J. Asiat. Soc. Bangladesh, Sci. 39(2): 129-138, December 2013

ASSESSMENT OF FOUR DIFFERENT MEDIA FOR THE MASS CULTURE OF *CERIODAPHNIA RETICULATA* (JURINE) AS A LIVE FISH FEED

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Abstract

Experiments on the mass culture of *Ceriodaphnia reticulata* (Jurine) were carried out in aquarium water for 54 days with different media like cowdung (1.5g/L), pulse bran water (50g/L), poultry manure (0.45g/L) and snail faeces (faeces of six apple snails). All the media were fertilized by 50-100% of the initial amount of feed in every 7 days. About 100 individuals of *C. reticulata* were inoculated as starter in 50 litres of water (2 individuals/ml).The temperature of the media ranged from 24-30^{o}C during study period. p^{H} of the culture media varied i.e., 9.1 ± 0.40 in cowdung; 8.72 ± 0.73 in pulse bran water, 8.82 ± 0.72 in poultry manure and 7.5 ± 0.55 in snail faeces. The highest average population of *C. reticulata* was observed in cowdung (8.56 ± 4.11individuals/ml), moderate in poultry manure (4.21 ± 2.97 individuals/ml) and snail faeces (2.52 ± 3.01individuals/ml). The lowest growth of *C. reticulata* was recorded in pulse water (0.37 ± 0.69 individuals/ml). The culture media with cowdung as well as poultry manure and snail faeces were found to be useful for artificial mass production of *C. reticulata*.

Key words: Assessment, Food media, Mass culture, Live fish feed, *Ceriodaphnia reticulata*

Introduction

Zooplanktons are important food item for the young and some adults of many freshwater fishes which represent a major component of the human diet (Kenneth 1990). Among freshwater zooplankton, rotifers, cladocerans and copepods are dominant groups throughout the year (Hutchinson 1967). Successful hatchery production of the fish fry and crustaceans for aquaculture depends on the availability of zooplankton of appropriate size of larval feeding. Freshly hatched *Artemia nauplii* has been popular larval feed used by the scientists and aquaculturists for a long time. But the high cost of *Artemia* cysts has led to the aquaculturists to search for alternative suitable zooplankton which could be easily reared in large scale. The rotifer *Brachionus plicatilis*, the cladoceran, *Moina* sp, the harpacticoid copepods such as *Tigriopus* spp and *Tispe* spp., nematodes, *Panagrellus* spp. and the ciliate *Fabrea salina* all of which have high reproductive rate, short generation time and the ability to live and grow in crowded culture conditions that are found to be useful as live feed for larval rearing of cultivable species of fish and crustaceans (Muthu 1982).

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Zooplankton play an important food item of omnivorous and carnivorous fishes (Alam *et al.*1987). Cladocerans often known as 'water fleas' because of their shape and "hop-sink" type of locomotion are the major group of zooplankton available in freshwater ponds. Larger fry and even adults of some fish species often selectively prey on the crustaceans (Ludwig 1999). *Ceriodaphnia* is a small cladoceran genus that has higher protein content than *Daphnia* and is an excellent feed for fish fry with minute mouths. The males range from 0.4-0.8mm in length, whereas the size of females is 0.4mm to 1.4 mm in length that varied depending upon various species (Balcer *et al*. 1984).

In the study, *Ceriodaphnia reticulata*, an important cladoceran as fish food had been selected for mass culture in aquaria with different types of media. Development of a suitable culture media for commercial production of *Ceriodaphnia* sp. will be an inexpensive alternative approach to live feeds needed for fish rearing.

Materials and Methods

The experiments were conducted over a period of 54 days in the Zoology Section of BCSIR Laboratories, Dhaka. *Ceriodaphnia reticulata* was collected from local water bodies of Dhaka city and this species was identified according to Brooks 1959. The culture media maintained in 12 aquarium tanks of 75 cm x 36 cm x 36 cm size with aeration for 24 hrs. Each tank was washed, left to dry and then filled with 30 litres of tap water. The tap water was kept for two days for seasoning. On the 3rd day, the tanks were fertilized by four different types of food media with three replicates for each treatment. The media were cowdung (44.88g dried manure + 30 litres of water) (Rottman 1992), pulse bran water with *Chlorella* (1.5g Urea+0.3g TSP + 3g salt + 600 ml pulse bran water + 100 ml *Chlorella* + 30 litres of water), poultry manure (13.45g dried manure + 30 litres of water) and snail faeces (six apple snails feed on 2-3 cabbage leaf daily). Additional feed, approximately 50-100% of the initial amount were added to 5 days later. On the third day of the experiment, about 100 individuals of *C. reticulata* were introduced to the culture medium of each tank as starter. Following initiation of different growth experiments, the number of living individuals of each tank was counted daily. These processes continued until population study in each replication that started to decline. The population of *C. reticulata* was recorded by using the Sedgewick-Rafter counter cell which is 50 mm long, 20 mm wide and 1 mm deep. Zooplankton number (no./ml) was calculated according to the formula outlined by Boyd and Lichktoppler (1979):

$$\text{Number of zooplankton/ml} = \frac{T \times 1000}{A \times N \times \text{Vol. of concentrate in ml/Vol. of sample}}$$

Where, T= total number of zooplankton counted
A=area of grid in mm^2
D=Number of grids counted
1000 = area of counting chambers in mm^2

Water temperature (^oC) of the culture media were recorded by using a mercury thermometer and pH was detected with the help of a portable pH meter (model-HI 98103) before sampling started at 10.00 a.m. once in every 3 days. The statistical analysis of different physico-chemical parameters were carried out by using one-way ANOVA and any difference at 5% level of significance by using the statistical package of SPSS-16(SYSTA, USA) to express the results.

Results and Discussion

Media, pH level and water temperature, affect the growth and reproduction of *Ceriodaphnia*. The growth of *Ceriodaphnia reticulata* varied from medium to medium used in this experiment (Fig. 1). Among the media, the highest average number of individuals were recorded in cowdung medium of 8.56 ± 4.11 no./ml whereas *C. reticulata* reared on pulse bran water medium exhibited the lowest density of 0.37 ± 0.69 no./ml. The other cultured media like poultry manure and snail faeces showed moderate population growth of 4.21 ± 2.97 and 2.52 ± 3.01 no./ml respectively on an average (Table 1).

Table 1. Average pH, temperature, survival and number of individuals of four different culture media.

Feeds	pH(Mean ± SD)	Temperature (^oC)	Days of survival	Number Individuals (Mean ± SD)
Cowdung	*9.1 ± 0.40^a	26.28 ± 1.88^c	29.08 ± 15.29^a	8.56 ± 4.11^a
Poultry manure	8.82 ± 0.72^b	27.52 ± 1.42^b	25.21 ± 16.94^b	4.21 ± 2.97^b
Pulse bran water	8.72 ± 0.73^b	26.42 ± 1.67^{bc}	26.22 ± 15.46^{ab}	0.37 ± 0.69^d
Snail faeces	7.5 ± 0.55^c	30.41 ± 0.88^a	27.43 ± 16.94^{ab}	2.52 ± 3.01^c

*means containing the same letters do not differ significantly at 5% level of significance.

Effects of days of survival: The parameter on the days of survival was studied to make a comparison of the effects of cultured food media in cultivation of *C. reticulata*. At the end of 54 days of experiment, average days of survival were highest in cowdung media (29.08 ± 15.29) and lowest in poultry manure media (25.21 ± 16.94).

There was a significant ($p < 0.01$) relationship between the number of individuals and days of survival ($r = 0.441$) and that the number of organisms increased with time in cowdung medium. The effects of days of survival on the growth of individual cultured in four types of media were not the same (Fig. 2). In cowdung, the population of *C. reticulata* showed more or less average growth rate over the period with two peaks abundances on the 15th and the 40th day. There was no figure for complete decline of this organism in cowdung medium. In poultry manure, the population started to grow from first day to fourth day and became steady till 10th day. It grew upto 6 individual/ml in

similar fashion till 23rd day and then suddenly increased to 10 individual/ml by 29th day which continued till 36th day and fell to 2 indi./ml by 40th day. In pulse bran water, the population of *C. reticulata* started to grow from 8th day with 1 indi./ml and became stable till 14th day and then sudden increase to 2 individual/ml by 15th to 20th day. After that population declined abruptly to 0 indi./ml. The number of individuals in snail faeces started to grow from 8th day and gradually increase till 32th day upto 3 indi./ml and then sudden increase to 14 indi./ml by 35th day and then fell to 4 indi./ml by 39th day. After that growth of *C. reticulata* continued with slight fluctuation from 1-3 indi./ml (Fig. 2).

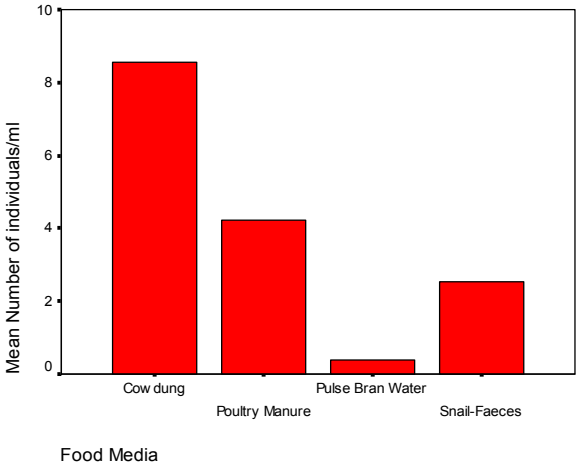

Fig.1. Average number of individuals produced in four types of feed media.

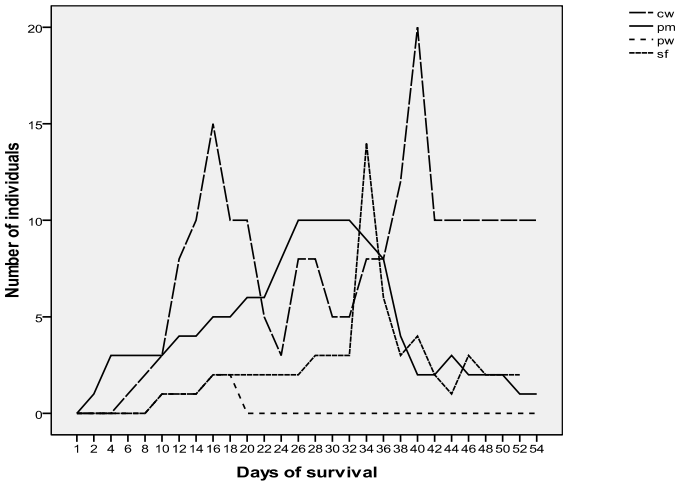

Fig. 2. Effects of days of survival on number of individuals in four types of feed media.

*cw = cowdung, pm = poultry manure, pw = pulse water, sf = snail feaces.

Effects of pH: pH of the culture media showed profound effects on the growth of individuals which ranged from 9.1 ± 0.40 (snail faeces) to 7.5 ± 0.55 (cowdung) on an average (Table 1). It is evident that pH of the poultry manure indicated a significant positive relationship (0.757) with the number of individuals up to a certain range (7.4-10). Result showed that there were no significant differences ($p > 0.05$) between the number of individuals and pH of the three culture media (Table 2). Fig. 3 showed the overall effects of pH comparatively in four cultured media under experiment. In cowdung, number of individuals increased with increase of pH up to a point between 8.4-9.1 and then population decreased sharply with short growth phase. In poultry manure, there was some extension period of population growth with few lag phases but showed optimum abundances within pH 9.2-9.8. The population of culture organism showed irregular pattern of growth in pulse bran water medium with few remarkable fluctuations at pH 8.2 to 9.4. Number of individuals showed a downward trend with the increase of pH values despite of two peak curves with certain pH level such as at pH 7.2 and 7.7 in snail faeces.

Table 2. Correlation of number of individuals with pH, temperature and days of survival in different culture media.

Food levels	p^H	Temperature	Days of survival
Cowdung	-0.248	0.408*	0.441*
Poultry manure	0.757**	0.286	0.020
Pulsebran water	0.024	-0.042	-0.378
Snail feaces	-0.356	-0.388	0.383

** significant at the 0.01 level, * significant at the 0.05 level

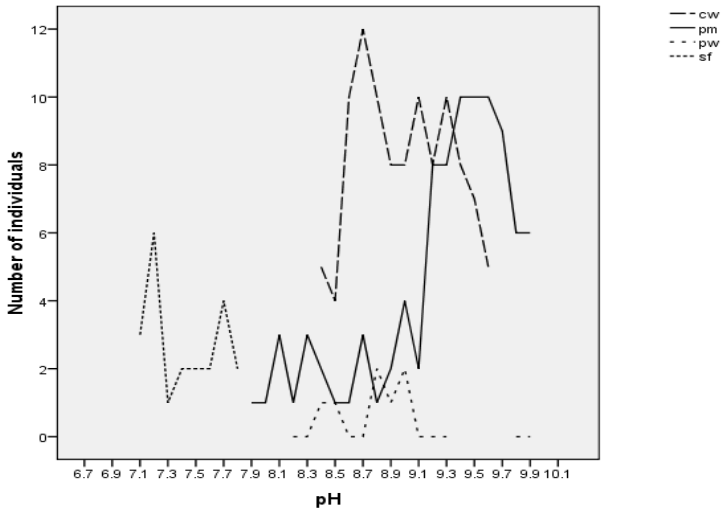

Fig. 3. Effect of pH on number of individuals in four types of feed media.
*cw = cowdung, pm = poultry manure, pw = pulse water, sf = snail feaces.

Effects of Temperature: Experimental results showed that the highest mean temperature was in snail faeces (30.41 ± 0.88^{o}C) whereas lowest in cowdung medium (26.28 ± 1.88^{o}C). The correlation between mean temperature (0.408) and population density of cowdung medium shows positive relationship (Table 2).

Fig. 4 showed the comparative effects of temperature on number of individuals cultured in four types of feed media with a great variation. In cowdung, number of individuals increased with the increase of temperature following few fluctuations. The figure reached at peak sharply at 23^{o}C, 25^{o}C and 26^{o}C temperature and dramatic declined at 23-24^{o}Cand 25.5^{o}C. After a short lag phase, growth of individuals continued its upward trend till 29^{o}C. In poultry manure, the number of individuals increased steadily from 25 to 28^{o}C and then declined abruptly at 28.5^{o}C. Then population increased sharply with little fluctuation within 29 to 30 ^{o}C. The number of individuals in pulse bran water medium did not increase beyond the inoculation density (2 ind./ml) and showed temperature range between 26^{o}C to 29.5^{o}C with a small peak at 27 ^{o}C. The temperature recorded in snail faeces culture medium was confined between 29 to 31^{o}C with a single peak at 30 ^{o}C (Fig. 4).

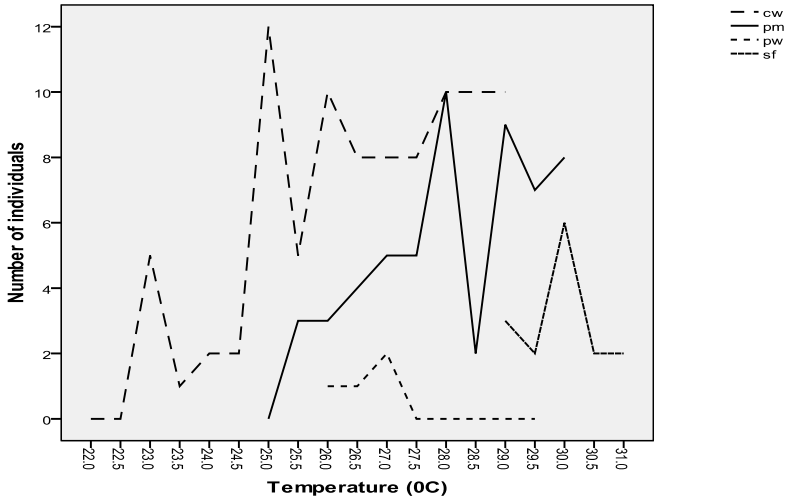

Fig.4. Effect of temperature on number of individuals in four types of feed media.
*cw = cowdung, pm = poultry manure, pw = pulse water, sf = snail feaces

Table 3 depicts the simple regression analysis with pH, water temperature and days of survival on the number of *C. reticulata* in different culture media. In cow dung media, the contribution of pH is inversely proportional to the production of number of individuals whereas temperature and days of survival are more contributing parameters for the production of *C. reticulata* than that of pH parameter. In poultry manure, pH and temperature are more important for the number of individuals than days of survival which is inversely proportional to the production of days of survival. Similar type of result was found in pulse bran water medium. In snail faeces, pH and temperature are more

inversely proportional to the production of number of individuals and days of survival are less prominent parameter for detecting the number of *C. reticulata.*

Table 3. Regression of pH, temperature and days of survival on number of individuals.

Food levels	Regression line	R^2	Adjusted R^2
Cow dung	No. of individuals = 23.531 - 3.948 pH + 0.602 Temperature + 6.72 Days of survival	0.309	0.210
Poultry manure	No. of individuals = - 46.21 + 3.49 pH + 0.810 Temperature - 0.106 Days of survival	0.736	0.704
Pulse bran water	No. of individuals = - 3.39 + 0.294 pH + 7.04 Temperature - 2.69 Days of survival	0.25	0.152
Snail faeces	No. of individuals = 31.908 - 1.05 pH -0.74 Temperature + 3.11 Days of survival	0.219	0.081

Comparison of the number of the culture organisms in four different media revealed that growth rate of *C. reticualta* was considerably good in cowdung medium, average in poultry manure and then snail faeces and pulse bran water respectively (Fig. 1). The highest growth rate (20 individuals/ml) was achieved in the cowdung medium might be the cause for rich nutrient component available than those of others. Muthupriya and Altaf (2009) have observed 3593 ± 258 to 9333 ± 203 individuals/litre of *C. cornuta* population in chicken manure medium which was more or less similar to the findings in present study for poultry manure (1-10 individuals/ml). In this medium, the peak population density was observed on the 24^{th} to the 34^{th} day which is in contradiction to the findings of Altaf and Mehraj-ud-Din (2010) who detected peak on the 17^{th} day. Malhotra and Langer (1993) studied on the four cladoceran species of importance as fish food organisms, viz. *Daphnia similes* (Claus), *Simocephalus vetulus* (Schodler). *Moina macrocopa* (Straus) and *Ceriodaphnia cornuta* (Sars) which were maintained in the laboratory on nutrient sources including manure, rice bran and *Chlorella.* The organisms responded better with rice bran and *Chlorella.* The findings of the present study indicate that *C. reticulata* cultured in pulse bran water medium with *Chlorella* inoculums had exhibited the lowest growth rate with an average (0.37 ± 0.69 indi./ml) that differed from the previous findings of Malhotra and Langer (1993).

Studies revealed that nutrient and temperature have significant effects on the life cycles of the planktonic species (Ebert *et al.* 1993, Gillooly 2000 and Savage *et al.* 2004) which in turn affected the population growth of zooplanktons. This was evident in *C. reticualta* cultured in cowdung medium up to a certain range (25-29^{o}C) of temperature. Normally under optimal range of culture conditions, the population growth rates of cladocerans are directly related to the food density and different temperature gradients (Nandini and Sarma 2003, Sarma *et al*. 2005, Xi *et al*. 2005). *C. reticulata* had optimal growth rates at 25^{o}C in cowdung medium, 28^{o}C in poultry manure, 27^{o}C in pulse bran water media and 30^{o}C in snail faeces medium.

Temperature is one of the major determinants of the feeding rate of *Ceriodaphnia*. Gopen (1976) showed that *Ceriodaphnia* feed at higher rates when the water temperature increased and it occurred upto a certain level. According to Gopen (1976), the optimum temperature for *C. reticulata* ranged from 20-22^{o}C. He also opined that reproduction and growth rates of *C. reticulata* decreased at temperature above 22^{o}C due to the increase in energy requirements released from increases in the respiration rates and slightly different results obtained from previous findings which support the results of Tauson (1930). He studied *Daphnia pulex* and observed the temperatures between 15 and 25^{o}C were favourable for egg production, but above and below these temperatures; there was a considerable decline in the number of egg production. Similar effects at higher temperature have also been recorded in *Ceriodaphnia* sp. (Green 1978) and *Moina macrocopa* (Malhothra and Langer 1990). Hall (1964) stated that temperature tolerance may be utilized to predict the frequency of molting, reproduction, and duration of the egg development. Bellosillo (1937) reported that temperature ranging from 26–31°C to be favourable for laboratory and outdoor cultures of zooplankter, *Moina macrocopa*.

It is apparent that temperature alone may not account for variations in plankton densities as other parameters such as high pH, alkalinity, carbon dioxide but nutrients are also responsible for the organic mass production (Pulle and Khan 2003). Hydrogen ion concentrations have great impact on the survival, growth and reproduction rates of cladocera (Walton *et al*. 1982 and Moustafa 2007). The results of the present study exhibited that pH recorded in the poultry manure medium have a significant effects on the growth of *C. reticulata* (Table 2).

C. reticualta as a live food is appeared to be the best food we can possibly feed to different adult fish, fish fry and fingerlings because they are natural and healthy. Live food gives healthier fry, more successful spawns, and better colouration than any prepared food available in the market. Hence, adequate research is needed before stepping into a large scale production of *C. reticulata* using different doses of applied food media as fertilizer and to obtain optimum growth rate and continuous production of live fish feed.

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(Received revised manuscript on 22 October 2013)