GROWTH AND REPRODUCTIVE PERFORMANCE OF ROTIFER (*BRACHIONUS* SP.) USING DIFFERENT DIETS

May Thu Rein Oo [1], Cho Cho Thin[2], Yu Yu Aye[3], Kay Lwin Tun[4]

Abstract

One of the rotifer species, Brachionus sp. plays an important role in aquaculture as live food for in the early larval stages of many marine and brackish water fish species. It is an excellent first food for larvae because of its relatively smaller size, slow swimming speed, habit of staying suspended in the water column, and ability propagation in captivity at high density and reproductive rate. Microalgae comprise the principal food component for most cultured rotifer. Many species of micro algae may be used for cultivation of the rotifers. In the present study, population growth and reproductive capacity of rotifer Brachionus sp. was evaluated for a period of eight day cultivation under three different feeding diet such as Nannochloropsis sp., Chlorella sp. and Chaetoceros sp.. The feeding density of each algae species was maintained similar as of ($4.5x\ 10^6$ cell/ml). The maximum mean population density of rotifer was observed in Treatment I (Chlorella sp.) (118.00 ± 1.00) compared to Treatment II (Nannochloropsis sp.) (45.00 ± 1.73) and Treatment III (Chaetoceros sp.) (42.00 ± 1.00) At the eight day of culture, the number of egg bearing rotifers was significantly highest ($p < 0.05$) in those fed on (Chlorella sp.) compared to Treatment II (Nannochloropsis sp.) and Treatment III (Chaetoceros sp.). According to the present results, the marine Chlorella sp. is the best food for rotifers for the mass production.

Keywords: *Nannochloropsis* sp., *Chlorella* sp., *Chaetoceros* sp., Rotifer *Brachionus* sp.

Introduction

Live food organisms include all plants (phytoplankton) and animal (zooplankton). Phytoplanktons (microalgae) are generally eaten by zooplankton. Phytoplankton forms the basis of the food chain in early stages of life cycle due to small sizes, easy digestions and enriched in nutrients.

Microalgae are cultured intensively for direct or indirect feeding through production of zooplanktons and Artemia nauplii. Seawater was supplemented with commercial nitrate and phosphate fertilizers, and a few other essential micro nutrients, are commonly used for growing marine microalgae. Microalgae are high in nutrient, not harmful to fish, not pollute the environment, suitable with the size of fish's mouth and has a high tolerance to environmental change.

Aquaculture is experiencing significant growth worldwide, leading to an increased demand for live food biomass (Lee, 2001). Hatcheries, with remarkable advancements in larval rearing technology, require suitable culture techniques for rotifers, which are essential as larval food. The success of any hatchery system heavily relies on the availability of suitable live food organisms (Dhert *et al.*, 2001).

The live food such as Artemia, rotifers, and some microalgae are commercially used as live feeds for fish and shellfish larval management. Among the commonly used live feeds for larviculture, rotifers are excellent as the first food for larvae. The rotifer species *Brachionus* sp. plays an important role in aquaculture as live food during the early larval stages of many marine and brackishwater fish species.

Rotifers are an excellent choice for fish larvae food due to their relatively smaller size, slow swimming speed, tendency to stay suspended in the water column, and their ability to propagate in captivity at high densities and reproductive rates (Lubzens *et al*., 2001). Microalgae serve as the primary food source for most cultured rotifers, and various species of algae can be used for cultivating rotifers.The most common microalgae species are *Nannochloropsis* sp.,

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Scenedesmus sp., *Pavlova* sp., *Dunaliella* sp., *Spirulina* sp., *Chlorella* sp., *Rhodomonas* sp., *Tetraselmis* sp., *Chaetoceros* sp. and *Skeletonema* sp., etc. (Parrish *et al*., 2012).

Among them, The *Chlorella* sp. is perfect food for shrimps, and all other ornamental fish, crustacean and also serves as a food for zoo-planktons such as daphnia, moina and rotifer. It is also used in food industry, cosmetics and pharmaceutical industry (Sergejevová and Masojidek, 2011).

Nannochloropsis sp. is a single-celled marine microalga that can be used as the live feed for larvae cultivation of shrimp, fish, and shellfish. *Nannochloropsis* sp. is used in aquaculture as a valuable feed, providing polyunsaturated fatty acids, essential vitamins, and amino acids, along with energy. *Nannochloropsis* sp. has high nutrition value, and it is used widely as aquaculture hatchery industry for food of larvae and juvenile of bivalve, rotifer, as well as fish larvae (Tawfiq *et al*., 1999). *Chaetoceros* sp. can also be used as rotifer feeds since they are easy to digest and are favoured by rotifers (Sutomo, 2005).

In Myanmar, fish farmers have limited experience in culturing the marine microalgae such as *Nannochloropsis* sp., *Chlorella* sp. and *Chaetoceros* sp. which is essential for the growth and hatching of rotifer *Brachionus. plicatilis*, for marine fish and shrimp rearing. Moreover, fish farmers lack technical knowledge in breeding and larval rearing, as well as knowledge of the culture and supply of appropriate live food for the first feeding fish larvae. One of the strategies to resolve this problem is the mass production of rotifer *Brachionus s*p. using three different microalgae which will be crucial for growth and survival of fish larvae, fry, and fingerlings in aquaculture.

The present study was conducted to investigate the feeding effect of three different diets on the growth of rotifer *Brachionus* sp. under culture conditions.

Materials and Methods

The present study was conducted in the Live Food Culture Laboratory of Fisheries and Aquaculture in the Research and Innovation Center, University of Yangon *Latitude*: 16° 49' 28.76" N *Longitude*: 96° 08’. The study period lasted from April 2022 to March 2023.

The initial microalgae seeds and rotifer were supported by Live Food Culture laboratory, Asia Institute of Technology (AIT), Thailand. The cultivation of microalgae seeds and rotifer were conducted in Live Food Culture Laboratory of Fisheries and Aquaculture, Center for Research and Innovation, University of Yangon.

Preparation of apparatus for cultivation of algae and rotifer

All apparatus (beakers, bottles and sea water) were covered by aluminum foil and autoclaved at 12°C for 25 mins to avoid contaminations. The seawater was prepared using natural salt to obtain 25‰ salinity and filtered with Millipore (0.45 µm) filter paper. The seawater was sterilized in an autoclave for 121°C at 20 mins pressure lb/in^2 to avoid contamination. The solution was then kept in a dark and cold place until used.

Preparation of agricultural fertilizers media for cultivation of algae

The agricultural fertilizers such as Urea, Ammonium Sulphate, and Triple Super Phosphate (TSP) were weighed by using a digital balance and added to the beaker that contained 1000ml of distilled water. After adding the substances to the beaker, the solution was stirred by using a magnetic stirrer. The solutions were sterilized in an autoclave for 121°C at 25 mins, the solutions were then taken out when the temperature was dropped to 80°C. Then, the solutions were stored in a sterilized bottle and kept in the refrigerator to avoid contamination for further use.

Experiment I .Cultivation of microalgae for rotifer feed

The sterilized plankton culture Septicity was filled with 1200 mL of sterilized 25 ppt seawater and 300 mL of pure strain microalgae (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.) were added into each glass bottle. A total of 1ml of the agriculture fertilizers media were added to glass bottle with *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.. Each culture bottle was sealed with aluminum foil and labeled with date and time. They were arranged on cultivation shelf and aerated with blower. All culture bottles were kept at air-conditions room at 25°C with light by fluorescent tubes. The experiments were extended for 10 days and population density of microalgae was calculated every day (Plate 1).

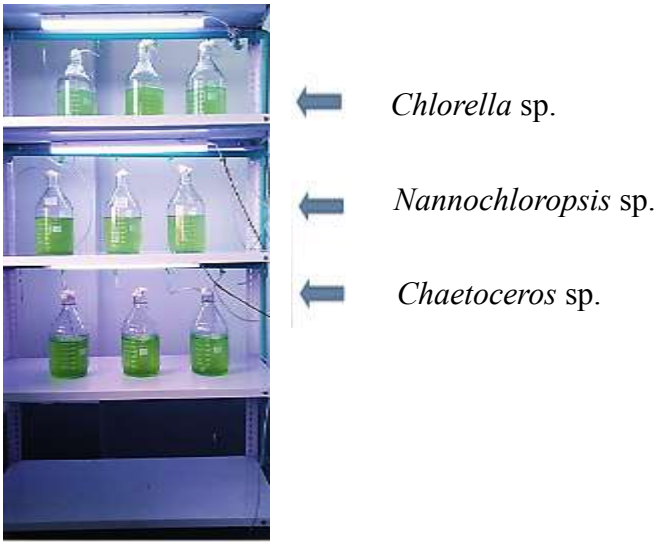

Plate 1 Cultivation of microalgae *Nannochloropsis* sp., *Chlorella* sp.,and *Chaetoceros* sp.

Determination of growth conditions and cell density of *Chlorella sp. Nannochloropsis* sp., and *Chaetoceros* sp.

The growth of *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp. were estimated by counting cell density using hemocytometer. The subsamples of 1-mL from each bottle were collected without replacement. One drop cell suspension was placed in the central counting chamber of hemocytometer (Thoma, Germany) and covered carefully with cover glass 22 mm to prevent the formation of bubbles between the cover glass and hemocytometer. The chamber was then positioned under light microscope (CX 31, Olympus) at 100× magnification. The counting of cell density was started from the first day of culture period until the 10th days and calculated using the formula (Taw, 1990) Plate 2.

$$\text{Cell count (cells/mL) for 25 squares} = \frac{\text{total number of cells counted}}{\text{Number of blocks}} \times 4 \times 10^{6}$$

Plate 2 Neubauer hemocytometer

Experiment II Cultivation of rotifer, *Brachionus* sp.

The pure strain rotifers, (*Brachionus* sp.) were fed with three different diets namely live microalgae *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp. were used as food to evaluate their effects on the growth of the rotifers, *Brachionus* sp..The experimental design of cultivation of rotifer was determined into three groups such as Treatment I (*Chlorella* sp.), Treatment II (*Nannochloropsis* sp.) and Treatment III (*Chaetoceros* sp.). The concentration of each algae reached to $4.5x10^6$ cells/ml, they were harvested to treat the rotifer. Three rotifer culture bottles were inoculated with *Brachionus* sp. at a density of 5 ind./ml. It was filled in 300 ml of each algal species. The salinity of culture bottles were kept 20 ppt.

Each culture bottle was labeled with the date. The culture bottles were arranged on the cultivation shelf and aerated with the blower and light by fluorescent tubes (Plate 3). The aeration and light were supplied with 24 hours. The experiments were extended for eight days and the growth of the rotifer was estimated every two days.

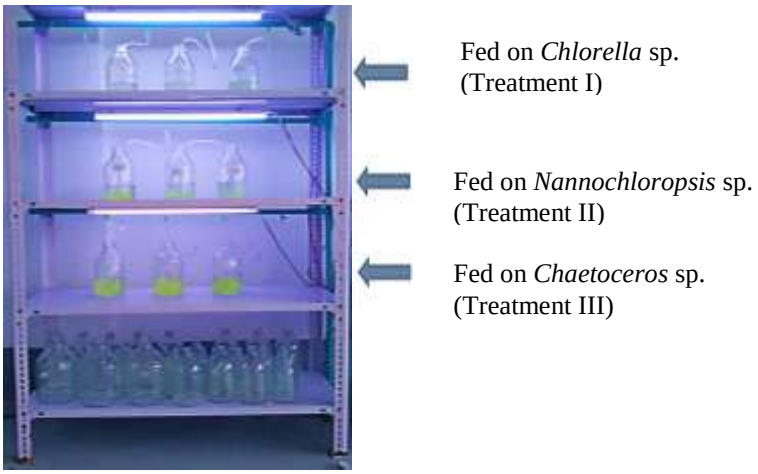

Plate 3 Cultivation of rotifer *Brachionus* sp. fed on *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.

Determination of growth of rotifer

The concentration of rotifers was counted every two days. Based on the density of rotifers, the rotifer culture was poured through a mesh size to collect a large number of rotifers on the screen. They were then rinsed with a small amount of filtered seawater and transferred to a petri dish. From each group, a total of 1 ml of rotifers was sampled using a bulbed Pasteur pipette and placed on the Sedgewick-Rafter counting cell. The rotifers were counted under the microscope (Braley, 1994) .

In addition, number of egg bearing was recorded to assess the reproductive capacity of them treated with three different diets. The reproductive capacity of rotifer was calculated using the following formula.

$$\text{Reproductive capacity of rotifer (\%)} = \frac{\text{Number of egg bearing rotifers}}{\text{Total number of rotifers}} \times 100$$

Determination of water quality

The water quality including temperature, salinity, pH and dissolved oxygen were measured every day.

Data Analysis

Cell densities of algae and rotifers were expressed as the average number of cell ml^{-1} ± standard deviation. Cell densities of the rotifer, as well as growth curves, were measured and analyzed using a one-way analysis of variance (ANOVA). The significance of the result was determined at a p-value of 0.05. Growth curves for each treatment were generated by plotting the average cell density *vs* corresponding cultivation time. These curves were created using the EXCEL computer program.

Results

Population density of *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp. agricultural fertilizer media

The population density of *Nannochloropsis* sp., *Chlorella* sp. and *Chaetoceros* sp.increased during the culture period. The colour of culture bottle intensified as the experiment progressed increasing from day 1 to day 10 (Plate 4).

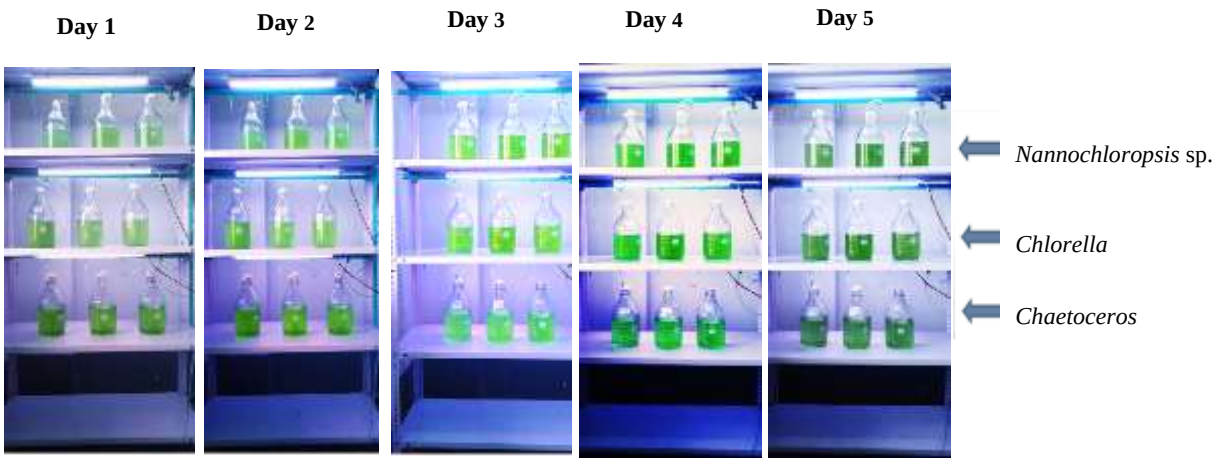

Plate 4 Cultivation of microalgae *Nannochloropsis* sp., *Chlorella* sp., *Chaetoceros* sp. (Day 1 to Day 5)

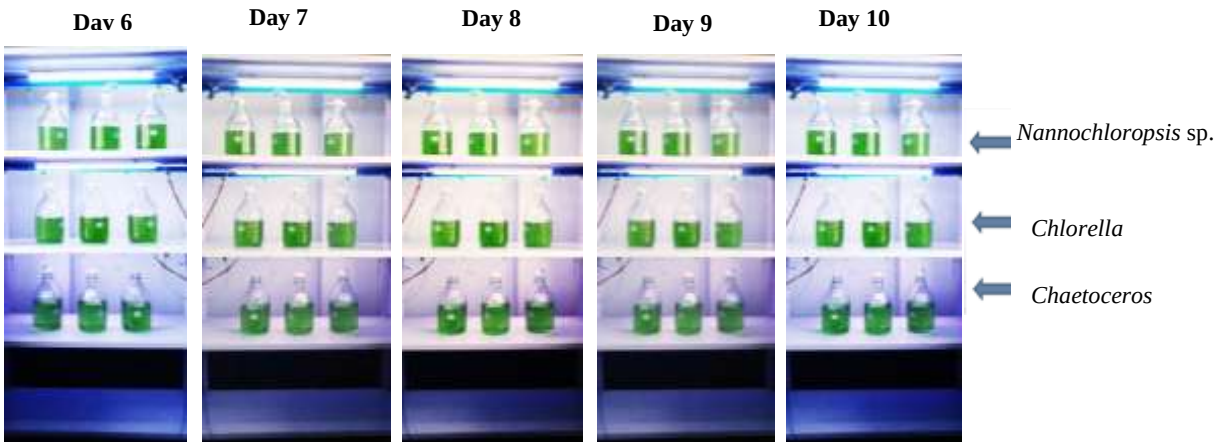

Plate 4 Cont. Cultivation of microalgae *Nannochloropsis* sp., *Chlorella* sp., *Chaetoceros* sp. (Day 6 to Day 10)

The population density of *Nannochloropsis* sp., *Chlorella* sp. and *Chaetoceros* sp. cultured with agricultural fertilizer media was shown in (Table 5). The maximum population density of *Chlorella* sp. and *Nannochloropsis* sp., were found on the fourth day of cultivation. The highest population densities of *Chaetoceros* sp. were reached in seventh day during the cultivation period. The highest population density of *Chlorella* sp. was observed in (6.83 x 10^6 cell/ml), followed by *Nannochloropsis* sp. (6.19 x 10^6 cell/ml) and *Chaetoceros* sp. (7.07 x 10^6 cell/ml) during the culture period (Table 1 and Figure 1, 2, 3,4.).The density decreased starting from 5th day in *Nannochloropsis* sp., *Chlorella* sp. While it decreased in 8th day in *Chaetoceros* sp..

Table 1. Population density of *Chlorella* sp. *Nannochloropsis* sp., and *Chaetoceros* sp. during the culture period

Time (Day)	*Chlorella* sp.	*Nannochloropsis* sp.	*Chaetoceros* sp.
Day 1	1.03±00	1±00	1.02±00
Day 2	1.76±0.23	1.5±0.1	1.33±0.05
Day 3	3.4±0.06	3±0.2	2.35±0.51
Day 4	6.83±0.06	6.19±0.36	3.72±0.15
Day 5	5.17±0.06	4.86±0.05	4.15±0.1
Day 6	4.23±0.13	4.11±0.07	5.87±0.17
Day 7	3.27±0.1	3.32±0.41	7.07±-0.26
Day 8	2.34±0.2	2.18±0.15	4.21±0.1
Day 9	1.48±0.22	1.41±0.05	2.74±0.2
Day 10	0.42±0.35	0.69±0.01	1.77±0.51

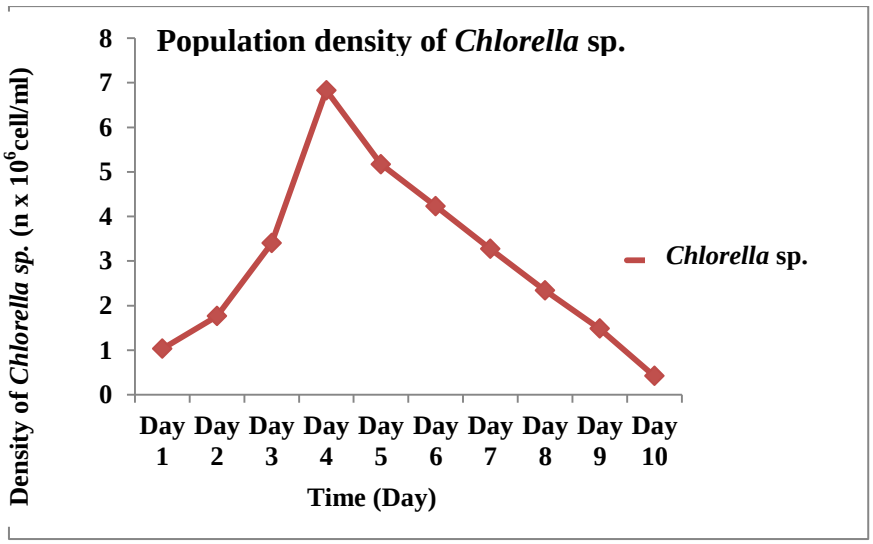

Figure. 2 Growth curve for *Chlorella* sp. cultured in agriculture fertilizer media

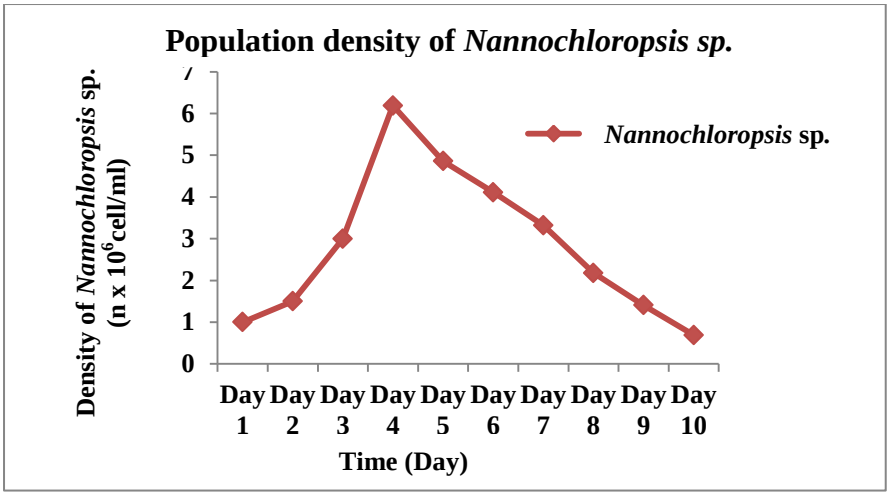

Figure 3 Growth curve for *Nannochloropsis* sp. cultured in agriculture fertilizer media

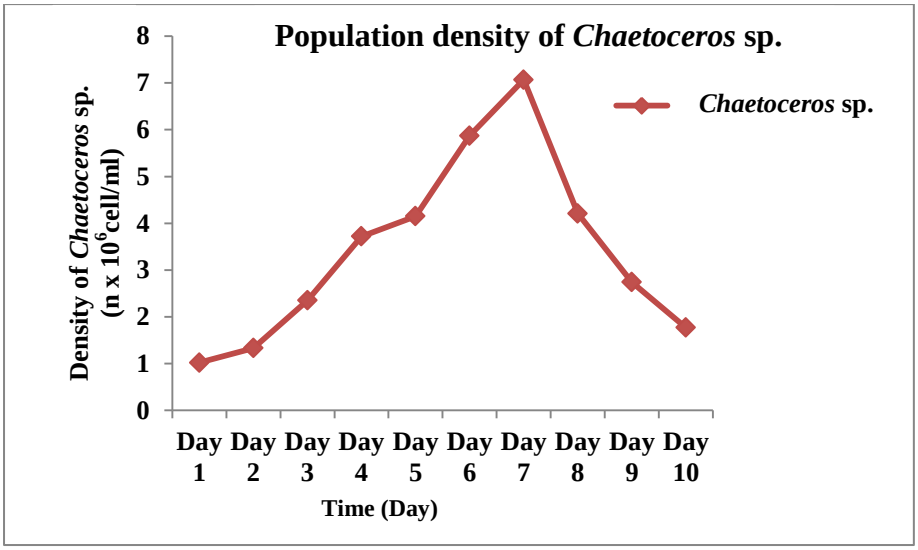

Figure 4 Growth curve for *Chaetoceros* sp. cultured in agriculture fertilizer media

Morphology of rotifer

The body of the rotifers was spherical, flattened, microscopic, multicellular, mostly aquatic organisms that are found in water. They had specialized organ systems and a complete digestive tract that included both a mouth and an anus. The rotifer's body was divided into three distinct parts: the head, trunk, and foot. The head had the corona, a rotatory organ responsible for creating whirling water movements that facilitated in the intake of small particles. The trunk contained the digestive tract, while the foot was a non-segmented, retractable ring-like structure. The rotifer observed in this study was assumed to the S Type due to its length of 130 - 210 µm (Plate 5).

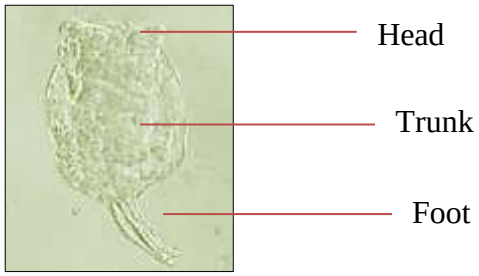

Plate 5 . Rotifer on microscope (400 x magnification)

Population density of rotifer, *Brachionus* sp. treated with three different algae

The present experiments were performed to cultivate the rotifer, *Brachionus* sp. in laboratory conditions using three different diets live microalgae (*Chlorella* sp., *Nannochloropsis sp.* and *Chaetoceros sp.*). The maximum mean population growth of the rotifer occurred on the 8th day of the culture period. The maximum mean population density of rotifer was observed in Treatment I (*Chlorella* sp.) (118.00 ± 1.00) ind./ml, followed by Treatment II (*Nannochloropsis* sp.) (45.00 ± 1.73) ind./ml and finally Treatment III (*Chaetoceros* sp.) (42.00 ± 1.00) ind./ml during the cultivation of *Brachionus* sp**.** after 8 days of the culture period (Table 2 and Figure 4).

Table 2. Population density of rotifer, *Brachionus* sp. fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

Time (Day)	Density of rotifer feed on *Chlorella* sp.(ind./ mL)	Density of rotifer feed on *Nannochloropsis* sp. (ind./ mL)	Density of rotifer feed on *Chaetoceros* sp. (ind./ mL)
Day 2	24.67 ± 0.58	10.33 ± 0.58	7.67 ± 0.58
Day 4	59.67 ± 1.53	20.67 ± 2.52	18.33 ± 0.58
Day 6	80.67 ± 1.15	28.00 ± 1.00	24.33 ± 4.04
Day 8	118.00 ± 1.00	45.00 ± 1.73	42.00 ± 1.00

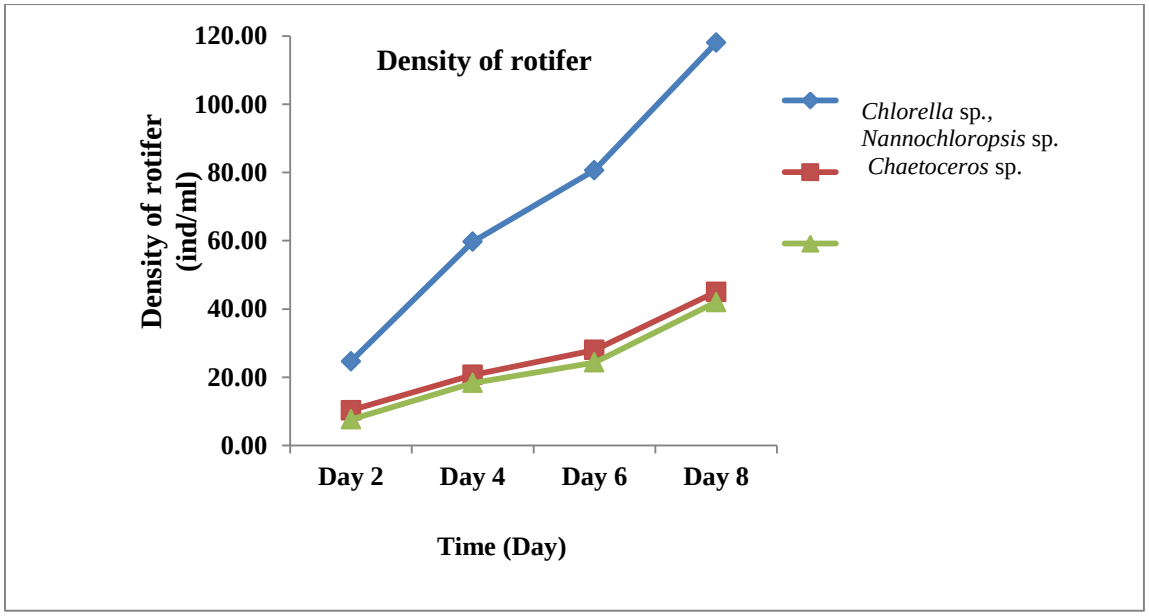

Figure. 4. Density of rotifer, *Brachionus* sp. fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

Number of egg bearing rotifer fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

The maximum number of eggs bearing rotifer was observed in Treatment I (*Chlorella* sp.) (44.33 ±2.10), followed by Treatment II (*Nannochloropsis* sp.) (11.00 ± 1.73) and finally Treatment III (*Chaetoceros* sp.) (10.00 ±1.50) in culturing *Brachionus* sp. (Table 3 and Figure 5).

Table 3. Number of egg bearing rotifer fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

Time (Day)	Number of egg bearing rotifer feed on *Chlorella* sp.	Number of egg bearing rotifer feed on *Nannochloropsis* sp.	Number of egg bearing rotifer feed on *Chaetoceros* sp. .
Day 2	5.67 ± 0.58	1.67 ± 0.58	0.67 ± 0.58
Day 4	16.67 ± 0.58	4.00 ± 1.00	2.00± 1.00
Day 6	24.33± 2.08	6.33 ± 0.58	4.00 ± 1.73
Day 8	44.33 ± 2.10	11 .00 ± 1.73	10.00 ± 1.50

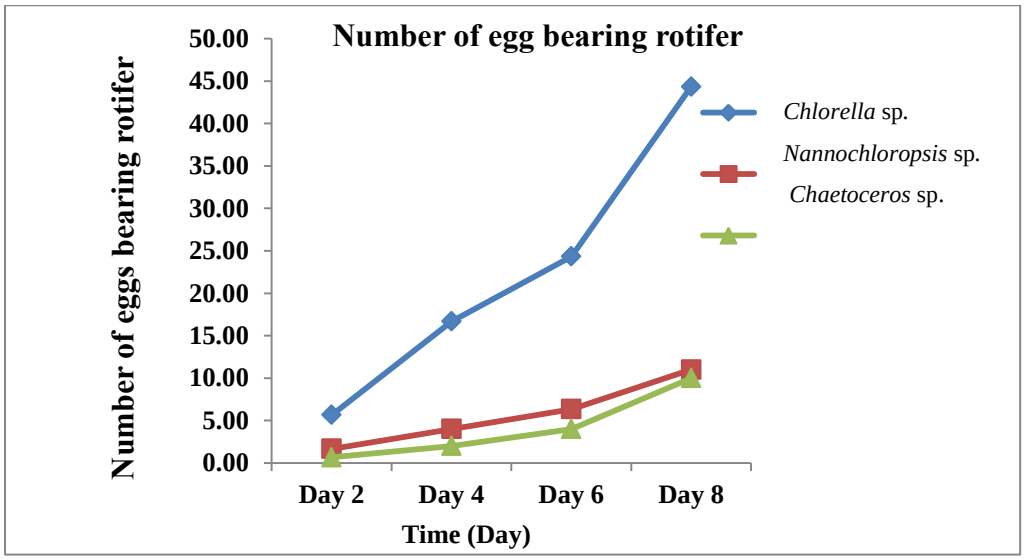

Figure. 5 Number of eggs bearing rotifer fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

Reproductive capacity of rotifer fed on different live feeds *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.

The highest reproductive capacity of rotifer was observed in Treatment I (*Chlorella* sp.) (37.57%), followed by Treatment II (*Nannochloropsis* sp.) (24.44%) and Treatment III (*Chaetoceros* sp.) (23.81 %) during the eighth day culture period (Table 4).

Table 4. Reproductive capacity of rotifer fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

Time (Day)	Reproductive capacity of rotifer feed on *Chlorella* sp. (%)	Reproductive capacity of rotifer feed on *Nannochloropsis* sp. (%)	Reproductive capacity of rotifer feed on *Chaetoceros* sp. (%)
Day 2	22.97	16.13	8.70
Day 4	27.93	19.35	10.91
Day 6	30.17	22.62	16.44
Day 8	37.57	24.44	23.81

Water Parameters

The water parameters of Treatment I, Treatment II, and Treatment III were measured during the study period. The results for the water parameters were as follows: temperature ranging from 26.6 to 27.8°C, pH ranging from 7.3 to 7.8, dissolved oxygen (DO) ranging from 5.1 to 5.3, and salinity ranging from 25 to 26.2 ppt for Treatment I; temperature ranging from 26.6 to 27.8°C, pH ranging from 7.43 to 7.9, DO ranging from 5.2 to 5.4, and salinity ranging from 25 to 26.4 ppt for Treatment II; and temperature ranging from 26.6 to 27.87°C, pH ranging from 7.2 to 8, DO ranging from 5.2 to 5.5, and salinity ranging from 25 to 26.3 ppt for Treatment III, respectively. These values are presented in Table 5.

Table 5 Water Parameter during the culture period

No	Cultivation of rotifer fed on algae	Temperature °C	Salinity ppt	pH	DO
1	Treatment I (*Chlorella* sp.)	26.6 - 27.8	25 - 26.2	7.30 – 7.8	5.1 - 5.3
2	Treatment II (*Nannochloropsis* sp.)	26.6 - 27.8	25 - 26.4	7.43 – 7.9	5.2 - 5.4
3	Treatment III (*Chaetoceros* sp.)	26.6 -27.87	25 - 26.3	7.2 - 8.0	5.2 - 5.5

Discussion

The maximum population density of *Nannochloropsis* sp*.,* and *Chlorella* sp. were found on the fourth day of cultivation period in the present study. *Chaetoceros* sp. reached its maximum density on the seventh day during the cultivation period. The factors affecting the growth of microalgae in the cultivation include light intensity, dissolved oxygen, temperature and nutrients. The microalgae require nutrients for their growth because nitrogen is a major nutrient for microalgal cultivation and marine environment. The highest population density of different live feeds (*Nannochloropsis* sp*., Chlorella* sp*.,* and *Chaetoceros* sp.) for feeding rotifer *Brachionus* sp.was obtained by using cheapest agriculture fertilizer media in this experiment.

The present study was observed the variations in survival and growth rates among different live feeds. The maximum population density of rotifers was observed in Treatment I (*Chlorella* sp.) (118.00 ±1.00), compared with Treatment II (*Nannochloropsis* sp.) (45.00 ± 1.73) and Treatment III (*Chaetoceros* sp.) (42.00 ± 1.00) in culturing *Brachionus* sp. According to the present results, among three different diets, Treatment I (*Chlorella* sp.) showed significantly highest mean population growth ($p < 0.05$) at the end of the cultured period. The egg-bearing rotifers were significantly highest ($p < 0.05$) in those fed on (*Chlorella* sp.) compared to Treatment II (*Nannochloropsis* sp.) and Treatment III (*Chaetoceros* sp.) throughout the cultured period.

Alam (2004) reported that reproductive capacity of the rotifers fed on Treatment I (*Chlorella* sp.) was 38% compared to Treatment II (*Nannochloropsis* sp.) at 24%. The reproductive capacity of the rotifers fed Treatment I (*Chlorella* sp.) showed a significantly higher value ($p < 0.05$) compared to Treatment II (*Nannochloropsis* sp.) and Treatment III (*Chaetoceros* sp.). The number of eggs produced by a female is dependent on the species of food algae (Hirayama *et al.*, 1979).

Epp and Winston (1978) described that changes in pH from 6.5 to 8.5 had no effect on rotifer activity or metabolism effect on rotifer activity or metabolism. The result of the present study indicated that temperature, pH, dissolved oxygen, and salinity remained consistently stable throughout the rotifer culture period. These parameters showed no significant changes. Therefore, the water quality in the present study was considered suitable for rotifer culture.

The effects of different microalgae diets on the culture of rotifer *Brachionus* sp. indicated that the suitability of a particular microalgae species in rotifer culture largely depends on its nutritional quality. Hirata (1979) described that marine *Chlorella* sp. was considered to have better nutritional value than other species in Japan. In the present study, marine *Chlorella* significantly enhanced the population growth of rotifers."

Conclusion

The present study showed that the rotifer *Brachionus* sp. grows best when fed *Chlorella* sp.. Successful fingerling production in fish hatcheries, aimed at stocking grow-out production systems, depends heavily on having suitable zooplankton. *Chlorella* sp., serving as a marine rotifer food source, is preferred not only for its easy mass cultivation but also because it imparts beneficial nutritional characteristics to marine rotifers. This makes them excellent food for rearing various marine fish larvae. Mass-producing the rotifer *Brachionus* sp. will enhance the optimal growth and survival of fish larvae, fry, and fingerlings. The successful production of early fish and shrimp larvae in hatchery largely depend on the availability of suitable live food. The mass production of the rotifer by the use of *Chlorella* sp. will be benefitted for the production of fish and shrimp larvae in aquaculture farms.

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