



Full Length Research Paper

A comparative study on the population growth of rotifer *B. calyciflorus* produced with algae

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In this research, mass production of freshwater rotifer, *Branchionus calyciflorus*, for aquaculture in south-eastern Nigeria, using different food types (*Chlorella* sp., *Spirulina* sp and Baker's yeast) was investigated. Rotifer stock culture was prepared using Nitrogen phosphorus Potassium, (NPK) + urea (50 : 2.5 g) and chicken droppings (25 g) with freshly grown alga. Different food concentrations (150, 300, 650 and 1000 mg/ml) and feeding intervals (2x and 3x per day) were used to grow *B. calyciflorus* in a Batch culture method. The highest population growth was attained with 650 mg/ml of *Chlorella* sp. (213.81±9.94 individuals/ml), followed by Baker's yeast (196.67±8.18 individuals/ml) and 300 mg/ml of *Spirulina* sp. (151.90±7.98 individuals/ml). The least population growth of *B. calyciflorus* was recorded with 150 mg/ml concentration of *Chlorella* sp. and *Spirulina* sp. (81.43±6.19 and 75.71±5.12 individuals/ml, respectively), and 1000 mg/ml of Baker's yeast (from local market) (65.24 ±3.86 individuals/ml). The peak population growth was attained on day 8 of experiment ($p < 0.05$). This study indicated that both the quantity and quality of food type have significant role on mass production of rotifer for aquaculture.

Key words: *Branchionus calyciflorus*, mass production, aquaculture, fish farm, Nigeria.

INTRODUCTION

The hatchery sector has played an immense role in aquaculture practice in Nigeria, provided fish for our societal consumption, promoted social, economic and health indices. Sequel to this important contribution of fish rearing to agriculture in Nigeria there is the urgent need for improvement in fish rearing techniques. Fish contain essential nutrient called fatty acid in which the popular known omega-3 and 6 is obtained from. Unfortunately, fish cannot synthesize this nutritive component (n-6 and n-3) by itself. Hence one or both of

these fatty acids must be supplied preformed in the diet, depending on the Essential Fatty Acid (EFA) requirements (NRC, 1993). The preferred live food organisms for fish larvae are those in their natural diets, such as algae; however, Rotifer and Brine shrimp (*Artemia*) are the only zooplanktons produced in mass quantities (Kazi et al., 2010). Thus, the composition and quantity of fatty acids in zooplanktonic food affects growth and survival of larvae fish. Ashraf et al. (2010) stated that fish larvae depend on the nutrients stored in

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the yolk sac for the first few days and on completion of yolk sac absorption, they demand immediate external food for nourishment. They respond best to motile prey organisms, which are important food as well as enzyme source to digest food (Ashraf et al., 2010). Hence culture of live food is an important component of a successful fish hatchery (Lee et al., 2002). Non-availability of appropriate food at this stage is a major cause of larval losses.

Rotifers are micro-zooplanktons that have been widely used as essential food source in raising freshwater and marine fish larvae due to its unique characteristics (Lubzens, 1987). It is easily digestible, has appropriate size, can survive in high stocking densities and swims slowly giving an ample opportunity to its predator for prey (Qie et al., 1997; Lubzens et al., 2001). Monogonont rotifers are simple morphologically, with a body consisting of three parts: a head, trunk and foot. Locomotion and food capture is done by a circular band of cilia surrounding the head called a corona (Hoff and Snell, 2007).

Moreover, it has the potential for enrichment with fatty acids and vitamins and various therapeutants for production of healthy fish. Therefore successful culture of fish and shrimp in various parts of the world can be attributed partly if not totally to successful mass cultivation of rotifers.

Rotifers by themselves have little nutritional value, they serve as a “nutrient container” in other words, and they get the essential nutrient from algae on which they feed. Lubzens et al. (2001) stated that, to the fish grower, rotifers are live food capsules that deliver essential nutrients for growth and survival of fish larva. This means that the nutritional value of rotifer for fish larva, shrimp and crab depends on the rotifers food source. Rotifers actively graze the water column feeding on particles approximately 1 to 30 microns in sizes. Apart from pure algae, there is a number of yeast or algae-based rations suitable for culturing rotifers that are commercially available (Watanabe et al., 1979; Watanabe, 1993; Chew and Lim, 2006).

Rotifers have a life span of 12 to 15 days but are only reproductive on the first 2 to 5 days. They reproduce both sexually and asexually but under optimal culture conditions, reproduce by parthenogenesis.

They are cultured using a wide variety of culture systems, including batch, semi-continuous and continuous (feedback) culture. In this work, batch culture was applied. Shiri et al. (2003) reported that *Branchionus calyciflorus* could be used efficiently as starter feed for burbot *Lota lota*. Nevertheless very little is known about the culture, nutrition and population dynamics of the *B. calyciflorus*. The rotifers *B. calyciflorus* and *B. rubens* have become the most commonly cultured rotifers in freshwater aquaculture. Several studies have examined rotifer performance in freshwater mass cultures in an attempt to identify optimal culture conditions (Schluter,

1980; Schluter et al., 1981) in Nigeria.

Larviculture is yet to be stabilized due to insufficient supply of reliable live food within the locality. More so since larviculture is one of the main bottlenecks in the promotion of the production of fish and crustaceans, the need for stable source of live feed for fish larvae became imperative. The production of live food within the locality will reduce high loss of fish larvae and input cost, which are the main inherent challenges confronting aquaculture production. The main purpose of this research was to compare the population growth of rotifer (*B. calyciflorus*) produced with algae (*Spirulina* sp. and *Chlorella* sp.) and that produced with commercial food (Baker's yeast). A further aim was to check the influence on other parameters such as feed rate.

MATERIALS AND METHODS

Procurement of rotifer specimens

The resting eggs of *B. calyciflorus* were obtained from Nigeria Institute for Oceanography and Marine Research (NIOMR) Victoria Island Lagos State, Nigeria. The species of algae used in this study, were obtained from Department of Microbiology University of Nigeria, Nsukka, Enugu State and the Baker's yeast which the nutritional quality of the yeast-fed rotifers can be improved by enrichment with live microalgae or Omega-3 fatty acid emulsions prior to feeding fish larva. In addition, the reproductive rate of yeast fed rotifers is lower than algae or yeast/algae mixture) was purchased from the local market. Mass production of the freshwater rotifer *B. calyciflorus* carried out in Fisheries and hydrobiology unit of Department of Zoology, University of Nigeria, Nsukka, Enugu State Nigeria between March and April, 2013.

Preparation of stock culture

This was before the experiment in order to produce egg-bearing rotifers for mass production of rotifers. Mass production of algae was achieved by continuous resubculturing of *Spirulina* sp., raised on Bold's basal medium

The culture medium was prepared using Nitrogen phosphorus Potassium (NPK) + Urea A (50 g) and B (2.5 g) media, D media with 25 g of chicken dropping and E medium with *Chlorella* sp. only. There was constant aeration throughout the period of experiment. After 7 h, the rotifer cyst was inoculated into 5 culture media. Two days after inoculation, egg bearing rotifer was harvested (5 ml containing 4 rotifers per ml) into 13 L of transparent buckets used for mass production. There was constant aeration throughout the period of experiment. After 7 h, the rotifer cyst was inoculated into 5 culture media. Two days after inoculation, egg bearing rotifer was harvested (5 ml containing 4 rotifers per ml) into 13 L of transparent buckets used for mass production.

Experimental setup

The experiment was carried out using Batch culture method under laboratory condition and maintained for 14 days. Batch culture is a method used for cultivation of micro-organism in the laboratory in which inadequate supply of nutrients for development is provided. Once the nutrients supplied are exhausted, the culture reduces. The setups had *Chlorella* sp., *Spirulina* sp., and Baker's yeast as diets; *Spirulina* sp served as alga medium while the baker's yeast

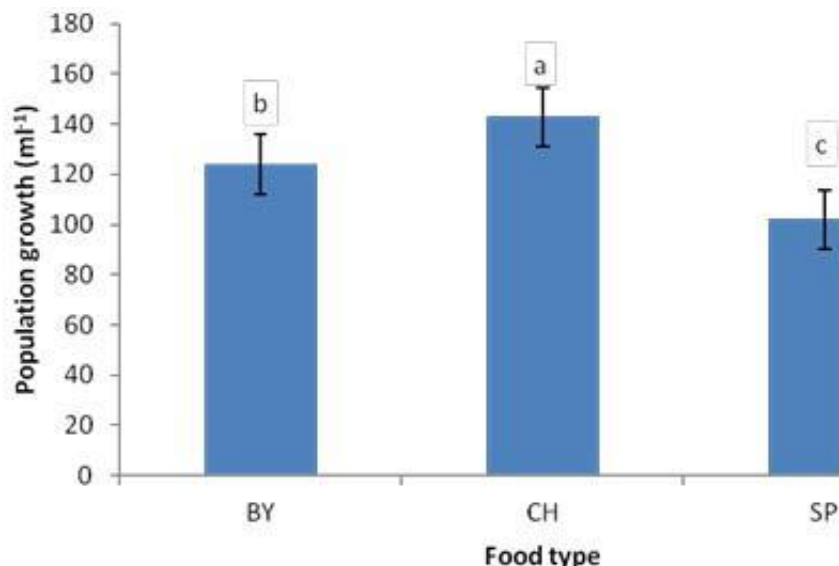


Figure 1. Overall response of *B. calyciflorus* to food type. BY = Baker's yeast; (Control) CH = *Chlorella* sp.; SP = *Spirulina* sp. Mean values represented as bars with different alphabets are significant ($p < 0.05$).

served as the control diet. Each of the set ups was further divided into four subgroups that received 150, 300, 650 and 1000 (mg/L) in a 13 L capacity aquaria (Rajendiran and Subramanian, 2007). Each of the sub groups had two different feeding intervals 2 and 3x per day each of which had three replicates. The water quality parameters of the test solution were determined in the experimental tanks following the standard method (APHA, 2005). These were monitored at 8 am and 6 pm daily.

The physico-chemical parameters monitored were pH, conductivity, total hardness, alkalinity, Temperature, and DO. These were monitored at 8am and 6pm daily. Mass production of algae was achieved by continuous resubculturing of *Chlorella* sp and *Spirulina* sp., raised on Bond's basal medium (Borowitzka, 1988) using 1000 ml capacity glass flask. The algae in log phase of growth were harvested and centrifuged at 3000 rpm for 10 min and then the pellet was weighed and mixed with water. The green alga, *Chlorella* sp. can be easily quantified by numbers, however, in the present study, to maintain the uniformity of the experimental diets weight basis was used.

Sampling of rotifer

Rotifer (*B. calyciflorus*) was sampled every 2 days to determine their population growth. The sampling was done in the morning between 8.00 to 10.00 am to minimize heat stress. A micropipette of 50 μ l was used to collect micro sample for counting using calibrated petri-dish under Stereomicroscope. Number of rotifer per ml were obtained by multiplying whatever number of rotifer in 50 μ l x 20 which is equivalent to 1ml since 1000 μ l = 1 ml.

Data analysis

Data obtained were subjected to analysis using GENSTAT (VSN international, Hemel Hempstead, Herts, UK). Analysis of variance (ANOVA) was used to test significance level while Least significant difference (LSD) was used to compare the mean population density

of rotifer. Analysis was done considering the 5% ($p < 0.05$) level of significance.

RESULTS

During the experimental period the test water pH ranged from 6.8 to 7.7, temperature was from 26 to 26.2°C while dissolved oxygen varied from 6 to 7 mg L⁻¹. Also conductivity ranged from 68.33 to 71.00 μ M cm⁻¹, while total hardness and alkalinity recorded 5.99 to 6.28 mg L⁻¹ and 136.5 to 180.5 mg L⁻¹ as CaCO₃, respectively.

Overall effect of food type on rotifer population

The effects of *Chlorella* sp., Baker's yeast and *Spirulina* (*alga medium*) sp on *B. calyciflorus* (with initial population growth of 20 *B. calyciflorus* in each medium) revealed that *Chlorella* sp. significantly increased ($p < 0.05$) the population growth of *B. calyciflorus* to 142.92 \pm 5.63 individuals/ml when compared to the population density (123.93 \pm 5.46 individuals/ml) recorded with *Spirulina* sp. (Figure 1).

Combined effects of the different food type and concentrations on the population growth of rotifer

The effects of food type and concentration level on the population growth of rotifers are shown in Figure 2. Comparing the concentration of Baker's yeast used in treatment, 650 mg/ml gave the highest population growth

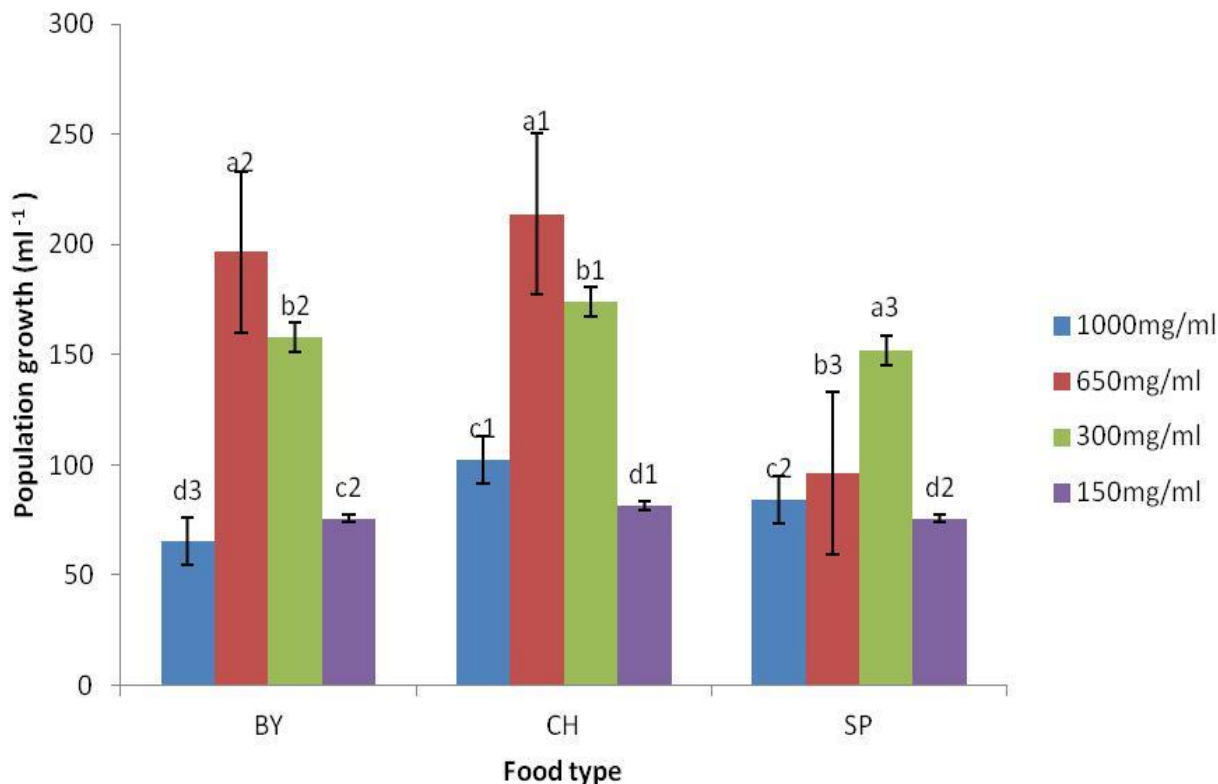


Figure 2. Combined effects of food type and concentration on rotifer production. BY = Baker's yeast (Control); CH = *Chlorella* sp.; SP = *Spirulina* sp. Bars of different colours with different alphabets on top within a treatment are significant ($p < 0.05$). Bars of same colour among treatments with different numbers on top are significant ($p < 0.05$).

of rotifer (196.67 ± 8.18 individuals/ml). With *Spirulina* sp., the 300 mg/ml concentration gave the highest population.

Combined effect of food type and feeding interval on population growth of rotifer

Rotifer fed 3x per day with baker's yeast and *Chlorella* sp. significantly ($p < 0.05$) had higher population growth than those fed 2x per day with same food type (Figure 3). The population growth in 3x feeding interval varied significantly ($p < 0.05$) in all the food type. The 3x per day feeding interval was best with *Chlorella* sp. followed by baker's yeast and *Spirulina* sp.

Combined effect of food type and duration on population growth of rotifer

The population growth of rotifers in all food type attained significant maximum growth at day 8 (Figure 4). For baker's yeast, there was no significant difference in the population density of rotifer at days 6 and 10. The population growth of rotifer was significantly low at day 2 and 4. At Day 2 of treatment, the population growth

of rotifer fed *Chlorella* sp. was significantly higher ($p < 0.05$) than those of the other two food types: There was no significant ($p < 0.05$) difference in the population growth of rotifer fed with Baker's yeast and *Spirulina* sp. At days 4, 6, 8 and 10 of treatment, the population growth of rotifer increased significantly ($p < 0.05$) in all food types used, with the population growth of rotifer being highest in those that received *Chlorella* sp.

The impact of feeding rotifer with 650 mg/ml of the three food types is shown in Table 1. This concentration also had it peak rotifer growth at day 8. The 2x per day feeding interval produced insignificant higher ($P > 0.05$) population growth of rotifer when fed with baker's yeast and *Chlorella* sp. (Table 1). The population growth of rotifer fed *Spirulina* sp. was significantly lower ($p < 0.05$) when compared to population growth of rotifer fed baker's yeast and *Chlorella* sp. The population growth of rotifer fed *Spirulina* sp. 2x and 3x per day was statistically similar ($p > 0.05$) at the peak in day 8. As the experiment progressed, no marked difference ($p > 0.05$) was found in the population growth of rotifer fed *Spirulina* sp. 2x or 3x per day. The population growth of rotifer fed 300 mg/ml concentration of the three food types increased significantly ($p < 0.05$) at 3x per day feeding interval compared to the 2x per day feeding interval (Table 2).

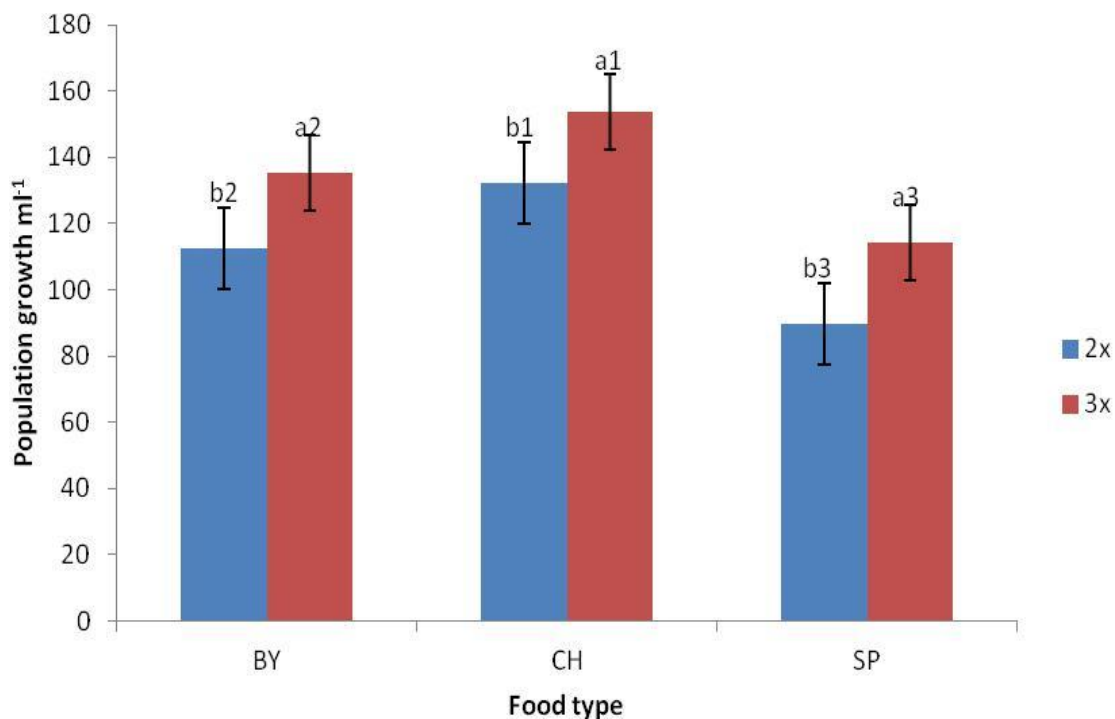


Figure 3. Combined effects of food type and feeding interval on rotifer production. BY = Baker's yeast (Control); CH = *Chlorella* sp.; SP = *Spirulina* sp. Bars of different colours with different alphabets on top within a treatment are significant ($p < 0.05$) Bars of same colour among treatments with different numbers on top are significant ($p < 0.05$).

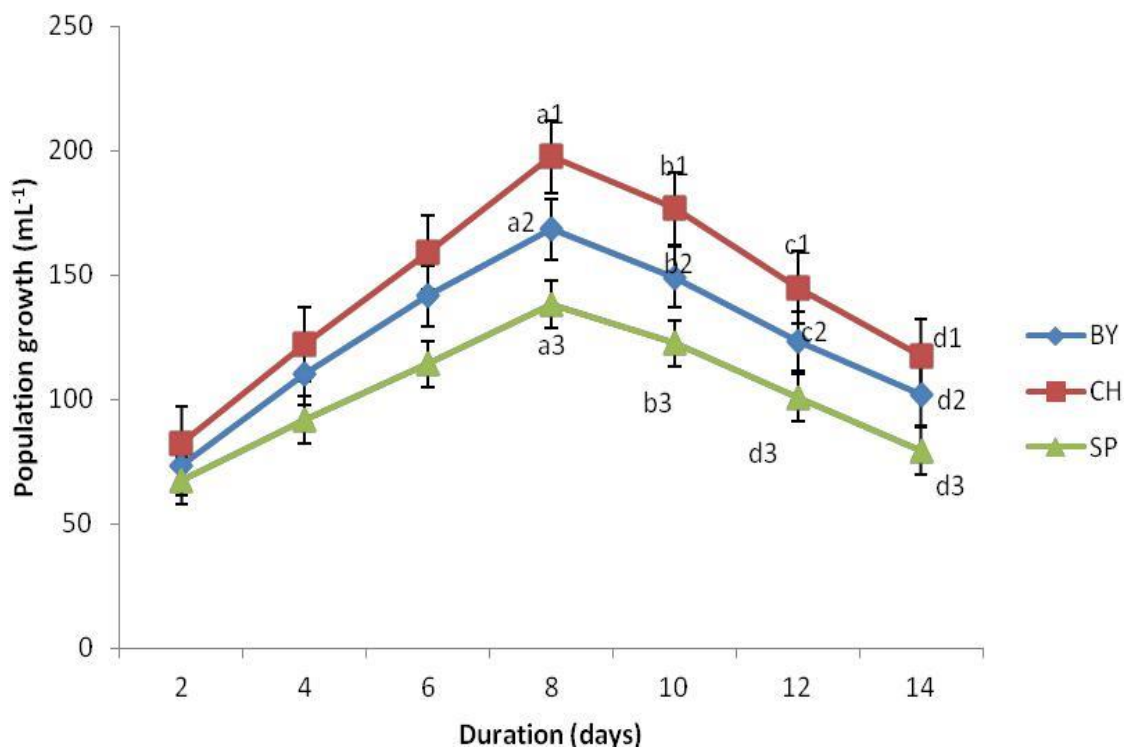


Figure 4. Combined effects of food type and feeding duration on rotifer production. Points of different colours with different alphabets on top within a treatment are significant ($p < 0.05$) Points of same colour among treatments with different numbers on top are significant ($p < 0.05$).

Table 1. Combined effects of concentration (650 mg/ml), feeding interval, food type and duration on rotifer production.

Food type	Feeding interval	Duration (days)						
		2	4	6	8	10	12	14
Baker's yeast	2x	100.00±0.00 ^{e1}	173.33±6.67 ^{d1}	233.33±17.64 ^{ab1}	266.67±13.33 ^{a1}	253.33±3.33 ^{a1}	213.33±13.33 ^{bc1}	193.33±13.33 ^{cd1}
	3x	100.00±11.55 ^{f1}	160.00±11.55 ^{e1}	206.67±13.33 ^{bc2}	246.67±13.33 ^{a1}	226.67±13.33 ^{ab2}	200.00±11.55 ^{cd1}	180.00±11.55 ^{de1}
<i>Chlorella</i> sp.	2x	120.00±11.55 ^{cd1}	146.67±17.64 ^{bc1}	226.67±13.33 ^{cd1}	293.33±6.67 ^{a1}	293.33±6.67 ^{a1}	266.67±6.67 ^{b1}	226.67±6.67 ^{cd1}
	3x	106.67±6.67 ^{f1}	153.33±29.06 ^{e1}	233.33±17.64 ^{bc1}	280.00±11.55 ^{a1}	246.67±6.67 ^{b2}	220.00±11.55 ^{c2}	180.00±11.55 ^{d2}
<i>Spirulina</i> sp.	2x	73.33±6.67 ^{c1}	93.33±6.67 ^{bc1}	113.33±6.67 ^{ab1}	133.33±6.67 ^{ab1}	133.33±6.67 ^{ab1}	93.33±6.67 ^{bc1}	73.33±6.67 ^{c1}
	3x	60.00±0.00 ^{c1}	86.67±6.67 ^{b1}	113.33±6.67 ^{ab1}	133.33±6.67 ^{a1}	113.33±6.67 ^{ab1}	86.67±6.67 ^{b1}	60.00±0.00 ^{c1}

Mean values with different alphabets as superscripts in a row are significant ($p < 0.05$); Mean values with different numbers as superscripts in a column are significant ($p < 0.05$).Key: 2x = 2x per day; 3x = 3x per day.

Table 2. Combined effects of concentration (300 mg/ml), feeding interval, food type and duration on rotifer production

Food type	Feeding interval	Duration (days)						
		2	4	6	8	10	12	14
Baker's yeast	2x	80.00±0.00 ^{e1}	106.67±6.67 ^{cd2}	126.67±6.67 ^{c2}	166.67±13.33 ^{a1}	153.33±6.67 ^{b1}	120.00±11.55 ^{cd1}	93.33±6.67 ^{de2}
	3x	100.00±0.00 ^{e1}	166.67±6.67 ^{d1}	213.33±6.67 ^{bc1}	253.33±6.57 ^{a1}	233.33±6.67 ^{ab1}	206.67±6.67 ^{c1}	193.33±6.67 ^{cd1}
<i>Chlorella</i> sp.	2x	93.33±6.67 ^{e1}	126.67±17.64 ^{cd2}	160.00±20.00 ^{b2}	193.33±13.33 ^{a2}	180.00±11.55 ^{ab2}	146.67±6.67 ^{c2}	113.33±6.67 ^{e2}
	3x	100.00±0.00 ^{e1}	180.00±11.55 ^{d1}	220.00±11.55 ^{c1}	260.00±11.55 ^{a1}	246.67±6.67 ^{b1}	220.00±11.55 ^{c1}	200.00±11.55 ^{cd1}
<i>Spirulina</i> sp.	2x	73.33±6.67 ^{c2}	93.33±6.67 ^{c2}	120.00±11.55 ^{b2}	160.00±11.55 ^{a2}	153.33±6.67 ^{a2}	126.67±6.67 ^{b2}	93.33±6.67 ^{c2}
	3x	106.67±6.67 ^{d1}	166.67±6.67 ^{c1}	200.00±11.55 ^{b2}	233.33±17.64 ^{a1}	220.00±11.55 ^{ab1}	200.00±11.55 ^{b1}	180.00±6.67 ^{bc1}

Mean values with different alphabets as superscripts in a row are significant ($p < 0.05$)Mean values with different numbers as superscripts in a column are significant ($p < 0.05$).Key: 2x = 2x per day; 3x = 3x per day.

At this food concentration, it was observed that the population growth of rotifer was similar for the three food types at the two different feeding interval used.

The 3x per day feeding interval recorded significantly higher ($p < 0.05$) population growth than the 2x per day feeding interval in 150 mg/ml

concentration of the three food type (Table 3). At this concentration, the population growth of rotifer fed 3x per day at peak in day 8 for *Chlorella* sp. was significantly higher ($p < 0.05$) than the density of rotifer fed *Spirulina* sp. at this peak. Comparing the population growth at peak in day 8 for *Chlorella* sp., food type with that of baker's yeast

revealed that the population of the two food type at this feeding interval was not significantly different ($p > 0.05$). Similarly, no significant difference ($p > 0.05$) was observed in the population growth of rotifer fed 3x per day with *Spirulina* sp. and baker's yeast at peak (day 8).

The population growth of rotifer fed 2 x per

Table 3. Combined effects of concentration (150 mg/ml), feeding interval, food type and duration on rotifer production.

Food type	Feeding interval	Duration (days)						
		2	4	6	8	10	12	14
Baker's yeast	2x	40.00±0.00 ^{bc2}	53.33±6.67 ^{b2}	60.00±0.00 ^{b2}	80.00±0.00 ^{a2}	53.33±6.67 ^{b2}	33.33±6.67 ^{bc2}	20.00±0.00 ^{c2}
	3x	73.33±6.67 ^{cd1}	93.33±6.67 ^{c1}	133.33±13.33 ^{b1}	140.00±11.55 ^{a1}	120.00±11.55 ^{bc1}	93.33±6.67 ^{c1}	66.67±6.67 ^{d1}
<i>Chlorella</i> sp.	2x	40.00±0.00 ^{bc1}	53.33±6.67 ^{b2}	66.67±6.67 ^{a2}	86.67±6.67 ^{ab2}	66.67±6.67 ^{a2}	40.00±0.00 ^{b2}	26.67±6.67 ^{c2}
	3x	66.67±6.67 ^{d1}	100.00±11.55 ^{c1}	120.00±11.55 ^{bc1}	160.00±11.55 ^{a1}	140.00±11.55 ^{ab1}	106.67±6.67 ^{b1}	66.67±6.67 ^{d1}
<i>Spirulina</i> sp.	2x	40.00±0.00 ^{e2}	46.67±6.67 ^{cd2}	66.67±6.67 ^{a1}	86.67±6.67 ^{a2}	67.67±6.67 ^{bc2}	40.00±0.00 ^{de2}	20.00±0.00 ^{e1}
	3x	73.33±6.67 ^{cd1}	93.33±6.67 ^{c1}	113.33±6.67 ^{b1}	133.33±6.67 ^{a1}	120.00±0.00 ^{b1}	93.33±6.67 ^{c1}	66.67±6.67 ^{d1}

Mean values with different alphabets as superscripts in a row are significant ($p < 0.05$); Mean values with different numbers as superscripts in a column are significant ($p < 0.05$). Key: 2x = 2x per day; 3x = 3x per day.

day with the different food types did not differ significantly ($p > 0.05$).

DISCUSSION

In the present study, *B. calyciflorus* showed similar growth performance with all the three food types used, despite algal size differentiation. Almost equal selection of the three diets led to almost uniform productivity, irrespective of alga and food types. The two smaller morph types of *A. silvestril*, the saccate and the cruciform responded similarly to prey, except that the smallest morph type (Saccate) was unable to ingest relatively large prey (*Branchionus plicatilis*) (Starkweather and Keller, 1993), (Hampton and Starkweather, 1998). Significant increase in the various food items administered, implied that none of the food items had an objectionable odour, taste or an awkward shape unacceptable to *B. calyciflorus*. Prey size, shape, filaments, gelatinous coats, densities and surface projection were implicated as probable causes of the slight differences in

feeding behaviour (Nandini et al., 2003; Fileto et al., 2004).

In our present study, feeding of rotifer (*B. calyciflorus*) with *Chlorella* sp., Baker's yeast (*Saccharomyces cerevisiae*) and *Spirulina* sp. revealed a significant increase in population growth. *Chlorella* sp. had the maximum rotifer production followed by baker's yeast and *Spirulina* sp. Kennari et al. (2008) observed that *B. calyciflorus* had better population growth and fatty acid content when fed on *Chlorella* sp. when compared with *Scenedesmus obliquus*. This could be attributed to its size, shape and nutrient composition which made it more accessible to the rotifer due to their mouth diameter. Also rotifers feed or graze on water column, which make them prefer live food to that of artificial. It is also a fact that the algal nutrition depends on its type (Rothhaupt, 1995), biochemical composition (Ahlgren et al., 1990) and extent of uptake by rotifer. Therefore, it is understood that good-quality alga acted as excellent live food diet for rotifer population increase (Dhert et al., 2001).

In contrast, Ajah (2010) observed greater

preference for *Scenedesmus quadricauda* and *Eudorina elegans* to *Chlorella vulgaris* by Rotifer (*Branchionus quadridentatus*). Though he stated that, it was as a result of the rotifer size hence, prefer larger algae. Baker's yeast and this was also good in the production of *B. calyciflorus* when compared to *Spirulina* sp. that had the lowest rotifer density. Perhaps, *B. calyciflorus* preferred Baker's yeast to *Spirulina* sp. possibly due to problems related with feeding regime. The blue green alga due to its larger size, elongated and spiral shape may reduce the feeding ability and display resistance to uptake and subsequent digestion in the rotifers. There was also a difficulty in handling the filamentous algae when compared with the other simple, single-celled algae (Brett and Muller-Navarra, 1997; Ikawa, 2004) like *Chlorella*.

This result suggested that Baker's yeast even though was a good potential food for the rotifer, its lower performance compared with *Chlorella* sp. could be attributed to vitamin deficiency particularly vitamin B₁₂ (Hoff and Snell, 1989; Treece and Davis, 2000) and thus, the lower

production of *B. calyciflorus* as noted in this study. Watanabe et al. (1979) found that *Chlorella*-fed and yeast-fed rotifers differed in their proximate compositions.

Rotifers fed yeast alone lacked essential fatty acids required for proper development and survival of several marine fish species (Watanabe, 1993). Therefore, rotifers can be nutritionally improved when fed with good quality microalgae, another live feed that is extremely good in boosting the fatty acid content in rotifers. Many studies have also shown that the growth rates of rotifer populations are limited in bodies of water depending on food availability (Stemberger and Gilbert, 1985, 1987; Kirk, 1997, 2001; Sarma et al., 2001; Sarma and Nandini, 2001). On the other hand, at high food concentration, many species showed reduced growth rates, Nandini and Sarma (2000) showed that *Chlorella* concentrations higher than 4.5×10^6 cells/ml actually caused decreased growth rates for *Moinamacrocopa*.

This study recorded that *B. calyciflorus* fed *Chlorella* sp. and Baker's yeast had their highest population growth with 650 mg/ml of food concentration followed by 300 mg/ml while feeding with *Spirulina* sp. gave highest population growth with 300 mg/ml. From the result of this work, it seemed that 650 mg/ml of *Chlorella* sp. and Baker's yeast were the exact sufficient food needed for the population growth of the culture medium, for mass population of rotifers. This was in agreement with the report of Mohr and Adrian (2002), that the use of food densities above the incipient limiting level ensured optimal growth rates.

Sarma et al. (2001) noted that the highest density for *B. calyciflorus* and *B. patulus* fed with *Chlorella* at the concentration of 3×10^6 cells/ml was 103 ± 8 individuals/ml and 296 ± 20 individuals/ml, respectively. The fact that 300mg/ml was the best food concentration for *Spirulina* sp. may be as a result of difficulty in feeding, thereby increasing the quantity of leftover food in 650 mg and 1000 mg/ml after each feeding time leading to over population in their ecosystem that can cause struggle for survivorship and environmental space. Also as noticed in this study, the 150 mg/ml concentration decreased the population growth of *B. calyciflorus* in all the food types (*Chlorella* sp. and *Spirulina* sp.) used in the study significantly except for Baker's yeast in which 1000 mg/ml had the lowest population growth. The decrease in population growth of *B. calyciflorus*, fed 150 mg/ml of *Chlorella* sp. and *Spirulina* sp. could be as a result of insufficient food.

This result was in agreement with Sarma and Nandini (2001). Also, Lucia-Pavon et al. (2001) reported a population growth for *B. calyciflorus* ranging from 55 ± 1 individual/ml (under 0.5×10^6 cells/ml) to 471 ± 72 individuals/ml (under 4.5×10^6 cells/ml) with *Chlorella* sp. Generally in this study, it was observed that higher or maximum food concentrations used in this work did better with 2x per day feeding interval and, minimum or lower concentrations did their best with 3x per day feeding

interval.

This could be as a result of food level (quantity) needed to carry rotifer population availability either by food concentration or feeding interval. Food limitation caused both pressure on fecundity and an increase in mortality (Ozdemir and Ciltas, 2010). He pointed out that changes in the life history parameters of the members of the population (rotifer) and food availability will result in alterations of the population density. They also obtained from the group fed at 12 h intervals the highest population density (269 ± 40.12 individuals/ml) and the population density was decreased when the length of feeding interval were increased in rotifer groups.

This study recorded a significant highest population growth at day 8 and in all the food concentrations, food type and feeding levels used; after which the population growth of *B. calyciflorus* started to decline as the experimental duration progresses. This result could be attributed to the culture method used in this study (Batch culture method).

Krick (1997) attributed it to unidentified autotoxins, which reduced the rotifer population growth rates and individuals survival. The toxic effect increased with rotifer abundance, resulting in density dependent negative feedbacks (Shertzer et al., 2002). The rotifers produced toxins in the medium, thereby decreasing the rotifer fecundity or survival after the eighth day. Though Ozdmire and Ciltas (2010) observed their highest peak of rotifer population growth on the fifth day (5), Arimoro and Ofojekwu (2004) attained peak population density after 10 days of culture. Although food types, food concentrations and feeding level (interval) attained highest population growth at day 8, *Chlorella* sp. gave the highest rotifer population growth in this study

Conclusion

From a food type perspective, *Chlorella* sp. was the best and ideal food for *B. calyciflorus*, since its size, structure and nutrient composition were more appropriate for mass production of rotifer for aquaculture. However, the maximum rotifer population growth in all the three diets was achieved on the eighth day. Thus, the present study provided evidence that a complete harvest could be done on the 8th day instead of prolonging the culture until the 14th day. This work inferred that maximum production of *B. calyciflorus* rested on the optimum density of palatable quality of food. The quality and quantity of food together are mandatory for optimum mass rotifer production for aquaculture.

Conflict of Interest

The authors have not declared any conflict of interest.

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