ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2015; 3(1): 400-405

www.fisheriesjournal.com
Received: 21-07-2015
Accepted: 20-08-2015

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The effect of three different feed types on growth performance of *Ceriodaphnia reticulata* and *Bosmina* sp. culture as a live fish feed

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Abstract
Experiments were conducted to study the effect of three different food media on the mass culture of *Ceriodaphnia reticulata* and *Bosmina* sp. in aquarium water for 42 days. The culture media were poultry manure, pulse bran water with *Chlorella* and snail faeces. About 100 individuals of both *C. reticulata and Bosmina* sp. were introduced to the culture medium of each tank as starter. The range of temperature, pH and ammonia varied from media to media. The average temperature of poultry manure, pulse bran water and snail faeces media were 27.37 ± 1.18, 27.57 ± 0.78 and 27.51 ± 1.19 for *C. reticulata* and 27.88 ± 1.23, 27.98 ± 0.98 and 28.11 ± 1.21 for *Bosmina* sp. respectively. During the study period, mean pH of the culture media varied i.e., 8.84 ± 0.82 and 9.27 ± 0.43 in poultry manure, 8.92 ± 0.74 and 9.46 ± 0.027 in pulse bran water and 8.68 ± 0.80 and 8.69 ± 0.36 in snail faeces for *C. reticulata* and *Bosmina* sp. The ammonia content ranged from > 2.5 to 0.5 mg/l in the culture media. The highest average population of *C. reticulata* was observed in poultry manure (5.33 ± 3.01 individuals/ml), moderate in snail faeces (2.71 ± 2.72 individuals/ml) and lowest in pulse bran water (0.38 ± 0.74 individuals/ml). But in case of *Bosmina* sp. the highest average growth was recorded in snail faeces (3.59 ± 3.20 individuals/ml), medium in poultry manure (2.43 ± 3.23 individuals/ml) and lowest in pulse bran water (0.14 ± 0.48 individuals/ml). Therefore, the culture media with poultry manure and snail faeces were found to be useful for artificial mass production of *C. reticulata* and *Bosmina* sp. rather than pulse bran water.

Keywords: Feed types, Growth Performance, Live Fish Feed, *Ceriodaphnia reticulata*, *Bosmina* sp.

1. Introduction
Zooplankton are the initial prey item for almost all fish larvae, as they switch from their yolksacs to external feeding[1]. The survival rate of fish larvae (fry) is greatly enhanced if these 'baby fish' are maintained on a diet of zooplankton instead of artificially formulated feeds [2].
In fish farm operations, lack of suitable food is believed to be the main source of mortality of early larval and fry stages of fish. Such mortalities can be greatly reduced, if not completely prevented by provision of adequate amounts of natural food (plankton). The success of hatchery operations have been linked to the availability and supply of these natural feed, notably of zooplankton [3].
The larvae of carps feed mostly on zooplankton, because zooplankton provide the necessary amount of protein requires for the rapid growth and development of different organs specially the ground of fishes [4]. Brood fishes productivity depends on zooplankton as an ideal food source of them. Zooplankton are a valuable source of protein, amino acids, lipids, essential minerals and enzymes needed by fish larvae, for effective growth and survival[5,6]. Intensive fish culture in Bangladesh is hampered by crucial curtail of essential feed ingredients such as grains, root crops and tubers etc. They are all staple foods and are reserved for human consumption. Conventional feedstuffs such as fish meal and rice bran are in acute supply and aggressively sought after by the established livestock and poultry industries. Prospects for early development of artificial diets for intensive fish culture do not appear bright. Therefore, this study is aimed to develop the method of mass culture of live fish feed particularly for two cladoceran species, *Ceriodaphnia* sp. and *Bosmina* sp.
In the present study, *C. reticulata* and *Bosmina* sp. as an important cladoceran as fish food has been selected for intensive culture in aquaria with different types of food media. It is very important to develop a suitable culture media for commercial production of these two species

and make a comparison between them which will be an inexpensive alternative to other commercial feeds needed for fish rearing. Therefore, the major objectives of the study were - to make a comparative study of different culture methods; to find out the suitable food medium for the maximum growth of both *Ceriodaphnia* and *Bosmina* sp.; to develop an easy, simple and low cost method for supplying live fish feed; to find out the proper utilization of organic fertilizer for live fish feed culture.

2. Materials and methods

The experiments were conducted over a period of 42 days in the Zoology Section of BCSIR Laboratories, Dhaka. *C. reticulata* and *Bosmina* sp. were collected from local water bodies of Dhaka city and this species was identified according to Brooks [7]. 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 three different types of food media with three replicates for each treatment. The media were 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* and *Bosmina* sp. were introduced to the culture medium of each tank as starter. Water levels of aquaria were maintained by addition of water regularly. Following initiation of different growth experiments, the number of living individuals of each tank was counted at one day interval. These processes continued until population study in each replication had started to decline. The populations of *C. reticulata* and *Bosmina* sp. were 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 by Boyd and Lichktoppler [8]:

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

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

Physio-chemical parameters such as water temperature, pH (Oyster portable pH meter, pp-201), Ammonia (determined by a calorimetric method, APHA, 1985) of the culture media were recorded once in every 3 days.

2.1 Data analysis

Descriptive statistics of parameter of mass culture of *C. reticulata* and *Bosmina* sp. were computed at first. Then Pearson's Correlations were calculated to see the relation between the parameters. The regression analysis for different food media and culture were used to show the dependency of these parameters on mass culture of the live fish feed. In order to test the equality of number of individuals per milliliter in two different fish feeds (*C. reticulata* and *Bosmina* sp) and three cultivation media (poultry manure, pulse bran water and snail faeces) one way analysis of variance (ANOVA) test was used. Since parameters varies significantly ($p<0.05$) in different cultivation media, Duncan Multiple Rage Test (DMRT) of Post Hoc was performed. The statistical package namely SPSS (version 17.0) was used for data analysis.

3. Results

This study to compare the growth of Zooplankton-*Ceriodaphnia* and *Bosmina* cultured in three types of food media (Poultry manure/PM, Pulse bran water /PW and Snail faeces/ SF) showed great variation in every parameter. Water quality characteristics (Temperature, p^H, Ammonia) in the culture media and the number of individuals produced during 42 days experiment are summarized in Table 1. Among the media, the highest average number of individuals of *Ceriodaphnia* (5.25 ± 3.01) was recorded in poultry manure whereas *Bosmina* reared on snail faeces showed the highest density (3.68 ± 3.20) of new progeny. On the other hand, the lowest number of individuals was estimated in pulse bran water in both types of culture organisms- *Ceriodaphnia* (0.38±0.74) and *Bosmina* (0.06 ± 0.48) (Figure - 1). The highest mean temperature was recorded in SF medium (28.11 ± 1.21) for *Bosmina* culture and more or less same range in all other culture media (Table 1). The mean pH observed was more than 8.68 in all treatments. The average Ammonia estimated was between 0.50-2.42 in all the treatments. However, there was no poor water quality conditions observed throughout the study.

In poultry manure, there was a significant positive relationship among number of individuals with culture period($r=0.493$) and pH ($r=0.783$) of *Ceriodaphnia* culture. On the other hand, there was a significant negative relationship between number of individuals and Ammonia($r=0.730$) of *Bosmina* culture but has significant positive relationship with recorded pH ($r=0.651$).The least number of individuals (0-1/ml) recorded in PW showed a significant positive relationship ($r=0.458$) with Ammonia in *Bosmina* culture. In SF medium, growth of *Bosmina* exhibited a significant negative relationship ($r= -0.475$) with pH (Table 2).

Table 3 depicts the simple regression analysis with pH, temperature, ammonia and days of culture period on the number of *Ceriodaphnia* and *Bosmina* in three types of food media. For *Bosmina*, the contribution of culture period, pH, Ammonia is inversely proportional to the production of number of individuals whereas temperature is more contributing parameter. For *Ceriodaphnia* temperature and pH are more important for the number of progeny which is inversely proportional to the days and ammonia of culture media.

It evident from table 4 that, growth performance of organisms in culture media (PM, SF and PW) are significantly different ($p<0.05$) but growth performance of culture organisms (*Bosmina* and *Cariodaphia*) are not significantly different ($p>0.05$).

Table 5 shows that the culture media with poultry manure and snail faeces are found to be useful for artificial mass production of *C. reticulata* and *Bosmina* sp. rather than pulse bran water.

Figure 2 - 4 showing the comparative growth performance of *Ceriodaphnia* and *Bosmina* in three types of food media. In PM medium, the population density of *C. reticulata* gradually increased and reached the optimum level (10ind/ml) on 20th day and then decrease steadily to a lower level (0ind/ml) after 26th day. On the other hand, *Bosmina* showed more or less

average growth rate over the period with peak abundances (10ind/ml) from 28 to 32th days. There was no figure for complete declined of this organism (Fig.-2). In PW medium, the growth of *Ceriodaphnia* was not satisfactory as no (0ind/ml) organism was recorded after 6th day of culture. *Bosmina* sp. showed more or less similar pattern with remarkable fluctuations (up to 2ind/ml) from 10th to 18th days and decreased abruptly to decline (Fig.3). The number of individual of *Ceriodaphnia* in SF medium showed gradual increase up to 18th day and reached the highest level (12ind/ml) and then decreased gradually with little fluctuation (Fig. 4). The abundances of culture organisms in poultry manure was steadily increased and reached the optimum level (10ind/ml) on 18th to 20th days. After that the population density decreased gradually up to base level (Fig. 4).

Table 1: Average p^{H}, temperature, ammonia and number of individuals of three different culture media

Food media / Parameter	Poultry manure (PM)		Pulse bran water (PW)		Snail faeces (SF)	
	***Bosmina* sp.**	***Cerio-daphnia***	***Bosmina* sp.**	***Cerio-daphnia***	***Bosmina* sp.**	***Cerio-daphnia***
Temperature (Mean ± SD)	27.88 ±1.23	27.37±1.18	27.98±0.98	27.57±0.78	28.11±1.21	27.51±1.19
p^{H} (Mean ± SD)	9.27 ± 0.43	8.84± 0.82	9.46±.027	8.92±0.747	8.69±0.36	8.68±0.80
Ammonia (Mean ± SD)	2.0 ± 0.45	1.92±0.59	1.50±0.63	2.42±0.20	0.50±0.00	0.50±0.00
Number Individuals (Mean ± SD)	2.34 ± 3.23	5.25±3.01	0.06±0.48	0.38±0.74	3.68 ±3.20	2.63±2.72

Table 2: Correlation of number of individuals with p^{H}, temperature and ammonia in different culture media

Food media / Parameter	Poultry manure		Pulse bran water		Snail faeces	
	***Bosmina* sp.**	***Cerio-daphnia***	***Bosmina* sp.**	***Cerio-daphnia***	***Bosmina* sp.**	***Cerio-daphnia***
Day	-0.257	0.493*	-0.366	-0.262	-0.227	-0.149
Temperature	0.005	0.311	-0.276	-0.155	-0.271	0.260
p^{H}	0.651**	0.783**	-0.098	-0.100	-0.475*	-0.328
Ammonia	-0.730**	-0.198	0.458*	0.383	-	-

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Table 3: Regression of number of individuals with p^{H}, temperature and ammonia in three culture media

Culture	Regression model	R^{2}	Adjusted R^{2}	Sig.
Bosmina sp.	Y=-3.997-.097D+0.658T-0.956P-1.164A	0.493	0.191	0.002
C. reticulata	Y=-13.065-0.011D+0.248T+1.063P-0.121A	0.221	-0.170	0.565
Media				
Poultry Manure	Y=-1.221-0.049D-0.10T+1.887P-2.539A	0.531	0.204	0.014
Pulse water	Y=0.057-.006D+0.028T-0.114P+0.282A	0.438	0.105	0.088
Snails	Y=11.131-0.019D+890T-3.723P	0.542	0.238	0.004

Y: Individual/ml, D: Days, T: Temperature (^{0}c), P: pH, A: Ammonia (mg/l)

Table 4: One way Analysis of Variance (ANOVA) of culture organisms and culture media.

	Variations	Sum of Squares	df	Mean Square	F	Sig.
Culture Media	Between Groups	313.159	2	156.579	21.772	0.000
	Within Groups	884.600	123	7.192		
	Total	1197.759	125			
Culture Organisms (*Bosmina* and *Ceriodephina*)	Between Groups	15.365	1	15.365	1.611	0.207
	Within Groups	1182.394	124	9.535		
	Total	1197.759	125			

Table 5: Duncan Multiple Range Test (DMRT)

Media	Sample size (n)	Subset for alpha=0.05	
		Subgroup-1	Subgroup-2
Pulse water	42	0.176	
Snails	42		3.152
Poultry manure	42		3.795
Sig.		1.000	.274

Means for groups in homogeneous subsets are displayed.

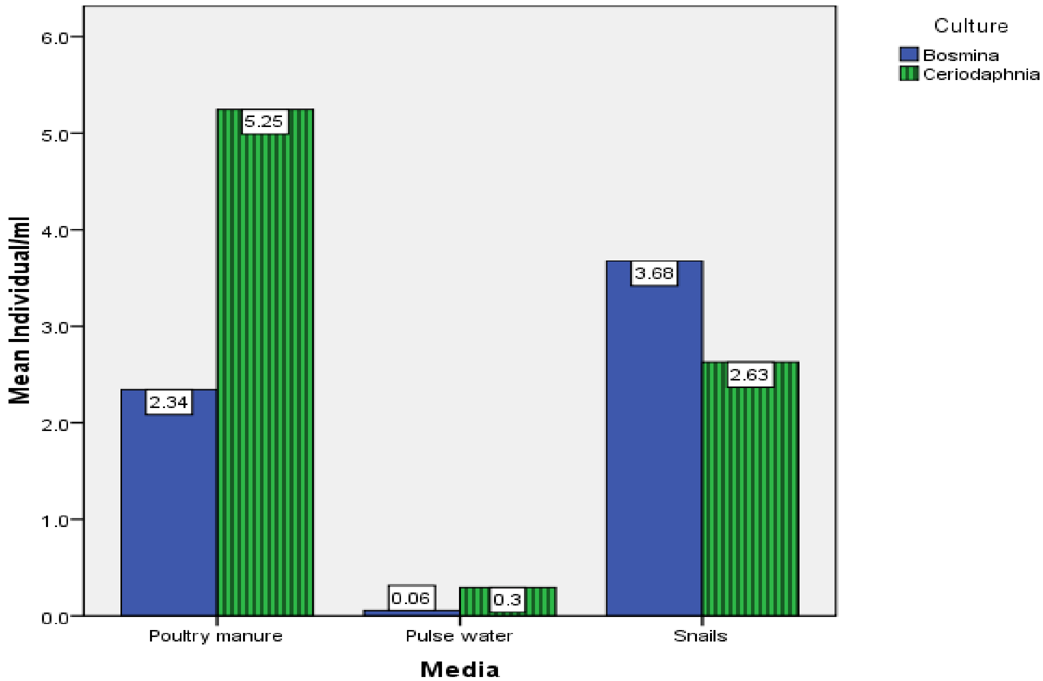

Fig 1: **Mean number of individuals of *Bosmina* sp. and *Ceriodaphnia* sp. in different culture media.**

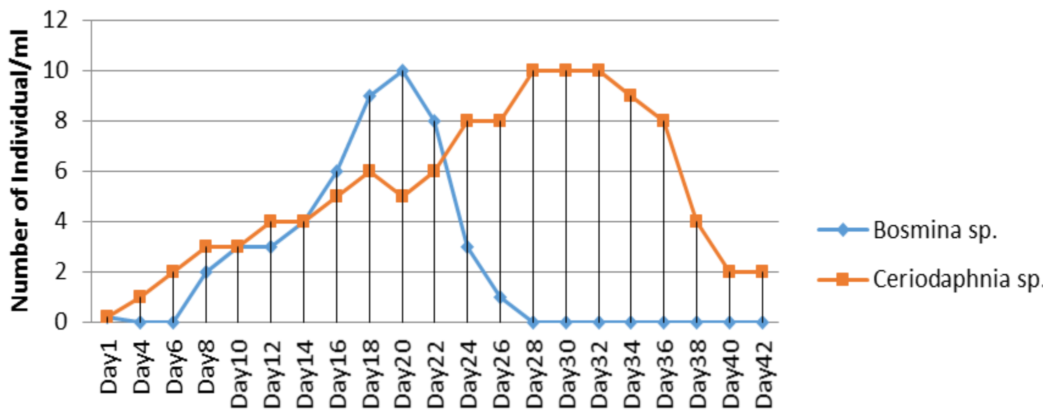

Fig.2 Comparative growth analysis of Ceriodaphnia sp. and Bosmina sp. in Poultry Manure medium

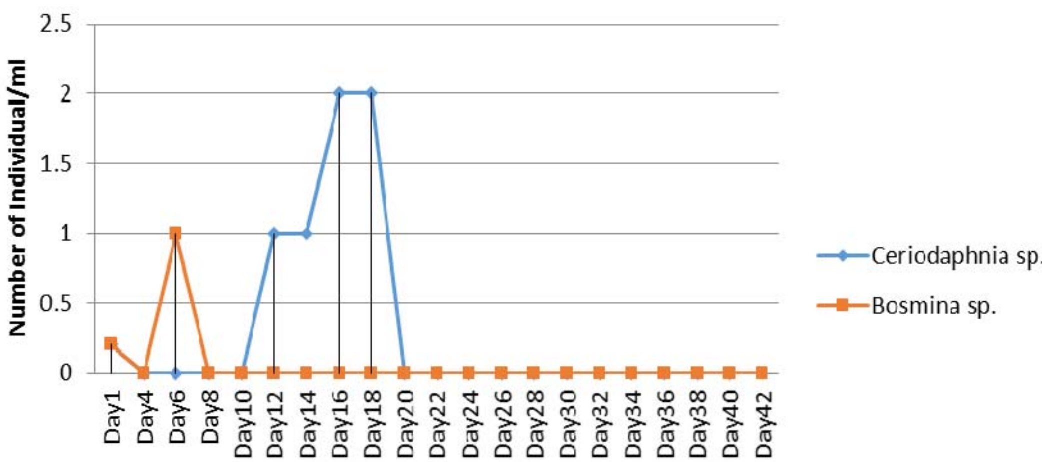

Fig.3 Comparative growth analysis of Ceriodaphnia sp. and Bosmina sp. in pulse water medium

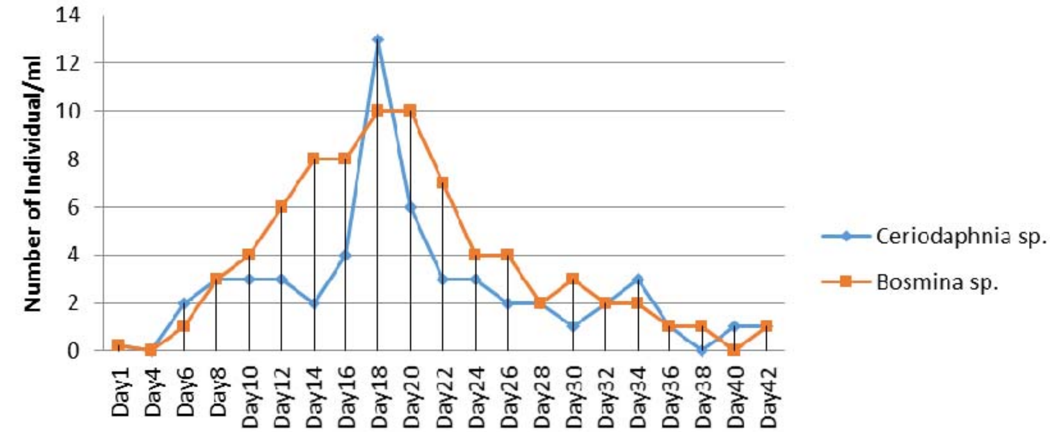

Fig.4 Comparative growth analysis of Ceriodaphnia sp. and Bosmina sp. in Snail feaces medium

4. Discussion

Growth of cultured organisms *Ceriodaphnia* and *Bosmina* sp. comparatively in three types of media revealed that poultry manure medium and snail faeces were considerably good than pulse water medium. For cladocerans, it can be observed that results due to culture medium variations, like water hardness, ([9, 10, 11] are very similar to those obtained from food quality variations [12, 13]. For studying maximum production level of these two species, it was found that *Ceriodaphnia* reared in poultry manure showed the highest survival rate (1-10indi/ml) throughout the experimental period compared to snail feaces and pulse water media. On the other hand, *Bosmina* cultured in snail faeces and poultry manure media showed more or less similar pattern of highest abundance (10 indi/ml) from 14-24th days but survival rate was better throughout the culture period in snail faeces medium (Fig.2 - 4).

The findings of the present study indicate that *C. reticulata* and *Bosmina* cultures in pulse bran water medium with *Chlorella* inoculums had exhibited the lowest growth and survival rate with an average of 0.38±0.74 and 0.06±0.48 respectively. It is well established that some species of *Chlorella* can liberate toxins in the water, thus altering growth and reproduction of cladocerans [14, 15, 16]. Infante and Litt [16] obtained poor growth and reproduction culturing *Daphnia pulicaria* and *Daphnia thorata* with *Chlorella* sp. These results were attributed to inhibitory effects and decreasing of filtering rates caused by *Chlorella* toxins. This findings support the poor growth rate of both organisms cultured in present study with *Chlorella* mixed pulse water.

According to Vijverberg, [17] and Fonseca, [18], some of the most important environmental factors controlling zooplankton growing and reproduction are temperature, food quantity and quality, and the physical environment suitability. Several studies revealed that nutrient and temperature have significant effects on the life cycles of the planktonic species [19, 20, 21] which in turn affected the population growth of zooplanktons.

Temperature is responsible for decreasing development time, instar intervals, time between clutches and longevity of Zooplanktons [22, 23, 24, 25]. Vanni and Lampert [26] verified that *Daphnia galeata* reacts similarly to resource depression, regardless if it results from low food quantity or low food quality. Temperature is one of the major determinants of the feeding rate of *Ceriodaphnia*. Gopen [27] showed that *Ceriodaphnia* feed at higher rates when the water temperature increased and it occur up to a certain level. The optimum temperature for *C. reticulata* ranged from 20-22 ^{0}C [27]. The findings of the present study oppose this study as maximum growth (10indi/ml) of *Ceriodaphnia* and *Bosmina* sp. observed at higher temperature i.e., 28-30 ^{0}C which is quite similar to findings of [28] who reported that temperature ranging from 26–31 ^{0}C to be favorable 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 [29]. Hydrogen ion concentrations have great impact on the survival, growth and reproduction rates of cladocera [30, 31]. The results of the present study exhibited that pH recorded in the poultry manure medium have a significant effects on the growth of both *C. reticulata* and *Bosmina* sp. Ammonia recorded in poultry manure medium has a significantly negative relationship for *C. reticulata* but significantly positive relationship in pulse bran water for *Bosmina* culture (Table 2).

5. Conclusion

Poultry manure was found to be a relatively better food source for *C. reticulata* than snail faeces and pulse water with *Chlorella* inoculums in laboratory cultures. Snail faeces produced naturally by feeding cabbage leaves could be an excellent medium for mass culture of *Bosmina* sp. in controlled environment. The study therefore reveals poultry manure as a better source of organic manure compared to other organic manure. However, further research is needed before stepping into a large scale production of *C. reticulata* and *Bosmina* sp. 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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