*

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Abstract

The rotifer *Brachionus plicatilis* was cultured with powdered dried *Chlorella* in treatment 1, live or fresh cultured *Chlorella* in treatment 2, and baker's yeast in treatment 3. All the jars under three treatments were stocked with *B. plicatilis* at the initial density of 10 individuals per ml. The water temperature, air temperature, pH and dissolved oxygen were within the suitable range for *B. plicatilis* culture. The highest population densities of *B. plicatilis* in treatments 1, 2 and 3 were 60000, 50000 and 30000 (individual/L), respectively. The powered dried *Chlorella* was comparable with live *Chlorella* and may be used successfully as a feed for *B. plicatilis*. (*Bangl. vet*. 2010. Vol. 27, No. 2, 91 – 98)

Introduction

Brachionus plicatilis is a brackishwater rotifer, which has been used as food for marine fish larvae and planktonic crustaceans throughout the world (Watanable *et al.,* 1983). But several authors have demonstrated the importance of rotifers as food for freshwater larvae (Hale and Carlson, 1972). *B. plicatilis* has been recognized as a potential food for shrimp larvae in addition to or as a replacement for *Artemia* (Hirata *et al.,* 1985). 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. *Chlorella* is suitable for rotifer culture because it can be grown with an organic carbon source such as glucose or acetic acid without light (Hirayama *et al.,* 1989). Attempts to use other food sources for growing rotifers have been successful, but rotifers produced with algae have a higher nutritive value for young fish (Kitajima *et al.,* 1979). Sometimes algal cultures crash (Suminto and Hirayama, 1997) which may, in turn, lead to problems of finding adequate food for rotifers. Therefore, stored algae such as dried algae could be used as substitute. On the other hand, at times there may be excessive production of micro-alga which could be stored for future use (Martinez and Chavez, 1994). The nutritional quality as well as the digestibility of the stored micro-alga may vary considerably and consequently the growth responses of zooplankton feeding on them (Dobberfuhl and Elser, 1999). In this context, population level responses of rotifers to stored micro-algae have not been well documented (Lubzens *et al.,* 1995).

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Initiating mass cultivation of rotifers, a rotifer strain can be selected with appropriate size for the fish larvae and suitable to the culture condition with adequate quality food. The physico-chemical properties of water affect the growth and production of rotifers. The present study was undertaken to prepare an inexpensive culture medium using pulse bran to culture *Chlorella* for making powder to use as diet for rotifer and to compare the culture of rotifers with fresh *Chlorella*, powdered *Chlorella* and with baker's yeast.

Materials and Methods

Maskalai (*Vigna mungo*) bran 700g was mixed with 20 litre tap water in three plastic 30 litre buckets. After one week 11g urea was added to each bucket. After four weeks partially decomposed bran mixture was filtered through thin clean cloth to remove solid particles. After a week the supernatant was siphoned to another bucket and 2g lime (Calcium oxide) per litre of medium was mixed to make it clear and pH was adjusted to seven using sulphuric acid. After a week the clear supernatant was again siphoned to another bucket as algae culture medium. The prepared medium was sterilized in an autoclave (121°C) under 15 1b/cm^2 pressures for 15-20 minutes.

Chlorella was cultured in this inexpensive organic medium for 18 days in 18 conical flasks (500 ml) arbitrarily marked 1-18, and each containing 200 ml of medium and *Chlorella* seed (*Chlorella ellipsoidea*, 20% or *Chlorella ellipsoidea* is 40 ml). Nine plastic jars were stocked with *B. plicatilis* at the initial density of 10 individuals/ml. Three treatments were used: in jars 1, 2, 3 (T-1) powdered dried *Chlorella* was used as feed for rotifer; in jars 4, 5, 6 (T-2) fresh cultured *Chlorell*a was used; in jars 7, 8, 9 (T-3) baker's yeast was used as feed. A lay-out of the experiment is shown in Table 1.

Table 1. Experiment layout for rotifer culture

Treatment	Feed for B plicatilis	Replications
T_1	Powdered dried *Chlorella*	3 (T-1a, T-1b, T-1c)
T_2	Fresh cultured *Chlorella*	3 (T-2a, T-2b, T-2c)
T_3	Baker's yeast	3 (T-3a, T-3b, T-3c)

Pond water was collected in plastic bottles and centrifuged (2000 rpm) for 5 minutes to collect dense sample of phytoplankton. This was examined under a compound microscope to check for micro-alga. One or two drops of algae sample were taken using micropipette as inoculums in sterilized medium (5 or 10 ml) in test tubes. After about five days prospective cultures were examined and dominant culturable micro-algae were isolated using micropipette to culture in sterilized medium in glass tubes. After repeated cultures, seeds of *Chlorella* sp were collected.

C. ellipsoidea was cultured in the inexpensive organic medium. Twenty percent seeds of C. *ellipsoidea* were used as inoculum in 200 ml culture medium in 500 ml conical flask. The culture experiment was done in natural light and temperature on a glass shelf at 2nd floor facing the north where there was sufficient sunlight. Cell density of C. *ellipsoidea* collected from different conical flasks was estimated daily using haemocytometer as described by Rahman (1992). During the period of *Chlorella* culture the environmental factors were recorded daily.

Cultured *Chlorella* was centrifuged (2000 rpm) for 5 minutes around 13th day of culture to collect the dense sample of *Chlorella*, which was preserved in the refrigerator. Supernatant was mixed with medium to use as *Chlorella* seed. The frozen *Chlorella* after thawing was dried in a microwave oven (SHARP, Japan), powdered with a mortar and pestle and kept in a glass tube with stopper for use as feed for rotifer. The seeds of rotifer were collected from different ponds of the Bangladesh Agricultural University campus through zooplankton nets. They were cultured in four plastic jars (5L) for two months, with continuous aeration by air pumps. Fresh cultured *Chlorella* was used as food. Samples were taken every day from each plastic jar for preservation (5% formalin) and daily analysed under a compound microscope using a special zooplankton counting cell until the concentration of rotifer was high without other contaminants.

In T-1 (Jars 1, 2, 3) powdered dried *Chlorella* was given daily at the rate of 0.1g/L of water. In T-2 (Jars 4, 5, 6) fresh cultured *Chlorella* was given at the rate of 1.2×10^6 cells/ml and in T-3 (Jars 7, 8, 9) baker's yeast was given daily at the rate of 0.2g/L. The *Chlorella* powder and the baker's yeast were suspended in small amounts of water and homogenized. Continuous aeration was provided by aerators (SIGMA 4000 SW, Japan) connected by a narrow plastic pipe. Continuous lighting was provided. Physico-chemical parameters were recorded daily. *B. plicatilis* density was determined daily using a special zooplankton counting cell under a compound microscope.

Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) of the experimental data were done by a computer.

Results and Discussion

The cell density of *Chlorella* and environmental factors are presented in Table 2. Maximum cell density of *Chlorella* was 4.5×10^6 cells/ml and on 13th day of culture the range was 1.3 to 4.5×10^6, cells/ml. The range of light intensity was 2850 to 4120 lux: the mean was 337.6 ± 388.0 lux. The range of water temperature was 21 to 29°C and the mean 25.2 ± 0.72. The range of air temperature was 20 to 32°C and the mean 26.4 ± 0.6°C. The mean sunshine period and rainfall were 8.7 ± 1.1 hours and 0.0 mm, respectively.

Table 2. Environmental factors and mean density of *Chlorella ellipsoidea* cultured in inexpensive organic medium for 18 days

Factors	Mean values
Cell density (×10^6 cells/ml)	2.9 ± 0.9 (1.3 - 4.5)
Light intensity (Lac)	337.6 ± 388.0 (2850 - 4120)
Water temperature (°C)	25.2 ± 0.7 (21 - 29)
Air temperature (°C)	26.4 ± 0.6 (20.0 - 32.0)
Sunshine period (hours)	8.7 ± 1.1 (5.2 - 9.5)
Rainfall (mm)	0.0 (0.0 - 0.0)

The cell density of *B. plicatilis*, air and water temperature (°C), pH and dissolved oxygen (mg/L) are presented in Table 3. The mean density of *B. plicatilis* cultured under T-1, 2 and 3 were 30.1 ± 12.0, 37.4 ± 14.6 and 21.1 ± 6.1 (×10^3 individual/L), respectively: the ranges were 14 to 52, 16 to 64, and 12 to 30 (×10^3 individual/L), respectively. The range of water temperature for rotifer culture was 20 to 26°C and the mean value was 22.5 ± 0.9°C. The ranges and mean air temperature during the culture period was 22 to 27.5°C and 24.7 ± 0.3, respectively. The mean pH of water in T-1, 2 and 3 were 7.0 ± 0.0, 7.0 ± 0.1 and 7.0 ± 0.1, respectively. The range of pH of water in T-1, 2 and 3 were 7.9 to 7.0, 6.9 to 7.1 and 6.9 to 7.0, respectively: the minimum and maximum pH was 6.9 and 7.1, respectively. The mean dissolved oxygen of the water in T-1, 2 and 3 were 6.2 ± 0.5, 6.1 ± 0.6 and 6.1 ± 0.6 mg/L, respectively: the range in T-1, 2 and 3 were 5.1 to 7, 5.2 to 7.1 and 5.2 to 7.0 mg/L, respectively.

Table 3. Air and water temperature, pH and dissolved oxygen in three treatments and mean (± s.d) density of *B. plicatilis* cultured for nine days

Factor	T-1 (Powdered dried *Chlorella*)	T-2 (Fresh cultured *Chlorella*)	T-3 (Baker's yeast)
B. plicatilis (×10^3 individual/L)	30.1 ± 12.0 (14-52)	37.4 ± 14.6 (16-64)	21.1 ± 6.1 (12-30)
Water temperature (°C)	22.5 ± 1.0 (20.0-26.0)	22.5 ± 0.9 (20.0 - 26.0)	22.5 ± 0.9 (20.0- 26.0)
Air temperature (°C)	24.7 ± 0.9 (22.0-27.5)	24. 7± 0.3 (22.0 - 27.5)	24.7 ± 0.3 (22.0 - 27.5)
pH	7.0 ± 0.0 (6.9 - 7.0)	7.0 ± 0.1 (6.9 - 7.1)	7.0 ± 0.1 (6.9 - 7.0)
Dissolved oxygen (mg/L)	6.2 ± 0.5 (5.1 - 7.0)	6.1 ± 0.6 (5.2 -7.1)	6.1 ± 0.6 (5.2 - 7.0)

The result of Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) of mean population densities of *B. plicatilis* cultured under three treatments are presented in Tables 4 and 5, respectively. The range of cell density of *Chlorella* was 1.3 × 10^3 to 4.5 × 10^3 cells/ml, which was similar to that of Rahman (2000). From the beginning to 13th day, the culture was in the logarithmic phase and then the death phase started. The maximum cell density was 4.489 × 10^3 cells/ml on 13th day of

culture. James *et al*. (1998) observed that the range of cell density of *Chlorella* was 20 × 10^6 to 80 × 10^6 cells/ml, higher than in the present study. The environmental factors during the culture period were similar to those reported by Rahman (2000); Hossain (1996); Fatema (1996) but the light intensity was slightly higher.

Table 4. ANOVA of mean population density of *B. plicatilis* cultured with 3 treatments fewer than 3 replications

Sources	DF	SS	MS	F-value	Probability
Between treatments	2	1204666666.667	602333333.333	4.569□	0.0208
Within treatments	24	3164000000.000	131833333.333		
Total	26	4368666666.667			

* Significant at 5% level

Table 5. DMRT of mean population density of *B. plicatilis* cultured with 3 treatments fewer than 3 replications

Original order		Ranked order	
Treatment	Mean value	Treatment	Mean value
1	30111.111[b]	2	37444.444[a]
2	37444.444[a]	1	30111.111[b]
3	21111.111[c]	3	21111.111[c]

The mean density of rotifer, *B. plicatilis* under 3 treatments reveals that F value (F = 4.569) is significant at 5% level, which indicates significant differences of population density of *B. plicatilis* among the three treatments.

The optimum water temperature for rotifer growth was 22 to 30°C as reported by McVey and Moore (1983). In the present study, the lowest and highest temperature was 20°C and 26°C, respectively. Hirayama and Nakamura (1976) cultured *B. plicatilis* with feeding of dry powder of *Chlorella* at an air temperature of 22 to 30°C, similar to the present study. Most pH values of the water under three treatments were about neutral (6.9 - 7.1). Lubzens (1987) reported that rotifers lived at pH levels of above 6.6; although in their natural environmental the best results were obtained at a pH above 7.5. The optimum range of pH for rotifer culture is 7.5 to 8.0 (McVey and Moore, 1983). Therefore, the pH of the water in three treatments was suitable. Continuous aeration is essential for rotifer culture. It was observed that if aeration stopped for 12 to 16 hours then mass mortality of rotifer happens (Rahman and Hossain, 1992). The range of dissolved oxygen of the water of rotifer culture in T-1, 2 and 3 were 5.1 to 7.0, 5.2 to 7.1 and 5.2 to 7.0, respectively, similar to those of Rahman and Hossain (1992).

Studies on the use of preserved algae for zooplankton growth are rare because of a general idea that non-living algae do not support their growth (Baer and Goulden,

1998). In order to maintain rotifer populations during periods of low algal production, it is necessary to offer alternative diets, some of which include dried algae. However the mean value of *B. plicatilis* density fed on powdered dried *Chlorella* under treatment 1 was 30.1 ± 12.0 ($\times 10^3$) individual/L. Hirayama and Nakamura (1976) found 400 individuals/ml during mass culture of *B. plicatilis* fed with powdered *Chlorella*. The culture was continued for 41 days. The population density if *B. plicatilis* found by them was much higher than in the present study. Lucia *et al.* (2001) found the population density for *B. calyciflorus* cultured with heat-killed *Chlorella* ranged from 6.0 ± 1.0 to 26 .0 ± 6.0 ($\times 10^3$) individual/L. These results are similar to those of the present experiment although the species of rotifer is different.

Live C*hlorella* is one of the most widely used foods for culturing planktonic rotifers (Pourriot and Rougier, 1997). Live algae support the best growth of rotifer (Hirayama and Nakamura, 1976). The mean value of *B. plicatilis* density fed on fresh cultured *Chlorella* under treatment 2 of the present experiment was 37.4 ± 14.6 ($\times 10^3$) individuals/L. This result is similar to that of Rezeq and James (1987).

In order to supplement the algal quantity, Hirata and Mori (1967) used baker's yeast as food for *B. plicatilis*. Since then a number of investigators used baker's yeast (Lie *et al*., 1997). The mean value of *B. plicatilis* fed on baker's yeast under T-3 of the present experiment was 21.1 ± 6.1 ($\times 10^3$) individual/L. Rahman e*t al.* (1993) found that the mean value of *B. calyciflorus* fed on baker's yeast was 24.2 ± 59.4 ($\times 10^3$) individual/L, which is m similar to that of the present study although the species of rotifer is different.

It is concluded that the mean density of *B. plicatilis* was slightly higher in T-2 fed on fresh cultured *Chlorella* than in T-1 fed on dried *Chlorella*. The mean density of *B. plicatilis* in T-3 fed on baker's yeast was much lower than in T-1 and 2. This indicates that fresh cultured or live *Chlorella* is best as food for *B. plicatilis* and dried powder *Chlorella* is better than baker's yeast.

One reason for lower production of *B. plicatilis* fed on powdered dried *Chlorella* than that fed on fresh cultured or live *Chlorella* may be the acclimatisation of rotifer with live *Chlorella* in the stock culture. Another reason may be that dried alga may have poorer nutritional quality (Brown, 1995). The fact that *B. plicatilis* grew well on powdered dried *Chlorella* suggests good adaptation to changed conditions. This could also be due to the discriminatory capacity of rotifer species to live food versus dead food particles (DeMott, 1986).

Although baker's yeast alone was not comparable to *Chlorella*, it can be used at low concentration in rotifer culture. And as the production of *B. plicatilis* fed on powdered dried *Chlorella* is comparable to those fed on live *Chlorella*, dried *Chlorella* may be used when there is no live *Chlorella*.

It may be concluded that when live or fresh cultured *Chlorella* are not available then powdered dried *Chlorella* may be successfully used as a feed for *B. plicatilis* culture.

References

Baer KN, Goulden CE 1998: Evaluation of a high-hardness COMBO medium and frozen algae for *Daphnia magna*. *Ecotoxicology and Environmental Safety* **39** 201-206.

Brown MR 1995: Effect of storage and processing on the ascorbic acid content of concentrates prepared from *Chaetocero calcitrans*. *Journal of Applied Phycology* **7** 495-500.

DeMott WR 1986: The role of taste in food selection by freshwater zooplankton. *Oecologia* **69** 334-340.

Dobberfuhl DR, Elser JJ 1999: Use of dried algae as a food source for zooplankton growth and nutrient release experiments. *Journal of Plankton Research* **21** 957-970.

Fatema S 1996: *Culture of Phytoplankton in Inexpensive Medium*. MS Thesis, Department of Fisheries Biology and Genetics, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, pp. 67.

Hale JG, Carlson AR 1972: Culture of the yellow perch in the laboratory. *Progressive Fish-Culture* **34** 195-198.

Hirata H, Mori Y 1967: Cultivation of the rotifer *Brachionus plicatilis* fed on a mixed diet of marine *Chlorella* and baker's yeast. *Saibai Gyigo* **5** 36-40.

Hirata H, Anastasios M, Yamasaki S 1985: Evaluation of the use of *Brachionus plicatilis* and Artemia for rearing prawn *Penaeus japonicus* larvae on a laboratory scale. *Memoirs of the Faculty of Fisheries Kagoshima University,* Japan **34** 27-36.

Hirayama K, Nakamura K 1976: Fundamental studies on the physiology of rotifers in mass culture. V. Dry *Chlorella* powder as a food for rotifers. *Aquaculture* **8** 301-307.

Hirayama K, Maruyama I, Maeda T 1989: Nutritional effect of freshwater *Chlorella* on growth of the rotifer *Brachionus plicatilis*. *Hydrobiologia* **186** 39-42.

Hossain MA 1996: *Culture of Microalgae (Scenedesmus quadricanda and Chlorella ellipsoidea) in Inexpensive Medium.* MS Thesis, Department of Fisheries Biology and Genetics, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, pp. 81.

James CM, Al-Khars AM, Chorbani P 1998: pH dependent Growth of *Chlorella* in a Continuous Culture System. *Journal of the Worlds Aquaculture Society* **19** 27-35.

Kitajima C, Fujita S, Ohwa F, Yone Y, Watanabe T 1979: Improvement of dietary value for red sea bream larvae of rotifer *Brachionus plicatilis* cultured with baker's yeast *Saccharomyces cervisiae*. *Bulletin of the Japanese Society of Fisheries Science,* Japan **45** 469-471.

Lie O, Haaland H, Hemre GI, Maage A, Lied E, Rosenlund G, Sandness K, Olsen Y 1997: Nutritional composition of rotifers following a change in diet from yeast and emulsified oil to microalgae. *Aquaculture* **5** 427-438.

Lubzens E 1987: Raising rotifers for use in aquaculture. *Hydrobiologia* **104** 77-84.

Lubzens E, Gibson O, Zmora O, Sukenik A 1995: Potential advantages of frozen algae (*Nannochloropsis* sp) for rotifer (*Brachionus plicatilis*) culture. *Aquaculture* **133** 295-309.

Lucia PE, Sarma SSS, Nandini S 2001: Effect of different densities of live and dead *Chlorella vulgaris* on the population growth of rotifers *Brachionus calycif*lorus patulus (Rotifera). *Revista de Zoología Tropical* **49** 77-83.

Martinez JF, Chavez FE 1994: A laboratory-scale system for mass culture of freshwater microalgae in polyethelene bags. *Journal of Applied Phycology* **6** 423-425.

McVey JP, Moore JR 1983: CRC Hand book of mariculture, Vol. 1 Crustaceans.CRC Press, *Inc Boca Raton, Florida*. pp. 3-101.

Pourriot R, Rougier C 1997: Reproduction rates in relation to feed concentration and temperature in three species of the genus *Brachionus* (Rotifera). *International Journal of Limnology* **33** 23-31.

Rahman MS 1992: *Water Quality Management in Aquaculture*. Published by BRAC Prokashana, 66 Mohakhali, Dhaka-1212, Bangladesh, pp. 71-72.

Rahman MS 2000: *Mass culture of phytoplankton in inexpensive medium*. Final Project Report, BAURES. Bangladesh Agricultural University, Mymensingh, pp. 15-25.

Rahman MS, Hossain MA 1992: Culture of rotifer, *Asplanchna priodonta* for feeding fish fry. *Baures Research Progress* **6** 409-416.

Rahman MS, Hossain MK, Habib MAB 1993: Culture of rotifer for feeding fish fry. *Baures Research Progress* **7** 676-682.

Rezeq TA, James CM 1987: Production and nutritional quality of the rotifer *B. plicatilis* fed marine *Chlorella* sp at different cell densities. *Hydrobiologia* **147** 257-261.

Suminto K, Hirayama K 1997: Application of growth-promoting bacteria for stable mass culture of three marine microalgae. *Hydrobiologia* **358** 223-230.

Watanable T, Kitajima C, Fujita S 1983: Nutritional values of live organisms used in Japan for mass propagation of fish. *Aquaculture* **34** 115-143.