I.J.A.B.R, VOL. 5(1) 2015: 69-72 ISSN 2250 – 3579

INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

ISOLATION AND MASS CULTURE OF FRESHWATER ROTIFER *(Branchionus calyciflorus)* USING DIFFERENT ORGANIC MEDIA

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ABSTRACT
This study on the isolation and culture of the rotifer *(Branchionus calyciflorus)* using different organic media was conducted for eight weeks using nine (9) culturing bowls, each with a diameter of 60 cm. The bowls were grouped in threes to form triplicate samples (A1 - A3, B1 – B3 and C1 - *C3)*. The study was in two parts. The first part involved collection of pond water, identifying the zooplankton present, eliminating all the zooplankton except *Branchionus calyciflorus,* by treating the water sample with 1.5 mg/l of Basudine and the water used in preparing a pure stock of *B. calyciflorus*. In the second part of the study each of the bowls was filled with 20 litres of distilled water and fertilized with 50 g of sterilized organic manure. Bowls A1- A3 were fertilized with pig dung, B1 - B3 with poultry droppings and C1 – C3 with cow dung. Each of the bowls was inoculated with 3 ml of the pure stock of *B. calyciflorus*. Dissolved oxygen, pH, water temperature and *B. calyciflorus* counts in the different culture media were determined weekly. Data collected were subjected to one-way analysis of variance and means separated using Duncan's multiple range tests. Result of the study showed pig dung to be sig. different ($P< 0.05$) and performed better than poultry droppings and cow dung in the early stages of the study. There was an initial lull in production using the poultry droppings, as it had the least performance. By the eighth week, it had picked up and there was no sig. difference ($P>0.05$) in production of *B. calyciflorus* using the poultry cow dung culture media. Thus any of the organic manures can be used for mass production of *B. calyciflorus.* Use of pig dung is however recommended because it has a higher production capacity from the beginning of the study to the end, using a giving quantity compared to the other two manure types.

KEYWORDS: rotifer, zooplankton, Basudine, poultry droppings, organic manures.

INTRODUCTION

Zooplankton is one of the primary food sources of fish larvae in fish culture (Arimoro, 2006). Mass production of catfish under controlled conditions depends on the provision of live plankton food for early fry and larval stages. The importance of live food in fry and larval rearing has been reported by a number of authors like Ovie *et al*. (1993) and Ajah (1997 & 1998), they established the advantages of fresh water plankton over artemia cysts. This is because artemia as a marine organism die in freshwater within two hours of introduction (Porticelli, 1987; Ovie, 1997). The commonly cultured zooplankton in fish Culture is Rotifer. Rotifer is the most dominant zooplankton in all the freshwater aquatic ecosystems and is considered an ideal food for fish larvae (Arimoro and Ofojekwu, 2004). The success in the hatchery production of fish fingerlings for stocking in the grow-out production system is largely dependent on the availability of suitable live food organisms *e.g.* rotifer for feeding fish larvae, fry and fingerlings (Lim, 2001). Advantages of rotifer as a culture organism are manifold. These among others, include their planktonic nature and tolerance to a wide range of environmental conditions (Dhert *et al*., 1995). Arimoro (2006) reported that *Branchionus calyciflorus* is the most commonly cultured rotifer in freshwater mass Culture. B. *calyciflorus* can thrive in temperature ranges of between 15 to 31°C. In their natural environment they thrive in waters of various ionic compositions. The optimal pH is 8 at 25 °C, Minimum oxygen level is 1.2 mg/L. Moreover, due to its small size and slow swimming velocity, the *B. calyciflorus* is a suitable prey for fish larvae that have just reabsorbed their yolk sac but cannot yet ingest larger food particles. However, the greatest potential for rotifer culture resides in the possibility of rearing these animals at very high densities. Densities of 2,000 individuals ml-1 have been reported by Harita (1979). Even at high densities, the animals reproduce rapidly and can thus contribute to the build up of large quantities of live food in a very short time. The filter-feeding nature of rotifers *(B. calyciflorus),* facilitates the incorporation into their body tissues of specific nutrients essential for the larval predators. In addition, the use of freshwater rotifers is likely to have an important impact on freshwater ornamental fish culture. (Lim, 2001). The use of rotifers would enable intensive pisciiculture of freshwater ornamental fish species with small larvae, which would eventually lead to exponential increase in the yield of the fry (Lim and Won, 1997). Arimoro *et al.* (2006) stated that a single rotifer can become thousands of rotifers in a few days. Its primary mode of reproduction is through parthenogenesis, which is a form of reproduction that does not involve the union a male and female. Usually when environmental conditions are favorable, female rotifers produce up to 7 eggs simultaneously without any genetic input from a male rotifer. These eggs are genetically identical and hatch to form new `daughter' rotifers within 12 hrs. By 18 hrs post hatching, the daughter rotifers begin to reproduce themselves and egg production is maintained for up to a week or more. Branchionid rotifers have a short life span. Female life span at 25°C is 6-8 days. Males live for about 2 days (Gilbert, 2004). Arimoro and Ofojekwu (2004) stated that to achieve pure culture of the rotifer *Brnnchionus calvciflorus*, 'Basudine' an organophosphoric acid ester, applied at the rate of 1.5 mgL-1 is used. This concentration was arrived at through series of toxicity experiments to determine the safe concentration for the rotifer (Agbon *et al*., 2002). At this concentration Crustaceans including copepods and cladocerans, aquatic insects, such as mosquito larvae will fail to survive thereby allowing the rotifers to multiply in the absence of predators. Mass production of rotifers can be performed using algae and yeast in a batch or semi-continuous culture system. Isolation of the desired rotifer to be cultured can be done through elimination, sub-culturing or pipetting method. Organic manure such as poultry manure, pig dung and cow dung could be used as fertilizers to promote algal blooms on which the rotifers feed. The preparation of this organic medium may be through broadcast, fermentation or sac method (Okoye, 1996). For a Successful culture, a temperature range of 20 - 30 °C is ideal. This temperature range enhances reproductive activity (Lubzens, 1987). Rotifers live at pH levels of above 6.6, outside their natural environment, under culture conditions. Best results have been obtained at pH above 7.5. They can survive in water containing as low as 2 mg/l of dissolved oxygen. The level of dissolved oxygen in the culture water depends on temperature, salinity, rotifer density and the type of food.

MATERIALS & METHOD

Nine plastic bowls, each of diameter 60 cm were used for the study. The bowls were grouped in threes to form triplicate samples Al- A3, BI - B3 and C1 - C3. The study was in two parts *viz.* a-priori and a-posteriori tests. The a-priori test, involved collecting 50.0 ml of pond water and sub- samples were viewed under the microscope to identify the zooplankton present, using the method described by Arimoro (2006). The most dominant zooplankton identified was the rotifer (*Branchionus caiyciflorus*). A pure culture of *B. calyciflorus* was obtained by adding 1.5 mg of Basudine per litre of the pond water in a round bottom flask. This eliminated all other zooplankton present except B. calyciflorus (Agbon *et al*., 2002). Thereafter, 3.0 g of yeast was added to the flask which served as food for the zooplankton. The mouth of the flask was covered and left exposed to light for three days, to enable the *B. calyciflorus* grow and reproduce. In the a-posteriori test, to each of the triplicate bowls containing 20 litres of distilled water was added 100.0g of organic manure tied in a sac. Before being added, the culture medium was sterilized in an autoclave for 15 minutes, at a temperature of 150 ºC and a pressure of one atmosphere, manure used were pig dung for bowls A1- A3, poultry droppings for bowls. BI - B3 and Cl - C3 Cow dung. Each bowl was inoculated with 3 nil of the pure culture *B. calyciflorus* and left for eight weeks, to grow and reproduce. Water temperature, pH and dissolved oxygen of the culture media were monitored weekly. Growth of the *B. calyciflorus* was determined weekly by using a slide with a counting chamber mounted on a microscope at a magnification of x10, to count the number present in 1 ml of the different culture media and multiplied by 1000 to give the number per litre.

Data Analysis

Data collected was subjected to analysis of variance (ANOVA) and means separated using DUNCAN's multiple range tests.

RESULTS

Results of the study on the production of *B. calyciflorus* using pig dung, poultry and cow dung, are presented in Tables 1- 4.

TABLE 1: Rotifer (*B. calyciflorus*) Count in the Different Culture Media ($x10^3$/l)

Weeks	Pig Dung	Poultrv Dropping	Cow Dung
1	$5.8333^a \pm 0.3333$	$3.1667^b \pm 0.4410$	$3.8333^b \pm 0.1667$
2	$9.1667^a \pm 1.0138$	$5.5000^b \pm 0.7638$	$7.000^{ab} \pm 0.7638$
3	$12.3333^a \pm 1.0138$	$8.1667^b \pm 0.4410$	$9.0000^b \pm 0.8660$
4	$15.5000^a \pm 1.0000$	$11.1667^b \pm 0.1667$	$10.3333^b \pm 0.4410$
5	$18.0000^a \pm 1.0408$	$14.3333^b \pm 0.1667$	$14.1667^b \pm 0.1667$
6	$20.3333^a \pm 1.0138$	$16.6667^b \pm 0.4410$	$17.8333^{ab} \pm 0.7265$
7	$22.8333^a \pm 1.4530$	$18.83333^b \pm 0.7264$	$20.0000^{ab} \pm 0.7638$
8	$24.3333^a \pm 1.7401$	$21.1667^b \pm 1.0929$	$22.3333^{ab} \pm 0.4410$

Means with the same alphabets as superscripts are not significantly Different (p>0.05).

The result of *B. calyciflorus* count across the weeks showed that rotifer production in pig dung was significantly different (P< 0.05) from that of poultry droppings, while that of the cow dung was not significantly different (P> 0.05) from those of pig dung and poultry droppings.

'**TABLE 2:** Weekly Temperature Level in the Different Culture Media (T ^{O}C)

Weeks	Pig Dung	Poultry Droppings	Cow Dung
1	29.5000±0.0000	29.3333 ±0.6009	28.1667±0.3333
2	27.8333±0.6009	29.3333±0.6009	27.8333±0.4410
3	26.1667±1.0138	28.333±0.3333	28.0000±0.7638
4	26.0000±0.2887	26.8333±0.4410	26.6667±0.4410
5	26.3333±0.4410	26.5000±0.5774	25.6667±_0.4410
6	25.8333 ^{a}±0.6009	24.5000^{b}±0.0000	24.004^{b}±0.0000
7	26.1667±0.8333	26.3333±0.6667	26.0000±0.7638
8	25.3333±0.6667	24.6667±0.1667	24.3333±0.4410

Means with the same alphabets as superscripts are not Significantly Difference (p>0.05).

The result of temperature measurements within the eight weeks of study showed that there was no significant difference (P> 0.05) among the different culture media.

TABLE 3: Weekly Dissolved Oxygen Concentrations (mg/l) in the Different Culture Media

Weeks	Pig Dung	Poultry Droppings	Cow Dung
1	5.9500±0.6265	6.3167 ±0.2892	6.0667±0.1364
2	6.4833±0.1202	6.0833±0.13G4	5.6333±0.4410
3	6.0000^{ab}±0.2363	5.6500^{b}±0.2517	6.3833^{a}±0.3333
4	6.2833±0.2587	5.9167±0.0441	6.1500±0.0289
5	6.2000 ±0.1000	6.4500 ±0.1323	5.916 ±0.1481
6	6.2000±0.1803	5.7000±0,0577	5.8667±0.1167
7	6.2333^{ab} ±0.2167	5.8833±0.1093	6.6000^{a}±0.2021
8	6.1500±0.2255	5.6500±0.1528	5.7500±0.2887

Means with the same alphabets as superscripts are not Significantly Difference (p>0.05).

Apart from weeks 3 and 7, where the dissolved oxygen concentrations (mg/1) in cow dung media was higher and significantly different (P< 0.05) from that of poultry droppings but similar to those of pig dung, dissolved oxygen concentrations in the three culture were generally not significantly different (P> 0.05).

TABLE 4: Weekly Hydrogen Ion Concentration (pH) in the Different Culture Media

Weeks	Pig Dung	Poultry Droppings	Cow Dung
1	8.4167^{a}± 0.0441	8.1333^{b}± 0.0833	8.3833^{ab} ±0.0333
2	8.1000 ±0.0866	7.9333 ±0.0167	7.9500 ±0.0395
3	7.9833 ±0.0928	8.1667±0.0882	7.9667 ±0.1167
4	8.1000 ±0.1000	7.9667±0.0601	8.1667 ±0.0882
5	7.9833 ±0.0333	8.1833±0.0333	8.0833 ±0.1093
6	7.8833 ±0.0601	8.0667±0.0928	8.1167 ±0.0601
7	7.9333±0.0601	7.9167±0.0441	7.9667 ±0.0667
8	8.1167 ±0.0833	8.0167±0.1481	8.1500 ±0.0764

Means with the same alphabets as Superscripts are not Significantly Difference (p>0.05)

The result of pH measurements for the different culture media showed that with the exception of week 1 where pH for pig dung was significantly different (P< 0.05) from that of poultry droppings but similar to that of cow dung, the pH value of the different culture media were not significantly different from each other (P> 0.05).

DISCUSSION

This study which was prompted by the need to develop an alternative to artemia for use in fish culture has revealed the possibility of mass production of rotifer (*B calyciflorus*) using organic sources. This is supported by the result of an earlier study by Ekelemu and Nwabueze (2011), Ovie and Ovie (2002) on the culture of zooplankton, using organic manure. The result of the study also showed that pig dung poultry droppings and cow dung can be successfully used to mass produce *B. calyciflorus*. However, production was observed to be faster using pig dung medium, compared to poultry droppings and cow dung at the early stages of the culture. This may be due to the pig dung being watery and decomposing faster to release nutrients for the growth of phytoplankton on which the rotifers feed. This result is supported by Arimoro (2006) in his work on the culture of *B. calyciflorus.* It was further observed that as the study progressed to the 8th week, production of *B. calyciflorus* which was least in the poultry dropping media, had picked up, almost leveling up with that of pig dung. This could be due to the Poultry droppings now decomposing and releasing its nutrients. This observation could be due to the poultry droppings having a higher carrying capacity for an equal weight of pig dung or cow dung. Thus while production was dropping in the pig dung and cow dung culture media, it was increasing in tile poultry droppings. Though *B. calyciflorus* production using poultry dropping culture media showed a lull at the beginning of the study, production leveled up with those of other culture media ($P > 0.05$) over time. The temperature, dissolved oxygen concentration and pH values, reported by Arimoro (2006) as being adequate for the growth of *B. calyciflorus* are in agreement with the ranges reported in this study.Conclusively *Branchionus. calyciflorus* easily reach large numbers and because of this, they can be used to substitute for wild zooplankton in feeding hatchery bred larval fish.

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