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Effect of feeding rate and feeding frequency in mass culture of *Brachionus plicatilis* in semi-continuous method with a yeast-based diet

Kalidoss Radhakrishnan[1], Samraj Aanand[2], Sethu Rameshkumar[3], Fernando Divya[3]

[1]*Central Institute of Fisheries Education, Mumbai– 400061, India*
[2]*Erode Centre for Sustainable Aquaculture, Tamil Nadu Fisheries University, Bhavanisagar – 638 451, India*
[3]*Bharathidasan University, Tiruchirappalli, 624 024, India*

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***CORRESPONDENCE**
theradhakrishnan@outlook.com

ABSTRACT

The present study was performed in semi-continuous method to calculate the optimum feeding rate and feeding frequency of *Brachionus plicatilis* with a yeast-based diet at 0.2g, 0.3g, and 0.4g yeast fed to a million rotifer in cement cisterns. Rotifer ind./ml, egg bearing ratio and population growth were evaluated in this study. The rotifer ind./ml was significantly ($p< 0.05$) greater in 0.3g of yeast (241 ind./ml) than that of the 0.2 of yeast (226 ind./ml) and 0.4g of yeast (178 ind./ml). Besides, the three-time feeding frequency had the highest rotifer (ind./ml) of 356 ind./ml and the lowest in 2 times feeding of 246 ind./ml as similarly kind of observation too recorded in egg-bearing ratio and population growth. Among the critical water quality parameters, ammonia was the only parameter that significantly ($p<0.05$) altered the rotifers density and egg bearing ratio.

INTRODUCTION

Aquaculture has an indispensable role in food fish production. Live feeds are an important nutritional source in the fish hatchery, as the first feeding fish fry does not accept the formulated diet due to poor enzymatic activity and non-functional stomach (Pedersen *et al.* 1987; Dhert *et al.* 2001). Therefore, successful seed production in any hatchery depends on the availability of live feed organisms (Lim *et al.* 2003) due to its easy acceptance and high nutritional quality (Mandal *et al.* 2009). Three live food organisms namely algae, rotifer and artemia, are commonly used as first food in larval rearing in marine fish hatcheries (Fu *et al.* 1997). Among these three live feed, rotifers retain the second place after the algae due to its continuous, stability, reliability, and nutritionally rich quality. The rotifer, *B. plicatilis* (L – type) is being used on the large scale as live feed (Maruyama *et al.*1997; Das *et al.* 2012) due to its characteristics such as slow movement, variable body size (90 - 350 µ) growth in high stocking density, faster reproduction rate, acceptance of algal feed and tolerance to higher salinity (Lubzens, 1987; Kailasam *et al.*2015).

In recent decades, the rotifer culture with baker yeast has increased due to an easy availability and less time consumption (as compared to algae), it was a breakthrough finding by Hirata and Mori (1967). In rotifer culture, the quantity of yeast usage vary hugely due to paucity information and documentation regarding the feeding rate and feeding frequency (James *et al.*, 1987; Reitan and Olsen, 1994; Dhert, 1996; Marini, 2002; Ajah, 2010). This investigation conducted with a hypothesis that is feeding rate and feeding frequency has no significant effect on rotifer (ind/ml) production. This paper tries to throw light about the rotifer culture in cement cistern by the semi-continuous method.

MATERIALS AND METHODS

Rotifer, *B. plicatilis* was cultured in rectangular cement tank by semi-continuous method. Each tank were of 15m x 2m x 1.5m (L x B x D) with volume of 15T. The tanks were fitted with proper inlet and outlet pipe to enusre proper water flow. Aereation was provided using oil fill compresser through perforator pipe at the tank bottom. To examine the optimum feeding quantity, three feeding rates 0.2, 0.3 and 0.4 g yeast/million were selected. The feeding frequency was fixed two times per day during the experiment, out of the three feeding rates the best feeding rate was take up to study different feeding frequency on rotifer population growth. The rotifer sampling was done daily morning between 07.00 am to 07.30 am from each tank. The rotifer density and number of egg-bearing rotifers were counted (duplicate sampling) by using the microscope (Magnus microscope). To feed the rotifer, the decided quantity of yeast was mixed with water kept in plastic bins (55L) for an hour. Prior to feeding, the rotifer feeding to

maintain the tank volume it is in practice to remove known volume of water, commonly known as discard. The discard is usually decided based on the egg-bearing ratio, and swimming activity. Finally, the tank is made of decided volume with fresh seawater.

The population growth rate of *B. plicatilis* was examined using the formula, $r - \frac{t}{T} \ln(N_t - N_0)$, where T is the duration of culture in the days, N_0 is the initial number of rotifer and their eggs, Nt is the total number of rotifer and their eggs after T days of culture. The number of egg-bearing rotifer ratio was estimated by RT = Nt / N x 100, RT is the egg-bearing rotifer ratio (%), N is the total number of *B. plicatilis*, Nt is the number of egg-bearing rotifers. The water quality parameters were estimated, temperature and dissolved oxygen were measured by using the YSI Pro ODO (Optical Dissolved Oxygen Instrument). The absorption of ammonium nitrogen was measured using a spectrophotometer. The pH was noticed through the analytical kit (Oakton Waterproof pH Tester 30 Pocket Tester). The one-way analysis of variance (Duncan multiple) test was taken to measure the influencing variable among different feeding rate and feeding frequency. The entire analysis was performed in SPSS v20. The level of significance among means was tested ($p<0.05$) by Duncan's multiple range.

RESULTS

The rotifer (ind./ml) were significantly greater in 0.3g of yeast than that of the 0.2 and 0.4g of yeast per million of rotifer ($p<0.05$) having an average number of 241, 226 and 178 ind./ml respectively (Table 1). Although the egg-bearing individual was significantly higher in 0.4g, but this closely followed in 0.3 g yeast per million of rotifer. The maximum percent of discarded was observed in experimental trail wherein the animal were fed in 0.2g of yeast/million. The water quality parameters had no statistically significant influence on the rotifer growth except ammonia. Besides, the least concentration of ammonia was recorded in the test wherein 0.3g yeast was fed a million of rotifer. However, the egg-bearing ratio was found to be higher in 0.3g yeast fed to a million individuals of rotifer than those of the 0.2 and 0.4g yeast fed to a million individuals of rotifer (Fig. 1). The poor population growth (r) of 0.038 was noticed in 0.4g yeast and higher r value 0.052 in 0.3g yeast fed to a million rotifer individuals.

Table 1. Rotifer production and water quality parameters among different feeding rate

Column Labels	0.2g / mi. rotifer	0.3g / mi. rotifer	0.4g / mi. rotifer
Rotifer (ind/ml)	178.3 ± 2.4^a	241.8 ± 4.5^c	226 ± 1.4^b
Egg bearing (ind/ml)	20.3 ± 2.5^a	45.2 ± 1.3^b	59.4 ± 1.2^c
Percent of rotifer discard	32.1 ± 3^b	15.8 ± 1^a	11.5 ± 0.7^a
Percent of water added	15.5 ± 1.6^a	11.4 ± 1.5^a	13.5 ± 0.8^a
Temperature (^oC)	28.6 ± 0.1^a	28.5 ± 0.4^a	28.4 ± 0.3^a
DO (mg/l)	4.9 ± 0.1^a	5 ± 0.6^a	6 ± 0.2^a
Salinity (ppm)	20.7 ± 0.2^a	20.4 ± 0.4^a	19 ± 1.4^a
pH	8 ± 0.1^a	8 ± 0.1^a	7.9 ± 0.1^a
Ammonia (mg/l)	3.3 ± 0.1^b	2.5 ± 0^a	3.2 ± 0.1^b

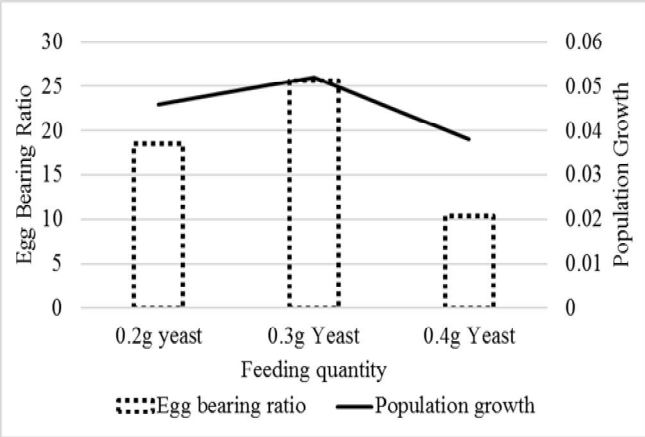

Fig.1: Population growth and egg-bearing ratio for the feeding rate

Table 2 shows the effect of feeding frequency in rotifer culture by the semi-continuous method. Three times feeding frequency achieved the highest rotifer individual per ml while two feeding frequency had the lowest egg-bearing individuals and this was found to be statistically significant ($p<0.05$). Among the water quality parameter studied only ammonia showed significant influence on the rotifer population.

Table 2. Effect of feeding frequency in rotifer culture (0.3 of yeast / million rotifer)

Column Labels	Two time feeding/day	Three time feeding/day
Rotifer (ind./ml)	246.2 ± 1.4^a	356.4 ± 8^b
Egg bearing (ind./ml)	47.2 ± 2.9^a	61.1 ± 1.5^b
Percent of rotifer discard	14.7 ± 1.7^a	13 ± 1.3^a
Percent of water added	10.9 ± 1.2^a	11.1 ± 2.3^a
Temperature (^oC)	26.9 ± 2.1^a	28.4 ± 0.2^a
DO (mg/l)	5.6 ± 0.1^a	6.5 ± 0.3^b
Salinity (ppm)	20.4 ± 0.1^a	20.3 ± 0.2^a
pH	8 ± 0.1^a	7.8 ± 0.2^a
Ammonia (mg/l)	17.2 ± 0.4^b	12.9 ± 0.6^a

The rotifer population growth was found to be lower (r-value 0.029) in two time feeding frequency and higher growth (r-value 0.099) in three time feeding frequency. Further, a two time feeding frequency had the least egg-bearing ratio (17.6%) than three time feeding frequency (20%).

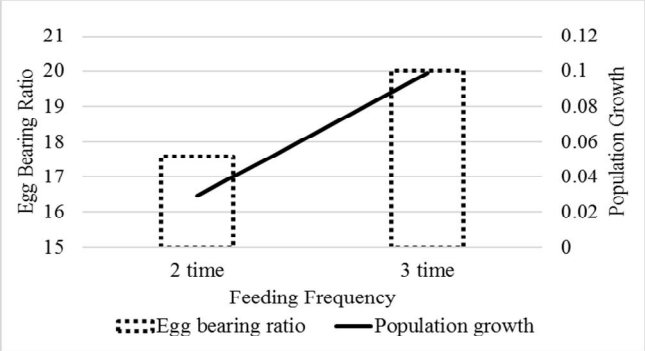

Fig. 2 Population growth and egg-bearing ratio for the feeding frequency

DISCUSSION

Rotifers vigorously graze the water column feeding on particles 1 to 10µm in size (Delbos and Schwarz, 2009). The greatest rotifer production has been recorded in 0.3g of yeast fed to rotifer, and the rotifer production increased in 3 times feeding. Contrary to the present observation, Hirano (1987) suggested that 1 to 4g of yeast is sufficient to feed a million rotifer. And, 1g of bread yeast was supplied to 106 S-type and L-type rotifers. With 2 times feeding in a day, it reached about 100 ind/ml from 20 ind./ml within 7 to 10 days period (Marini, 2002). Arnold and Holt (1991) and Liao *et al.,* (1991) examined the different kinds of rotifer culture, where 0.7 to 1g yeast was fed to a lakh of *B. plicatilis.* Morretti *et al.,* (1999), discussed the rotifer culture with Barker yeast, the feeding rate was according to rotifer density if it is less than 50 ind./ml 3g of yeast/million rotifer, 50 to 100 ind./ml 2g yeast/million rotifer and more than 100 1g ind./ million rotifer. He also argued that it should be the concentration of 100g/l. Situmorag and Suantika, sifted the effect of feeding quantity at 0.45 and 0.6 of g yeast/lakhs of *B. plicatilis* in RAS; the maximum 121 and 241 ind./ml of rotifer was recorded respectively but after 10 days it declined. Oda *et al.,* (2015) also studied the effect of feeding quantity; the maximum 200 ind./ml of rotifer was accounted in 650mg/l of yeast, followed by 150 ind./ml in 300 mg/l, 70 ind./ml in 150 mg/l and 60 ind./ml in 1000 mg/l. However, the lowest quality was utilized to production a million of rotifer. The *B. calyciflorus* density had been remarkably raised in three times feeding frequency (130 individual/ml) than that of two times feeding frequency (110 ind./ml). A coherent outcome was perceived in the present study as well. Nevertheless, Delbos and Schwarz (2009) stated that for rotifer grow out when fed at 0.5g is sufficient for a million rotifers. In the case of algal paste, 1 to 1.5 ml algae per million of rotifers. This study had relatively similar kind of observation to present study. Besides, the average percent of discarded rotifer was found to be slightly higher in the current study when it compared to Hirano (1987); suggested that 6-7% of rotifer elimination is appropriate.

Nandini *et al.* (2007) observed the population growth rate that ranged from 0.02 to 0.28/day. In present study population growth also fallen within this range, 0.048 for 0.2g, 0.054 for 0.3g and 0.039 for 0.4g yeast. A parallel line result had been noticed in this reconnaissance but less than the report of Sarma *et al.* (2001) and Serrania-Soto *et al.* (2011). The population growth influenced by the food density and food type (Sarma *et al.*2001; Lubzens and Zmora, 2003). The egg-bearing rotifers were used to foretell the population growth rate. The egg ratio is indicating the healthy status and population of rotifer culture (Edmondson, 1965). Serrania *et al.* (2011) study revealed that the egg ratio was decreased with increasing population density. Egg ratio was higher in the present study than the report of Serrania *et al.* (2011).

A sterile environment and excel water quality standard are requisite to culture high density for a sustained period. Water quality deterioration occurred mainly by a superfluous hoard of waste including the rotifer feces, urine, and uneaten feeds (Lubzens, 1987). These factors also affect the rotifer density. Among the water parameters, 5 – 15 ppt of salinity had the greater *B. plicatilis* density (Hirayama *et al.*1973; Joshi, 1988) which higher than the study. The consistent picture of temperature was observed by Ajah (2010) who cultured the rotifer by using different algal feed. A positive affiliation had noticed between the temperature and lifespan; a decline also noticed when it exceeds the ideal temperature 25^{o}C (Lubzens, 1987; Situmorag and Suantika. The optimum temperature could sustain the low bacterial and protozoans load in a unit that avoids the sudden loss. Rotifer also consumes the bacterial and protozoan, which also improved the reproduction rate (Lubzens, 1987) but do not suffice to sustain growth (Vadstein *et al.* 1993; Hotos, 2003). However, 25 to 28 is an appropriate temperature for rotifer mass culture.

Excellent dissolved oxygen (DO) insists the rich rotifer production; while practicing, the vigorous aeration should be avoided to prevent the physical damage of *B. plicatilis* (Dhert, 1996). Rotifer would be growth in the DO 1 mg/l (Yamasaki, 1987) but remarkable growth took place in 8mg/l (Lawrence, 2012). The temperature, salinity, rotifer population and type of food also were influenced the DO (Dhert, 1996). The pH is the potential factor, which affects the culture system when it exceeds the ideal level (Epp and Winston 1978). The optimum pH ranged 6.8 to 8.5 observed in semi-continuous culture system (Hirano 1987; Balompapueng *et al.* 1997) the present finding did not deviate from the recommended level. The optimum pH aids to keep lower ammonia concentration in culture rotifer system. Balompapueng *et al.* (1997) reported that the minimum concentration of ammonia (ranged 17.6 - 28.9 ppm) was noticed in the semi-continuous and the maximum (83.2 - 86.6 ppm) in a batch culture system. It was higher than the present study. Lubzens (1987) study revealed that even ammonia concentration 9 to 12 mg/l not affected the rotifer culture when the pH within 7.6 to 7.8.

CONCLUSION

From the present study, it could be concluded that there exists significant differences in feeding quantity as well as feeding rate by the rotifers. An average of 0.3g of Barker yeast is sufficient to feed a million number of rotifer and the population growth rate and egg-bearing ratio could be increased with rising feeding frequency from twice to thrice.

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