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Original Article

MASS CULTURE AND GROWTH RESPONSE OF ROTIFER (*Brachionus calyciflorus*) FED DIFFERENT COMBINATIONS OF MANURE FILTRATES AND ALGAE

Agbakimi, I .O[1]., Arimoro, F. O[1]., Ayanwale, A. V[1]., *Keke, U. N[1]., Gana, J[2] and Abafi, J[2].

[1]Applied Hydrobiology unit, Department of Biological Sciences, Federal University of Technology, P.M.B. 65, Minna, Niger State, Nigeria.
[2]Department of Science Laboratory Technology, Federal Polytechnic, Bida, Niger State, Nigeria

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ABSTRACT

The investigation of the effectiveness of algae and various combinations of manure filtrates on some growth characteristics and population densities of *Brachionus calyciflorus* was undertaken in order to ascertain their various potencies. Laboratory reared rotifer, *B. calyciflorus,* was fed with mixed populations of algae 4.00 ml day^{-1} at a density of 1.6 x 10^{6} cells L^{-1} and eight different types of filtrate of manure combinations comprising of cow dung, chicken droppings, groundnut cake, soyabean cake, rice bran and single super phosphate (3CM1,3CM2, 3CM3, 3CM4, 4CM1, 4CM2, 4CM3 & 4CM4, respectively) were combined in 1.00 ml/L, 2.00 ml/L, 4.00 ml/L, 8.00 ml/L and 16.00ml/L concentrations. The population densities were determined at 1, 3, 5, 7 and 9 days, respectively. Manure filtrates 4CM1 and 4CM2 at concentrations of 4.00 ml L^{-1} produced the highest population density of rotifer of 217 and 209 inds. ml^{-1}, respectively at day 5. Overall, the four different manure combinations filtrates supported better population density increase of *B. calyciflorus* than did the three different manure combination filtrates. However, concentrations less than 2.00ml/L and more than 8.00ml/L may not support a high population density of *B. calyciflorus.* In the light of the above, hatcheries, fish farmers and researchers are advised to use a concentration of 4.00ml/L to obtain a high population density of *B. calyciflorus*.

Keywords: Concentrations, Groundnut cakes, Population densities, Soya bean, Starter feed

***Corresponding author:** uniquekkn@gmail.com. +2347030379950

INTRODUCTION

The value of live food for fish larvae and fry cannot be overemphasized (Ajah, 2008). A comprehensive review of the importance of live food in aquaculture has been extensively shown by Ajitha (2016). Rotifers represent very fundamental live food organisms in the mass production of fish larvae and fish fry in outdoor hatcheries, especially during the early fry and larval stages. They are live food capsules that deliver essential nutrients for growth and survival of fish larvae (Ajitha, 2016; Arimoro and Ofojekwu, 2004; Lubzens *et al*., 1997).

The importance of supply of considerable rotifer culture with the adequate requisite nutritional quality for the survival of fry and larvae cultured in marine hatcheries has been established and emphasized (Luzbens, 2001; Ajah, 2008). Similarly, considerable information abound on the production, nutritional quality, reproductive rates, and the use of marine rotifer, *Brachionus plicatilis* and *B. calyciflorus* as valuable live food for larval fish and crustacean culture (Anitha *et al.,* 2016; Lubzens *et al.,* 2001; Arimoro and Ofojekwu, 2004). Luzbens *et al.* (2001) have earlier documented the culture of at least 10 species of marine rotifers, including *B. plicatilis*, *Brachionus rotundiformis*, *Brachionus pterodinoides*, *Brachionus satanicus and Hexarthra jenkinae*. Shiri *et al.* (2003) also demonstrated that *Brachionus calyciflorus* can be effectively used as starter feed for burbot, *Lota lota.* It was observed from the report of Hotos (2003) that *Brachionus plicatilis* ingested the larger cell diameter algae *Asteromonas gracilis* more efficiently that even the conventional, smaller cell *Chlorella* that was usually fed to it. Earlier, Abdul *et al.* (2015) indicated the importance of effects of Algae and other food types on population growth of rotifers. *Senecio quadridentatus* has been found to thrive better in freshwater cultures than *B. plicatilis* (Ajah, 2010). Furthermore, in search for suitable freshwater prey to sustain optimal growth in fish larvae for up to 30 days from yolk sac resorption, contrasted to 13 days with *Artemia* nauplii, *A. priodonta* was identified. Three species of algae, *Chlorella vulgaris, Eudorina elegans* and *Scenedesmus quadricauda* are capable of supporting large-scale production of this rotifer (Ajah, 2008). Anitha *et al.* (2016) evaluated the reproductive rate of *Brachionus calyciflorus* under the influence of salinity, temperature, feed type and feed concentration.

In as much as information abound on the culture, population dynamics and nutritional requirements of most marine rotifers, such published information is lacking for freshwater rotifers, especially *Brachionus calyciflorus* as regards its use for agriculturist. It was in the light of the above claims that this research was designed with the ultimate aim of finding out which of the readily available alternatives of food would be most capable for supporting large-scale, mass production of the rotifer, *Brachionus calyciflorus.*

MATERIALS AND METHODS

Experimental Site

This research was conducted at the hatchery unit of Water Resources, Aquaculture and Fisheries Technology (WAFT) department, and the

postgraduate laboratory of the Biological Sciences Department - both of Bosso Campus of Federal University of Technology (FUT) Minna, Nigeria.

Determination of Physico-chemical Parameters

The tanks were monitored daily for temperature, pH, dissolved oxygen, total dissolved solids, conductivity, and ammonia using APHA/AWWA/WPCF (1995) methods.

Determination of the Population Density of Algae

Determination of the phytoplankton population densities was calculated using the procedure as illustrated by Okunsebor (2014) and used to estimate the population density.

Before the samples were collected, the culture media was carefully stirred using battery aerators (AP – 800 AIR PUMP) to ensure even distribution of the organisms within the culture. A fixed volume of water samples of culture media were collected using a calibrated pipette and mounted on binocular microscope. Individual cells of organisms were counted carefully and recorded.

$$P_d = \frac{1000 \times B_x}{V\ ml}$$

Pd = Population density of organism in 1000ml of water.

V = Average volume of water sampled using automatic pipette

Bx = Average Number of organism counted in various random Samplings

Culture of Algae

Mass culture of algae was achieved using the Kamthorn and Jin (2006) sack fertilization method. The culture concrete tank (5 x 10 x 1.5 meters) was filled to about 80 % of pipe-borne water and then inoculated with green water got from aged fish pond water. The mixture was then filtered with plankton net of 100 µm mesh size before put into the basin to reduce the zooplankton population that would readily prey on the plankton community. The water was mixed with 2 kg of chicken and pig droppings, 200 grams of inorganic fertilizer (Nitrogen, Phosphorus and Potassium) (NPK), 200 grams of lime, 50 grams of finely ground fish meal (Kamthorn and Jin, 2006). After 3 days, there was an anticipated bloom of whitish swarm of plankton on the surface of water. The culture was maintained till microalgae reached a population density of 1.6 x 10^6/ L which was suitably ready for introduction into the rotifer culture (FAO, 2015). Algae were viewed with a Sony digital camera, cybershot model 7.2 mounted on a binocular microscope and identified with Janse *et al.* (2006) freshwater algae key. Culture was simultaneously duplicated (batch culture) to avoid any technical error that could have led lead to collapse of the system and also as a precaution to ensure rotifers do not starve as this would trigger the reproduction of degenerate male eggs.

Isolation and Culture of *B. Calyciflorus* into Mono specific culture

Mixed population of zooplankton community comprising of rotifers, copepods and cladocerans were collected from a stream at Dutsen Kura bridge, Western By-pass, Chanchanga Local Government, Niger State with a plankton net (50 µm mesh size) and then transferred to the Water Resources, Aquaculture and Fisheries Technology hatchery unit. Larger animals and other unwanted materials/sediments were removed by

physical observation and manual selection. Mono-specific culture of the rotifer, *B. calyciflorus* was achieved by repeated subculture according to the method described by Okunsebor (2014), adopted with slight modifications. A drop of zooplankton medium was put into several places in a glass slide mounted on a microscope with a graduated pipette. The various organisms were viewed and identified with a Sony digital camera, cybershot model 7.2 mounted on Olumpus binocular microscope, while desired organisms, *B. calyciflorus* were collected and transferred into a conical flask 'a' containing a borehole water (Okunsebor, 2014; Ovie and Egborge, 2002). After 3 days under intensive feeding with a mixture of algae and manure, a dominant population of *B. calyciflorus* appeared in the conical flask then the process was repeated in conical flasks 'b', 'c', 'd' and then'e'. *Brachionus calyciflorus* was cultured using the batch culture method of Arimoro, (2006) in plastic tanks of 5 x 10 x 1.5 meters. Mono-specific culture of *B. calyciflorus* was started at 10 rotifers/ tank (Arimoro, 2006; Frank, 2002) algae and manure combinations at different concentrations were fed to the rotifers daily. The culture media was gently aerated using portable battery aerators, (AP – 800 AIR PUMP).

Mass Culture of *B. calyciflorus* using Algae and Filtrate of Manure Combinations

Mono-specific culture of *B. calyciflorus* was treated with algae and filtrate from combinations of manure for 9 days to determine the population density of the organism. *B. calyciflorus* (10/ml) were placed into plastic tanks (55cm x 35cm x 25cm) containing aerated bore-hole water forming a total of 8 treatments each and 2 replicates. Rotifers were fed with 4.00ml of the algae culture per day at a density of 1.6 X 10^6 cells L^{-1} (Ovie and Ovie, 2004; FAO, 2015) and the filtrate of Manure combinations from grinded Cow dung (7.5 g L^{-1}), Chicken droppings (7.5 g L^{-1}), Single supper phosphate fertilizer (1.5 g L^{-1}), Groundnut cake (1.25 g L^{-1}), Soybean cake (1.25 g L^{-1}), Rice bran (1.25 g L^{-1}) (Okunsebor, 2014). The manure filtrates were fed at different concentrations: 1.00 ml L^{-1}, 2.00 m L^{-1}, 4.00 ml L^{-1}, 8.00 ml L^{-1} and 16.00 ml L^{-1} every morning to the *B. calyciflorus* culture for 9 days. Each treatment was aerated gently with battery aerators (AP – 800 AIR PUMP) to distribute the nutrient in the culture (Ovie and Ovie, 2004). *Brachionus calyciflorus* was collected into sample bottle which was gently inverted several to ensure even distribution of the rotifer and individual *B. calyciflorus* in the sample were determined using the method described by Okunsebor (2014). A graduated pipette was used to collect samples randomly from the sample bottle unto the microscope. One drop of diluted ethanol was used to paralyze the organisms in collected water sample so that counting will be easier and more accurate. The population density of *B. calyciflorus* of a known volume was counted under x10 objective of microscope and the organisms were returned back to the stock after counting.

Table 1: Manure Treatment Combinations.

Name	Combinations of manure (g L^{-1} of water)			
3CM1	Cowdung (7.5)	Soyabean cake (3.75)	Single Super Phosphate (1.5)	
3CM2	Chicken droppings (7.5)	Soyabean cake (3.75)	Single Super Phosphate (1.5)	
3CM3	Cowdung (7.5)	Groundnut cake (3.75)	Single Super Phosphate (1.5)	
3CM4	Chicken droppings (7.5)	Groundnut cake (3.75)	Single Super Phosphate (1.5)	
4CM1	Cowdung (7.5)	Soyabean cake (1.88)	Rice bran (1.87)	Single Super Phosphate (1.5)
4CM2	Chicken droppings (7.5)	Soyabean cake (1.88)	Rice bran (1.87)	Single Super Phosphate (1.5)
4CM3	Cowdung (7.5)	Groundnut cake (1.88)	Rice bran (1.87)	Single Super Phosphate (1.5)
4CM4	Chicken droppings (7.5)	Groundnut cake (1.88)	Rice bran (1.87)	Single Super Phosphate (1.5)

CM = Combination of Manure
Figure in parenthesis = dried weight in g L^{-1} of water.

Data Analysis

Data of physicochemical parameters of test tanks were subjected to analysis of variance (ANOVA) test. The least significant differences (LSD) ($p<0.05$) was used to separate the difference of various means using Tukey Honest multiple range test.

RESULTS

The results of the physico-chemical parameters of *B. calyciflorus* culture media is presented in Table 2. The water temperature ranged from 27.84±0.03 ^{0}C at Treatment 3CM4 to 27.88±0.41 ^{0}C at treatment 4CM3. The pH with a mean of 7.0 ranged from 6.97±0.02 at Treatment 3CM2 to 7.07±0.2 at Treatment 4CM2. The mean of dissolved oxygen was 5.76 mg L^{-1} while the value ranged from 5.73±0.02 mg L^{-1} at Treatment 4CM2 to 5.79±0.21 mg L^{-1} at Treatment 3CM2. Conductivity values ranged from 217.11±0.2 µS cm^{-1} at Treatment 3CM4 to 220.06±0.2 µS cm^{-1} at Treatment 4CM4 with a mean of 218.5 µS cm^{-1}. TDS measured ranged from 677.20±0.1 mg L^{-1} at 3CM3 to 680.25 ±0.2 mg L^{-1} at 4CM1 with a mean of 678.6 mg L^{-1}.

The effects of algae culture, three combinations of manure and various concentrations on the period of growth and population density of *B. calyciflorus* are shown in Table 3.

The interaction of the combination of manure, concentration, and period of the culture distinctly show that there was a significant pulled effect of the three factors. All the concentrations and manure combinations used supported population increase of *B. calyciflorus*.

Table 2: Physico-chemical parameters of *B. calyciflorus* Culture under the various manure combinations

Parameters	Treatment							
	3CM1	3CM2	3CM3	3CM4	4CM1	4CM2	4CM3	4CM4
Temperature (^{o}C)	27.86±0.02	27.87±0.02	27.88±0.02	27.84±0.03	27.86±0.21	27.87±0.3	27.88±0.41	27.88±0.06
pH	6.98±0.03	6.97±0.02	7.01±0.02	6.97±0.05	7.01±0.3	7.07±0.2	7.02±0.01	7.04±0.12
DO (mg L^{-1})	5.79±0.01	5.79±0.21	5.77±0.03	5.78±0.02	5.75±0.02	5.73±0.02	5.74±0.03	5.75±0.02
Conductivity (µS cm^{-1})	219.1±0.3	218.04±0.51	218.06±0.3	217.11±0.2	219.32±0.2	218.17±0.3	219.09±0.3	220.06±0.2
TDS (mg L^{-1})	677.58±0.4	678.23±0.3	677.20±0.1	679.18±0.4	680.25±0.2	679.07±0.2	679.09±0.3	680.21±0.2

Values are means and standard deviation of various treatments
Mean within the same row with different superscript differs significantly (P <0.05).
CM= Combinations of manure

Results showed that the population density at day 3 increased twice the initial stocking density from 10 individuals/ml to an average of 23 inds. ml^{-1} across all the treatments. There was no significant difference ($P > 0.05$) among all manure concentrations and among all combinations of manure. At day 5, it was observed that there was an exponential increase in the population density of *B. calyciflorus* in all the treatments and there was significant difference ($P < 0.05$) within all combinations and concentrations of manure within of all treatments. The optimal population density was obtained at day 7 for all manure combinations and concentrations and it was significantly different ($P < 0.05$) from other days of the experiment. Manure combinations 3CM1 and 3CM2 produced the highest population densities of 118 and 110 inds. ml^{-1} respectively (Figure 1). There was significant difference ($P < 0.05$) among manure combinations 3CM1, 3CM2, 3CM3 and 3CM4 and all concentrations at day 7 within their various categories. The results also showed that at day 7, concentrations 2.00 ml L^{-1} and 4.00 ml L^{-1} performed better in population density of *B. calyciflorus* across all manure combinations. There was an exponential decline (crashing) of the population density after day 7 for all manure combinations and concentrations.

Table 3: Effects of Algae and 3 manure combination filtrates on the population density of *B. calyciflorus.*

Phytoplankton culture (ml day^{-1})	Manure	Concentration (ml l^{-1})	*B. calyciflorus* population density (inds. ml^{-1}) Period of culture (days)				
			1	3	5	7	9
4	3CM1	1.00	10	24	66	87	11
4		2.00	10	20	85	104	15
4		4.00	10	24	90	108	8
4		8.00	10	23	91	118	12
4		16.00	10	26	54	69	10
4	3CM2	1.00	10	20	57	73	8
4		2.00	10	18	74	105	10
4		4.00	10	24	77	102	11
4		8.00	10	22	75	110	15
4		16.00	10	25	50	65	9
4	3CM3	1.00	10	22	58	77	7
4		2.00	10	20	81	104	10
4		4.00	10	24	84	105	10
4		8.00	10	26	84	107	14
4		16.00	10	24	48	64	6
4	3CM4	1.00	10	20	51	62	6
4		2.00	10	22	68	94	8
4		4.00	10	26	78	102	14
4		8.00	10	24	75	100	14
4		16.00	10	18	44	58	7
		SEM		0.54	0.40	0.12	0.04

3CM1=Cowdung, Soyabean cake & Single super phosphate, 3CM2=Chicken droppings, Soyabean cake & Single super phosphate, 3CM3=Cowdung, Groundnut cake & Single super phosphate, 3CM4 = Chicken droppings, Groundnut cake & Single super phosphate. SEM= Standard Error of Mean.

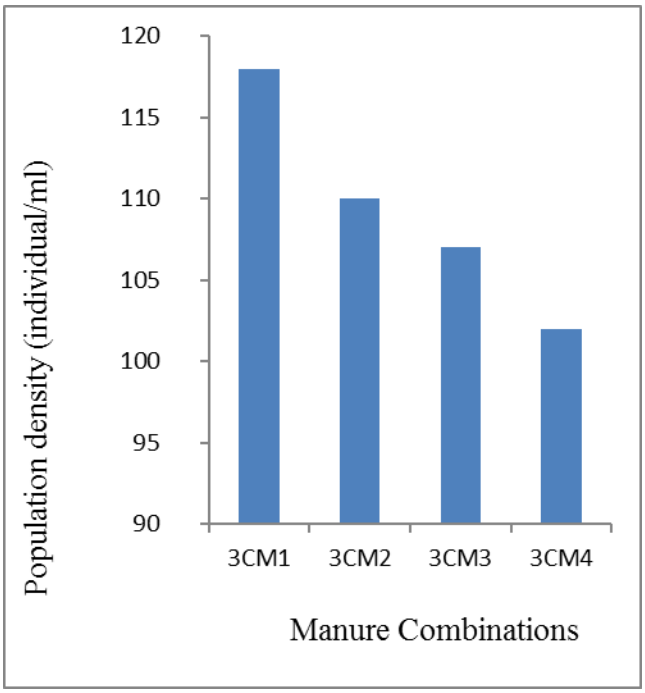


Figure 1: Effects of Algae and 3 Different Combinations of Manure at 4.00 ml L^{-1} concentration on the Population Density of *B. calyciflorus*

The result of the effect of algae culture, four combinations of manure, and various concentrations on the period of growth and population density of *B. calyciflorus* is presented in Table 4. The results showed that all the manure combinations and concentrations used supported population increase of *B. calyciflorus*. The population density of *B. calyciflorus* at day 3 increased more than twice the initial population but was not significantly different ($P > 0.05$) between all manure combinations and within various concentrations. The results further revealed that the population density grew exponentially at day 5 and the optimal population density was obtained at day 7 for all manure combinations and concentrations. The highest population densities of 217 and 209 inds. ml^{-1} were recorded from Treatments 4CM1 and 4CM2, respectively (Figure 2). The results also showed that concentrations 2.00 ml L^{-1} and 4.00 ml L^{-1} performed better in population density of *B. calyciflorus* across all manure combinations and they were significantly different ($P < 0.05$) than 1.00 ml L^{-1}, 8.00 ml L^{-1} and 16.00 ml L^{-1} used. Overall, the 4 various manure combinations filtrates supported better population density increase of *B. calyciflorus* than did the 3 various manure combination filtrates (Figure 3).

Table 4: Effects of Algae and 4 manure combinations filtrates on the population Density of *B. calyciflorus.*

Phytoplankton culture (ml day^{-1})	Manure	Concentration (ml l^{-1})	*B. calyciflorus* population density (inds. ml^{-1})				
			Period of culture (days)				
			1	3	5	7	9
4	4CM1	1.00	10	31	75	83	8
4		2.00	10	39	194	183	15
4		4.00	10	47	217	210	19
4		8.00	10	45	144	168	14
4		16.00	10	30	67	79	9
4	4CM2	1.00	10	29	66	82	8
4		2.00	10	36	188	170	16
4		4.00	10	46	209	184	14
4		8.00	10	43	141	153	14
4		16.00	10	30	53	70	10
4	4CM3	1.00	10	26	62	74	5
4		2.00	10	31	123	162	12
4		4.00	10	37	125	170	13
4		8.00	10	39	98	134	13
4		16.00	10	35	47	63	6
4	4CM4	1.00	10	25	54	67	5
4		2.00	10	34	114	153	18
4		4.00	10	37	111	157	15
4		8.00	10	38	87	120	14
4		16.00	10	33	45	56	8
		SEM		0.43	0.25	0.37	0.02

4CM1=Cowdung, Soyabean cake, Rice bran & Single super phosphate, 4CM2=Chicken droppings, Soyabean cake, Rice bran & Single super phosphate, 4CM3=Cowdung, Groundnut cake, Rice bran & Single super phosphate, 4CM4 = Chicken droppings, Groundnut cake, Rice bran & Single super phosphate. SEM= Standard Error of Mean.

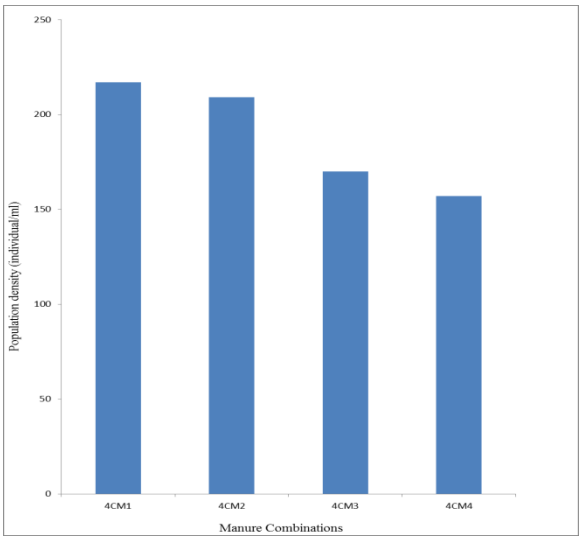
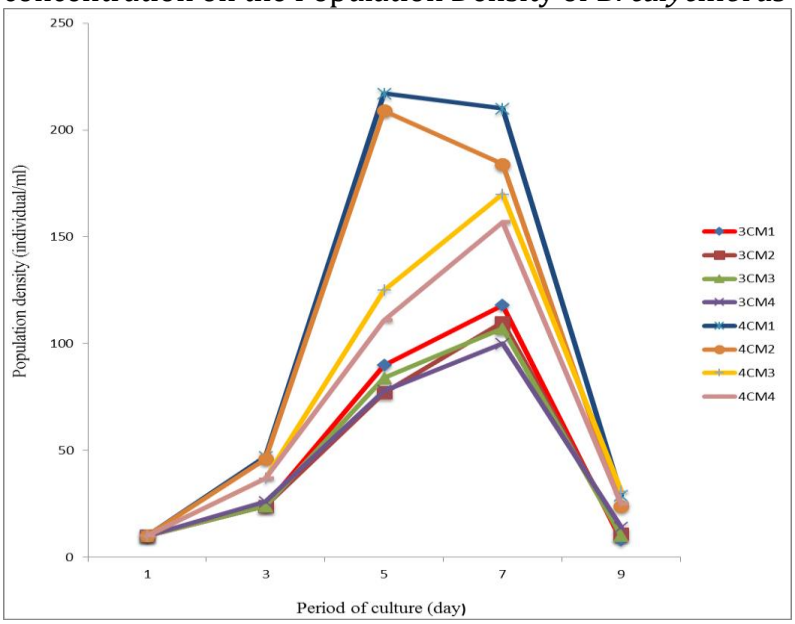


Figure 2: Effect of Algae and 4 Different Combinations of Manure at 4.00ml/L concentration on the Population Density of *B. calyciflorus*



3CM1=Cowdung, Soyabean cake & Single super phosphate,
3CM2=Chicken droppings, Soyabean cake & Single super phosphate,
3CM3=Cowdung, Groundnut cake & Single super phosphate,
3CM4 = Chicken droppings, Groundnut cake & Single super phosphate.
4CM1=Cowdung, Soyabean cake, Rice bran & Single super phosphate,
4CM2=Chicken droppings, Soyabean cake, Rice bran & Single super phosphate,
4CM3=Cowdung, Groundnut cake, Rice bran & Single super phosphate,
4CM4 = Chicken droppings, Groundnut cake, Rice bran & Single super phosphate.

Figure 3: Comparison of the effects of Algae and various 3 and 4 different Combinations of Manure filtrates on Population Density of *B. calyciflorus* at 4.00 ml/L

DISCUSSION

Physico-chemical Parameters of *B. calyciflorus* Culture

The monitoring of water temperature, pH and dissolved oxygen are very essential in the culturing of zooplankton because it enables laboratory manipulations of the particular strain of micro-organisms in the locality (Okunsebor, 2014). Average water temperature, pH of the water, dissolved oxygen, conductivity and total dissolved solids obtained in this study were within tolerable ranges for the culture of *B. calyciflorus* as reported by Lubzens *et al.* (2001), Shiri *et al.* (2003) and Ajepe *et al.* (2014). The slight differences in variation of the water parameters in the treatments for the culture of live food zooplankton was within acceptable ranges for the culture of *B. calyciflorus* as the water supplied was from the same source. Therefore, the results of each treatment were not altered by physico-chemical parameters in this experiment.

The value of live food for fish larvae and fry cannot be overemphasized (Ajah, 2008). Zooplankton in their natural environment feed on plankton species which provides their major nutritional requirements to enable them carry out all biological functions (Ajitha, 2016; Wang *et al.,* 2007). Algae and various combinations of manure filtrates were fed to *B. calyciflorus* in order to boost the nutrients contents of the rotifer which would be fed to fry as well as to enhance their daily feeding activity since rotifers are filter feeders. This was in tandem with Savas and Guan (2006) who worked extensively on the filtration and ingestion rates of *B. plicatilis* fed five species of Microalgae at different cell densities. These manure combinations were used because of their high composition of Nitrogen and Phosphorus which are known to stimulate the growth of rotifers (Anitha *et al.,* 2016; Guan, 2006). Okunsebor (2014) also opined that manure fed to cultured organism already in soluble form serves as an enrichment to the rotifer culture and also makes it easier for the organism to convert and utilize the nutrients for growth and reproduction more readily than phytoplankton which has hard cell wall (Khatun *et al.,* 2004), hence the combination of algae and manure filterate.

Manure combinations with the cowdung and soyabean cake portrayed a higher significant growth and population density capacity compared to manure combinations with chicken droppings. This might not be unconnected with the fact that cowdung and soyabean cake contains more nitrogen and phosphorus than that of chicken droppings. Nitrogen and phosphorus are very strong limiting factors to the growth and reproduction of *B. calyciflorus*. This result was in consonance with the findings of Thomas and Veschoor (2004) who worked extensively on the effects of food quality on the life history of *B. calyciflorus.* His findings revealed that diets low in nitrogen and phosphorus levels (ώ-3 PUFAs in both phosphorus- and nitrogen-depleted algae) reduced somatic growth and reproduction, whereas lifespan remained unaffected. Also in consonance with this present study are the findings of Guan (2006) who posited that rotifers thrive better and are higher in population densities in eutrophic waters, as cladocerans and copepods, which are predators to rotifers, cannot survive high concentrations of nitrogen and phosphorus and hence are few in numbers. Anitha *et al.* (2016) who examined the reproductive rates of *Brachionus calyciflorus* under the influence of salinity, temperature, feed type and feed concentration reported a similar outcome. The manure combinations of cowdung and soyabean

cake (3CM1 and 4CM1) produced outstanding populations compared to cowdung and groundnut cakes combinations (3CM3 and 4CM3). This was similar to the findings of Okunsebor (2014). The addition of rice bran (1.87 g L^{-1}) to the manure combinations also showed remarkable increase in the population density from 118 to 217 individuals at its peak as other combinations: cowdung, chicken droppings and single super phosphates were constant in the 3 combinations of manure. According to Najeeb *et al.* (2015), rice bran contains 50% Carbohydrates, 15% Proteins, 20% Fatty acids (linoleic and oleic acids), 5% Vitamin E (oryzanols, tocopherols, tocatrienols, phytosterols) and an array of other micronutrients.

Concentrations 2.00 ml L^{-1}, 4.00 ml L^{-1} and 8.0 ml L^{-1} of manure combination supported high population density of *B. calyciflorus*. However, concentrations of 4.00 ml L^{-1} had the highest population density at day 7, and this could possibly be due to the supply of needed nutrients at required levels of the cultured organisms without deleterious effect on the population density of *B. calyciflorus*. The most accurate dosage for obtaining optimal population growth of *B. calyciflorus* was obtained from 4.00 ml L^{-1} concentrations. The manure concentrations of 1.00 ml L^{-1} and 16.00 ml L^{-1} bordered between 2 extremes. Concentration 1.00 ml L^{-1} was too low while concentration 16.00 ml L^{-1} on the other hand was too high for the optimal production of *B. calyciflorus*. The low concentration could have been insufficient to carter for the nutritional needs of the cultured organisms to a very high population density. As the culture period increased, the population density also increased - although at a very slow rate - because of the high competition for little available nutrients. On the other hand, the population increase was also slowed down in concentrations of 16.00 ml L^{-1} probably because the high concentration of nutrients was beyond the threshold that *B. calyciflorus* could effectively breakdown daily and within the life span of the cultured organism making the culture media unconducive thereby producing a deleterious effect. At day 8, the population density stressed the available resources and survival was threatened by age and shortage of available resources (Lubzens, *et al.,* 2001; Arimoro, 2005; Okunsebor, 2014). The crash in population was high and sudden due to competition for space, available nutrient, pressure from waste products from the living organisms as well as high quantity of dead decaying organisms. The sudden population drop was less in treatments of less dense population as pressure and competition was lesser in the group.

From this research, it can be inferred that *B. calyciflorus* can be successfully produced under laboratory conditions using algae treatment and manure combinations at different concentrations. However, concentrations less than 2.00ml/L and more than 8.00ml/L may not support a high population density of *B. calyciflorus.* In the light of the above, hatcheries, fish farmers and researchers are advised to use a concentration of 4.00ml/L to obtain a high population density of *B. calyciflorus.*

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