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Comparison of growth performance of live feed microalgae and rotifer (*Brachionus sp.*) under different feeding medium in outdoor culture condition

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Abstract

Live feed is the basic food source and nutrient security for successful seed production of any commercially important aquaculture species of fishes, mollusks and crustaceans. Both plant and animal originated aquatic microscopic organisms are generally termed as live food. They are the basic food items in early stages (larval stage) of life cycle due to small sizes, easy digestibility and enriched in nutrients. The *Nannochloropsis sp.*; *Nannochlorum sp.* and *Tetraselmis sp.* are rich with relatively high content of essential fatty acids in comparison to other marine algae. Likelihood, the rotifer *Brachionus sp.* is ideal feed item for brackishwater finfish and mud crab larvae rearing due to its special features like rapid reproduction, slow movement, suitable size and easy digestion by the newly hatched larvae. The present study is the report on comparison of growth performance of live feed (microalgae and rotifer) in outdoor culture condition. The study was conducted at the hatchery complex of Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna. In outdoor culture condition the growth pattern obtained for three microalgae were *Tetraselmis sp.> Nannochlorum sp.> Nannochloropsis sp.* Highest average growth $6.87\times10^6\pm1.97\times10^6$, $6.91\times10^6\pm1.69\times10^6$ and $6.23\times10^6\pm1.53\times10^6$ was observed for *Nannochloropsis sp.*, *Nannochlorum sp.* and *Tetraselmis sp.* respectively in trial 3. Average growth of rotifer (*Brachionus sp.*) found highest 189±18.10 ind./ml at combined media of yeast and microalgae and lowest growth 119.67±17.60 ind./ml noticed with baker's yeast media. *Nannochloropsis sp.* contains more energy among all microalgae but *Tetraselmis sp.* carry highest level of protein. The highest level of protein 56.3±0.18% found in rotifer enriched with microalgae+fish oil and lowest protein content noticed with microalgae enrichment media. So, research finding suggests that, treatment T_3 would be the best recommendations for rotifer culture.

Keywords: Microalgae; Rotifer; Growth performance; Plastic jars; Bangladesh

1. Introduction

Live feeds are the chief item in the diet of cultured fish larvae and they are of particular significance when nurturing marine fish larvae of the altricial type. Altricial larvae are those that remain in a relatively immature state until the yolk sac is exhausted. At first-feeding the gastrointestinal system is still rudimentary, deficient of a stomach, and much of the protein digestion takes place in hindgut epithelial cells [1]. Such a digestive system is in most cases incompetent of processing articulated diets in a manner that allows survival and growth of the larvae comparable to those fed live feeds. In fact, despite recent progress in the development of inert diets for fish larvae [2-4], feeding of most species of interest for aquaculture still relies on live feeds during the early life stages. Brackishwater and marine hatcheries rely upon live foods as the main source of feeds for larvae of the target species being cultured. Though micro encapsulated and other inert diets have been developed for some commercial species (*e.g. Penaeus monodon, Scylla serrata*), there is still a

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requirement for microalgae in the early stages and a combination of inert diets along with Artemia [5]. Live food organisms include all plants (phytoplankton) and animal (zooplankton) lives grazed upon by economically important fishes. Phytoplanktons are generally eaten by zooplankton. Thus, phytoplankton forms the basis of the food chain. Live foods are able to swim in water column and are constantly available to fish and shellfish larvae are likely to stimulate larval feeding response [6]. In the natural food web, zooplankton constitutes a major part of the diet for marine fish larvae and it is generally believed that copepods can meet the nutritional requirements of fish larvae [7]. Both plant and animal originated aquatic microscopic organisms, also termed as live food. Live feed is the basic food source and nutrient security for successful seed production of any commercially important aquaculture species of fishes, mollusks and crustaceans. They are the basic food items in early stages of life cycle due to small sizes, easy digestions and enriched in nutrients. Plant originated live food are known as phytoplankton (microalgae) are primary producers in the food web [8]. Whereas, animal originated microorganism are the secondary producer those grazes on phytoplankton. Hence, the live food supports for better survival and growth of fish, prawn and crustacean larvae. They are also considered as water purifiers since they consume soluble nutrients, bacteria and detritus. The culture and production of adequate nutritive live food organisms is considered as the heart of the hatchery for sustainable seed production [9]. However, production of available nutritive live foods is a challenge for the operation of hatchery in a sustainable manner. The *Nannochloropsis sp.*; *Nannochlorum sp*. and *Tetraselmis sp.* are rich with relatively high content of essential fatty acids in comparison to other marine algae [10]. Likelihood, the rotifer *Brachionus sp.* is ideal feed item for brackishwater finfish and mud crab larvae rearing due to the suitable sizes and easy digestion by the newly hatched larvae [11]. Very often, ordinary grown live feeds did not contain available nutrients to support the survival and growth of larvae, especially for the crustaceans. However, live feeds are needed to be enriched to enhance the qualitative and quantitative nutrients, especially the essential fatty acids of the HUFA's [12]. So, scaling up of live feed culture is the prime need for successful operation of seed production of commercially important marine and brackishwater species like, mullet (*Mugil cephalus*), parse fish (*Chellon subviridis*), mud crab (*Scylla sp.*), etc.

2. Methodology

2.1. Study Location and Duration

The proposed research was carried out in the live feed laboratory of Bangladesh Fisheries Research Institute (BFRI), Brackishwater station, Paikgacha, Khulna 2017-18. A 10 days experiment was implemented in the hatchery complex of Brackishwater Station at Paikgacha, Khulna. The experiment was repeated for thrice. Performance of live feed were evaluated from the cell density and nutrient content especially proximate composition and levels of essential fatty acid contents such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

2.2. Experimental design and culture medium for live feed

Three live feed microalgae (*Nannochloropsis sp*., *Nannochlorum sp*. and *Tetraselmis sp.*) species were cultured under indoor and outdoor condition in order to compare their growth in F2 media and inorganic fertilizer. F2 media is a stock solution prepared by mixing the chemicals according to [13]. For outdoor culture of microalgae, 0.5 ml F2 medium/20 L filtered seawater (25-30 ppt), inoculated with microalgae (0.5×10^6/ml) applied at the rate of was 5-10% of culture volume. Light intensity was maintained from 1500 to 2000 lux for 24 hours with a constant temperature of 20-25 °C. Duration of culture was 6-14 days. For mass culture of microalgae (0.5×10^6/ml), 10-30% of total culture volume was inoculated in 200-2000 L of 25-30 ppt salt water for a period of 5-14 days under day-light photoperiod condition. Culture of live feed (microalgae and rotifer) were performed under the following experimental condition mentioned in Table 1.

Table 1 Experimental design for culture of live feed (microalgae)

Treatment	Replications	Species	Protocol	Culture Vessel	Inoculums Density	Media
T1	3	Nannochloropsis *sp.*	Outdoor	300L white fibre glass tank	0.5×10^6/ml	F2
T2	3	Nannochlorum *sp.*				
T3	3	Tetraselmis *sp.*				

For culture of rotifer, 15-20 ind./ml of culture volume was inoculated in 200-1000 L of 25-30 ppt salt water for a period of 7-10 days. Rotifers was fed with as per the design of experiment mentioned in Table 2. Harvesting of rotifer was done with 50-65 µm plankton net.

Table 2 Experimental design for culture of live feed (rotifer).

Treatment	Replication	Media/feeding	Protocol	Culture vessel	Inoculum Density
T1	3	Baker's yeast	outdoor	300L plastic jars	20/ml
T2	3	Microalgae			
T3	3	Yeast + microalgae			

2.3. Nutritional Value of live feed (microalgae and rotifer)

The nutritional value of any algal species for a particular organism depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. Proximate composition of three microalgae species were analyzed from Bangladesh council of scientific and industrial research (BCSIR), science laboratory, Dhaka. The nutritional composition of microalgae species were done according to the protocol of [14]. On the other hand, nutritive value of rotifer (*Brachionus sp.*) enriched with different enrichments media were evaluated as per design described in Table 3.

Table 3 Experimental design for enrichment of rotifers (*Brachionus sp.*)

Treatment	Replication	Media/feeding	Protocol	Culture vessel	Inoculum Density
T1	3	Microalgae	outdoor	300L plastic jars	300-400/ml
T2	3	Commercial diet			
T3	3	Fish oil (Selco)			
T4	3	Microalgae + fish oil			

Rotifer was cultured in baker's yeast, harvested and enriched with different enrichment media. After 6-8 hours of enrichment, rotifers were harvested further and the nutritive value (proximate composition and fatty acid contents) was investigated.

2.4. Data Analysis

The collected data and information has been statistically analyzed with MS Excel and presented as figures and tables to express the research findings in a meaningful way.

3. Results and discussion

3.1. Growth performance of microalgae species cultured in outdoor condition

For the microalgae species *Nannochloropsis sp.*, *Nannochlorum sp.* and *Tetraselmis sp.* were cultured under outdoor condition. All three species started cell division immediately after inoculation and reached to the peak on 9th day of culture. The stationary phase was observed for 9-14 days and then started to collapse. In outdoor condition highest average growth $6.87\times10^{6}\pm1.97\times10^{6}$, $6.91\times10^{6}\pm1.69\times10^{6}$ and $6.23\times10^{6}\pm1.53\times10^{6}$ was observed for *Nannochloropsis sp.*, *Nannochlorum sp.* and *Tetraselmis sp.* respectively in trial 3. And lowest average growth $2.99\times10^{6}\pm1.45\times10^{6}$, $3.40\times10^{6}\pm1.11\times10^{6}$ and $3.48\times10^{6}\pm1.36\times10^{6}$ was observed for *Nannochloropsis sp.*, *Nannochlorum sp.* and *Tetraselmis sp.* respectively (Table 4). The range of temperature: 20-30°C; light intensity: 2500-8000 lux and salinity: 0-36 ppt. are generally used to culture *Nannochloropsis oculata* [15].

Table 4 Growth performance (cell/ml) of microalgae species under outdoor culture condition**.**

Number of Trial	*Nannochloropsis sp.*	*Nannochlorum sp.*	*Tetraselmis sp.*
Trial 1	$2.99\times10^6\pm1.45\times10^6$	$3.40\times10^6\pm1.11\times10^6$	$3.48\times10^6\pm1.36\times10^6$
Trial 2	$4.99\times10^6\pm2.08\times10^6$	$5.01\times10^6\pm1.68\times10^6$	$5.08\times10^6\pm1.62\times10^6$
Trial 3	$6.87\times10^6\pm1.97\times10^6$	$6.91\times10^6\pm1.69\times10^6$	$6.23\times10^6\pm1.53\times10^6$

The optimal salinity is 22–25 ppt. for *N. oculata* [16]. The microalgae culture condition of our study was within that range. All the microalgae species were collapsed sharply at 14th days of culture period. Growth performance of *Tetraselmis sp.* was highest in all trial with respect to other two microalgae species (figure 1). From figure 1 it is clearly observable that the order of growth pattern of three microalgae were *Tetraselmis sp.>Nannochlorum sp.>Nannochloropsis sp.* In Singapore, [16] used an initial phytoplankton density of $3\text{-}4\times10^6$ cells/ml *N. oculata* to stock 3 L and 20 L bags. He found that culture of 3 L bags takes seven days, giving a final density of $2.00\text{-}2.50\times10^7$ cells/ml for *N. oculata*. He also found that 20 L cultures take five days to reach their maximum density of $1.80\text{-}2.00\times10^7$ cells/ml.

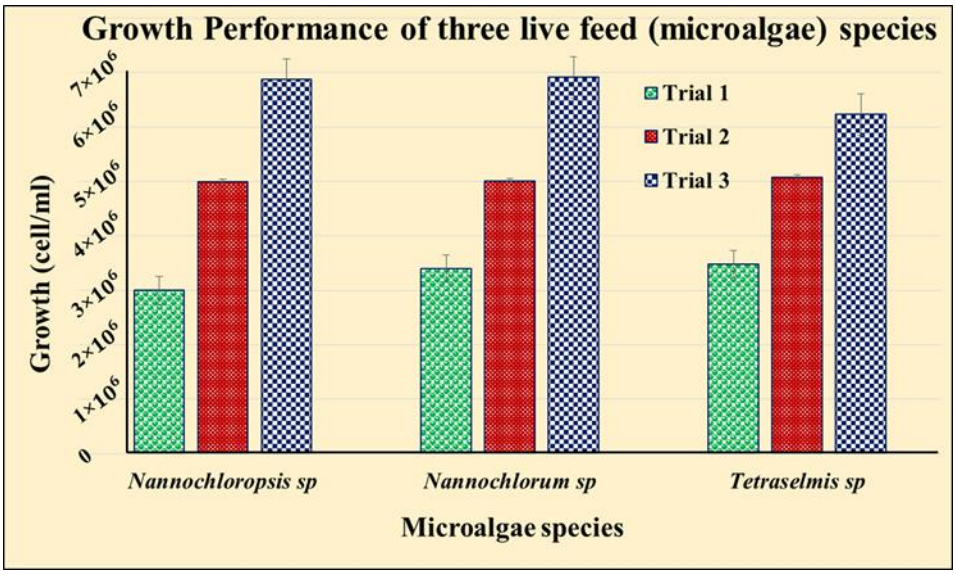

Figure 1 Growth Performance of three live feed (microalgae) under outdoor culture condition

3.2. Growth performance of Rotifer (*Brachionus sp.*) in different medium

Brachionus sp. was scaled up under outdoor culture condition in 300 liter plastic jars in 20/ml inoculum density with different media. Average growth of rotifer (*Brachionus sp.*) was highest 189±18.10 ind/ml at combined media of yeast and microalgae in trial 1 comparison with all treatment and trial (figure 2). Microalgae diet media yielded 158.33±21.60, 148.33±15.60 and 151.67±22.30 ind./ml in trial 1, trial 2 and trial 3 respectively, lowest growth rate was noticed with baker's yeast 119.67±17.60 ind./ml for trial 2. In 300 liter plastic jars with 20 /ml inoculum density, the growth performance of rotifer is better in the combined media of yeast and microalgae than microalgae diet and baker's yeast media. Growing rotifers (*Brachionus plicatilis*) at the temperature above 26°C and water salinity of 25 ppt for optimum growth suggested by [17]. A density of 20 rotifer/mL of culture should be introduced and fed with baker's yeast at a rate of 0.25-0.50 g per million of rotifers twice a day [17-18]. For up-scaling culture, rotifers (*Brachionus plicatilis*) are solely fed by *Nannochloropsis sp.* from 100 ml to 1000L and inoculated about 10-20 rotifer/ml [19].

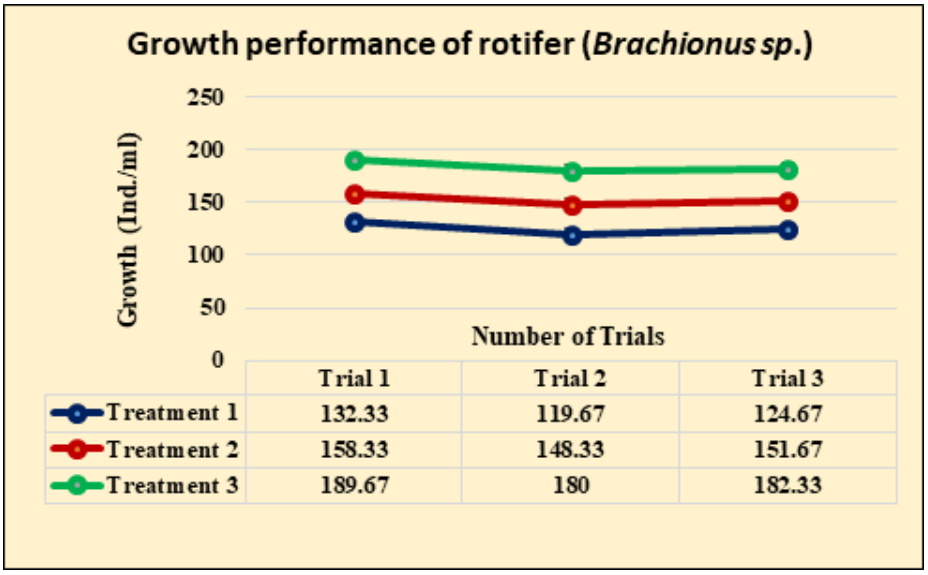

Figure 2 Growth performance of rotifer (*Brachionus sp*.) under different feedings.

3.3. Proximate composition of live feed (microalgae)

The nutritional value of any algal species for a particular organism depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. The gross composition of three species of micro-algae is compared in Table 5. Although there are marked differences in the compositions of the micro-algal classes and species, protein is always the major organic constituent, followed usually by lipid and then by carbohydrate. Expressed as percentage of dry weight, the range for the level of protein, lipid, and carbohydrate are 18-18.20%, 60-63%, 10-18% and 9-12% respectively. *Tetraselmis sp.* contains highest level of protein content 63±0.05% followed by *Nannochloropsis sp.* and *Nannochlorum sp.* (62±0.11 and 60±0.09)% respectively (Table 5). The highest lipid content obtained for *Nannochloropsis sp.* 18±0.02% followed by *Tetraselmis sp.* and *Nannochlorum sp.* (11±0.02 and 10±0.01)% respectively. *Nannochloropsis sp.* contains maximum calories in 100g dry weight of microalgae at a rate of 450±0.20 followed by *Tetraselmis sp.* and *Nannochlorum sp.* (390±0.18 and 385±0.16)% respectively (Table 5).

Table 5 Proximate composition of live feed (microalgae) cultured in outdoor condition**.**

Parameters	***Nannochloropsis sp.***	***Nannochlorum sp.***	***Tetraselmis sp.***
Dry Weight (%)	18.2±0.01	18.0±0.02	18.2±0.01
Calories (100g dry wt. algae)	450±0.20	385±0.16	390±0.18
Protein (%)	62±0.11	60±0.09	63±0.05
Lipid (%)	18±0.02	10±0.01	11±0.02
Carbohydrate (%)	9±0.01	12±0.01	11±0.01
Ash (%)	10±0.03	12±0.04	15±0.02
Vitamin C (%)	0.85±0.01	0.20±0.01	0.25±0.01
Chlorophyll A (%)	0.89±0.01	1.40±0.01	1.42±0.01

A considerable high protein percentage (62 and 55%, d.w.), respectively, in *Rhodomonas lens* obtained in semi-continuous culture found by [20-21]. Whenever microalgae are used as a direct food source or as an indirect food source, in the production of rotifers, Artemia or copepods, growth of the animals is usually superior when a mixture of several microalgal species is used [22]. The dry matter composition of microalgae is highly variable, even within a given species, with protein contents ranging from 12-35%, lipid from 7.2-23% and carbohydrates from 4.6-23% [22]. Microalgae grown to late-logarithmic growth phase typically contain 30-40% protein, 10-20% lipid and 5-15% carbohydrate [23]. The findings of our study correlates with the finding of above mentioned literature.

3.4. Nutritive value of rotifer (*Brachionus sp.*) enriched with different enrichment media

The nutritional value of rotifers for larval fish depends on the rotifer's food source. The feed of rotifers appears to the key element in their mass production as well as proximate composition. As in other zoo planktonic prey, rotifer biochemical composition is of primary importance for larval nutrition. The lipid and essential fatty acids profile is relatively modifiable by dietary manipulation [24-26]. Before the analysis of proximate composition the rotifer was enriched with different enrichment media as mentioned in methodology. The highest level of protein 56.3±0.18% found in rotifer enriched in combination with microalgae and fish oil followed by 51.4±0.29%, 40.5±0.23% and 38.5±0.83% for rotifer enriched with fish oil, commercial diet and microalgae respectively (Table 6). One the other hand, maximum lipid 28.5±0.16% found in rotifer enriched with fish oil followed by 24.6±0.22%, 22.6±0.27% and 21.2±0.19% for rotifer enriched with, commercial diet, microalgae+fish oil and microalgae respectively (Table 6).

Table 6 Proximate composition of live feed (rotifer) cultured in outdoor condition.

Parameters	Microalgae	Commercial diet	Fish oil (Selco)	Microalgae + fish
Protein (%)	38.5±0.83	40.5±0.23	51.4±0.29	56.3±0.18
Lipid (%)	21.2±0.19	24.6±0.22	28.5±0.16	22.6±0.27
Carbohydrate (%)	27.8±0.20	24.3±0.10	13.1±0.05	11.8±0.08
Ash (%)	7.9±0.08	6.8±0.12	3.06±0.04	3.9±0.10
Dry matter (%)	4.6±0.20	3.8±0.20	3.94±0.56	5.4±0.10
Moisture (%)	95.4±0.18	96.2±0.15	96.06±0.22	94.6±0.20

According to [27], rotifer's protein content ranges between 28-63%, lipid from 9- 28%, and carbohydrate from 10.5-27% of the dry weight (DW). Rotifer lipids have a high phospholipid content (34-43% of total lipid), and 20-55% of triacylglycerols [27]. The mean protein level in rotifers fed with different types of feeds varied between 29.19±0.23% and 46.02±0.23% with the highest protein percentages in rotifers cultured with *I. galbana* (46.02±0.23%) and the lowest protein level in rotifers fed *Nannochloropsis sp.* along with *B. licheniformis* (29.19±0.23%) reported by [28]. Protein content could vary with the type of food and growth phase and the values ranged from 34-52% showed by [29]. A lower lipid level for rotifers fed with yeast alone and a lipid content of 13.1% along with *Chlorella sp.* for 24 hrs reported by [30]. Proximate composition of rotifer in our study correlates with the above mentioned literature.

4. Conclusion

From this experiment, it can be concluded that treatment T_3 (yeast+microalgae) feeding media is advisable for rotifer production due to higher production and nutritional composition. Application of this findings might be developed the live feed production especially in the brackishwater hatcheries and extremely helpful for rearing of commercially important brackishwater finfishes and shellfishes.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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