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Comparative study of growth performance of three live feed (Microalgae) species in indoor culture condition

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ABSTRACT

The present study focused on the comparison of growth performance of three species of live feed (microalgae) in a fixed nutrient medium in indoor culture condition. The study was conducted at the hatchery complex of Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna. The order of growth pattern obtained for three microalgae were *Tetraselmis* sp.>*Nannochlorum* sp.>*Nannochloropsis* sp. In the three consecutive trials, highest average growth $3.47\times10^6 \pm 0.8\times10^6$, $5.08\times10^6 \pm 0.9\times10^6$ and $6.91\times10^6 \pm 1.0\times10^6$ (cells/ml) (Mean± SEM) were observed for *Nannochloropsis* sp., *Nannochlorum* sp. and *Tetraselmis* sp. respectively. Indoor culture condition is therefore, advantageous for *Tetraselmis* culture in comparison to the other studied species.

INTRODUCTION

Microalgae are microscopic unicellular phytoplankton having less than 10µm size. These floating planktons has got immense value as an aquaculture live feed because these are the predominant component of first tropic level in the aquatic food chain. 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 (larval stage) of life cycle due to small sizes, easy digestions and enriched in nutrients and so, brackishwater and marine hatcheries rely upon these as the main source of feeds for larvae of the target species being cultured. Most micro algae (live food) are rich sources of essential fatty acids; vitamins such as B_{12}, B_6, B_1, biotin, riboflavin, nicotinic acid, pantothenate, C, E and A; chlorophyll 'a' &'b' and carotenoids and these plankton plays a vital role in aquaculture to meet the nutritional requirements of the larvae as well as for bio-encapsulation (Madhu and Madhu, 2009). *Tetraselmis*and *Nannochloropsis* sp. contains high amounts of Omega 3 Fatty Acids (Chew et al., 2018). Protein in microalgae is considered to be of good quality with amino acid profiles comparable to those of other reference food proteins (Gallardo et al., 2002). Micro algae together with bacteria have an important role in oxygen balance and water purification in fish culture system (Madhu and Madhu, 2009). Microalgal use in the fish hatchery is known as the "green water technique" wherein microalgae are added directly to larval rearing tanks along with the zooplankton that serve as food for the larvae. Green water larviculture has been shown to increase larval quality of cultured fish species (Conceicao et al., 2010; Hemaiswarya et al., 2011; Sanchez et al., 2012). Live microalgae also inhibit bacterial growth (Mezriui et al., 1994) and this is an added advantage they have over artificial feeds such as microencapsulated feeds. Vibrio inhibitory activities of green algae *Tetraselmis suecica* has been reported in vitro (Regunathan and Wesley,

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2004). As the production of microalgae is considered expensive (Walsh et al., 1987), several approaches have been attempted to replace microalgae in the hatchery with artificial diets but microalgae still cannot be replaced fully with other types of feed, such as microbound and other enrichment diets (Sanchez et al., 2012; Ma and Qin, 2012). The culture and production of adequate nutritive live food organisms is considered as the heart of the hatchery for sustainable seed production. Gradual shipment of aquaculture practice towards selected marine species from the freshwater finfish, particularly in coastal water is also demanding the assurance of high quality target species like, mullet (*Mugil cephalus*), parse fish (*Chellon subviridis*), mud crab (*Scylla* sp.) and shrimps. Therefore scaling up of live feed culture and production has become a prime requirement for the last few years. On the other hand, the growth rate and the cell content of microalgae are highly dependent on the cultivation method and ultimately it plays a significant role in the productivity of microalgae Chew et al. 2018). So, Species specific culture method development for sustainable and cost effective production is a crying need today. The objective of the present study was to compare the growth performance of three species of live feed (microalgae) *Nannochloropsis* sp., *Nannochlorum* sp. and *Tetraselmis* sp. in a fixed nutrient medium in indoor culture condition.

METHODOLOGY

Study location and duration

The research was conducted in the live feed laboratory of Bangladesh Fisheries Research Institute (BFRI), Brackishwater station, Paikgacha, Khulnafrom 1st April 2017 to 30th September 2018. A 15 days experiment was implemented in the hatchery complex of Brackishwater Station at Paikgacha, Khulna. The experiment was repeated for thrice. Performance of live feed was evaluated from the cell density.

Experimental design and culture medium for live feed

Three live feed microalgae (*Nannochloropsis* sp., *Nannochlorum* sp. and *Tetraselmis* sp.) species were cultured under indoor condition in order to compare their growth in F_2 media. F_2 media is a stock solution prepared by mixing the chemicals according to Guillard (1975). 0.5 ml/L of F_2 media (Table 1) were added in 2L of filtered seawater (25-30 ppt) in the culture vessel. Light intensity was maintained from 1500 to 2000 Lux for 24 hours with a constant temperature of 20-25 °C. Duration of culture was 15 days. Cultures of live feeds (microalgae) were performed under the experimental condition mentioned in Table 1.

Table 1: Experimental design for culture of live feed (Microalgae).

Treatment	Replication	Species	Protocol	Culture vessel	Inoculum density/volume	Media
T_1	3	*Nannochloropsis* sp.	Indoor	2 liter conical flask	5-10% of culture volume	F_2 medium
T_2	3	*Nannochlorum* sp.				
T_3	3	*Tetraselmis* sp.				

F_2 media = Total 5 ml of A, B, C. Where,
A = [75 gm Sodium Nitrate+5 gm of Sodium Phosphate]
B = [(180 gm Manganese Chloride + 22 gm Zinc Sulphate+10 gm Copper Sulphate+10 gm Cobalt Chloride+ 6gmSodium Molybdate) = 1ml+4.36 gm EDTA+3.15 gm Ferric Chloride]
C = [20gmThiamine Hydrochloride+100gmD-Biotine+100gm Cianocobalamine]

Statistical Analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) v. 20.0 for windows (SPSS, SAS Institute Inc. Cary, USA). The data were analyzed to determine the descriptive statistics such as Mean, Standard Error of mean (SEM), Minimum &Maximum value and Ranges of variables. One way ANOVA was performed using 5% level of significance. Microsoft excel (2013) was used to plot graphs.

RESULTS AND DISCUSSION

The microalgae species *Nannochloropsis* sp., *Nannochlorum* sp. and *Tetraselmis* sp. were cultured under indoor condition. . All the three species started cell division immediate after the 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. *Nannochloropsis* sp. showed a growth range 1.33×10^6 to 4.0×10^6cells/ml (Figure 2) in 1st trial. In 2nd and 3rd trial, this species showed a growth range 2.13×10^6 to 4.23×10^6cells/ml and 2.13×10^6 to 4.85×10^6cells/ml, respectively. On the other hand, another observed species *Nannochlorum* sp. appeared at the growth range 3.33×10^6 to 7.33×10^6cells/ml, 3.95×10^6 to 6.95×10^6cells/ml and 3.76×10^6 to 6.9×10^6cells/ml (Figure 3) in 1st, 2nd and 3rd trial respectively. The 3rd experimental species *Tetraselmis* sp. flourished and showed a growth range 5.28×10^6 to 9×10^6cells/ml, 5.75×10^6 to 8.85×10^6cells/ml and 4.56×10^6 to 8.95×10^6cells/ml (Figure 4) in 1st, 2nd and 3rd trial respectively. Lim (1991) reported that, the culture grew from 5.00 x 10^6 cells/ ml to 6.70 x 10^7 cells/ ml in six days, and to 2.13 x 10^8 cells/ ml in 20 days.

All the microalgae species collapsed sharply at 14th days of culture period. Growth performance of *Tetraselmis* sp.was highest in all trials in comparison to other two microalgae species (Figure 1, Table 2). From figure 1, it is also clearly noticeable that the order of growth performance of three microalgae were *Tetraselmis* sp.>*Nannochlorum* sp. >*Nannochloropsis* sp. The highest cell density found in *Tetraselmis sp.* ranged from $6.23\times10^6 \pm 1.4\times10^6$cells/ml to $6.91\times10^6 \pm 1.0\times10^6$cells/ml. On the other hand, lowest cell density noticed in *Nannochloropsis* sp. fluctuated from $3.00\times10^6\pm 0.8\times10^6$ cells/ml to $3.47\times10^6 \pm 0.8\times10^6$cells/ml. There found no significant difference among the species growth (cells/ml) in all the three trials (ANOVA, $p>0.05$). In a study conducted by Lee et al. (2011), the potential of 5 different marine microalgae species was researched by measuring the dry cell weight, specific growth rate, biomass productivity, oil content and fatty acid composition of the microalgae. Overall, *Tetraselmis suecica*s howed the most potential with rapid growth rate and nutrient uptake for biomass production. The reason why *T. suecica* is able to perform well in saltwater is due to the presence of Na + pumps and its highly adaptable osmoregulatory mechanism to cope with rapid and gradual changes in salinity over a wide range (Kumar et al, 2015). In laboratory condition, cell count 5.7 ± 0.40 (cells/mlx 10^6) was found in the species *Nannochloropsis oculata* which was less than the cell count found in outdoor condition (Kumar et al, 2015). 308.0 ± 11.5 (cells/ml x 10^6) cell count was found after 21 days at 30 ppt salinity and 25˚C in the species *Nannochloropsis oculata* (Cho et al., 2007). *Tetraselmis striata* had a higher areal biomass productivity, specific growth in modified medium (Boopathy et al., 2020) which coincides with our findings that this species can be economically cultivated feasibly compared to the other two studied species in simple inorganic seawater media using industrial-grade chemicals.

Table 2: Average Growth performance (cells/ml) (Mean± SEM) of studied species in three trial under indoor culture condition

Number of Trial	*Nannochloropsis* sp. (cells/ml)	*Nannochlorum* sp.(cells/ml)	*Tetraselmis* sp.(cells/ml)
Trial 1	$3.00\times10^6\pm 0.8\times10^6$	$5.00\times10^6\pm 1.2\times10^6$	$6.87\times10^6\pm 1.1\times10^6$
Trial 2	$3.40\times10^6\pm 0.6\times10^6$	$5.01\times10^6\pm 1.0\times10^6$	$6.91\times10^6\pm 1.0\times10^6$
Trial 3	$3.47\times10^6\pm 0.8\times10^6$	$5.08\times10^6\pm 0.9\times10^6$	$6.23\times10^6\pm 1.4\times10^6$

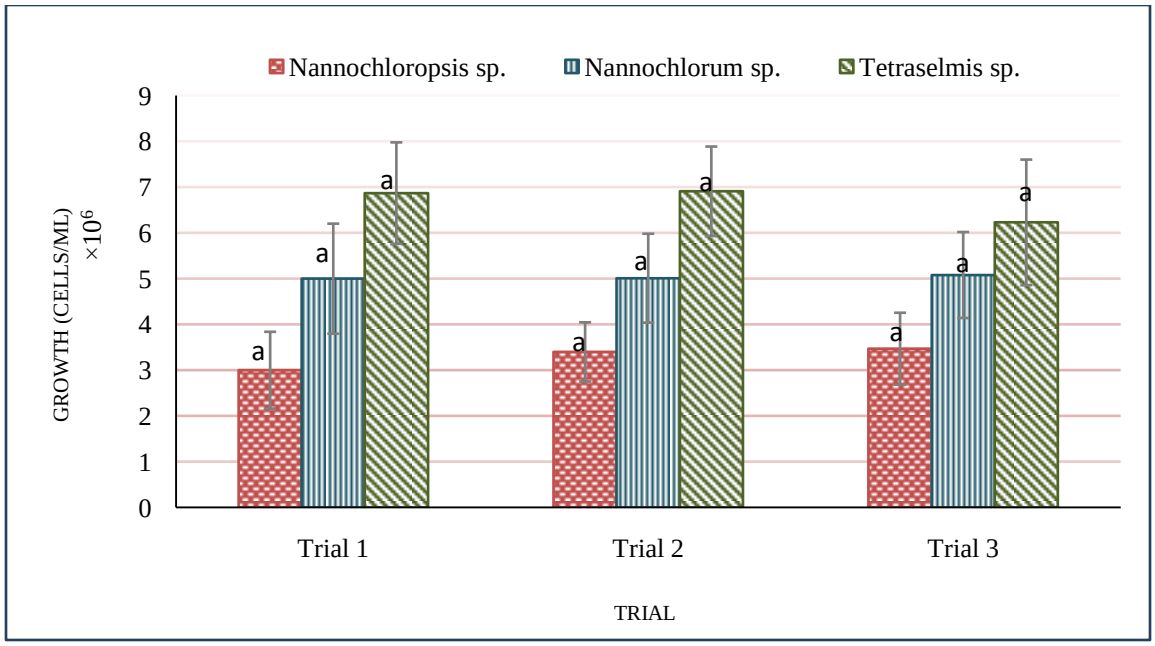

Figure 1: Comparison of the Average Growth performance of three microalgae species in three trial under indoor culture condition.Bars (Mean± SEM) with similar letters illustrated no significant difference (ANOVA, p>0.05).

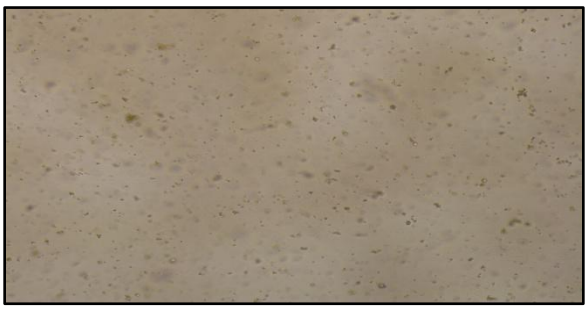

Figure 2: Microscopic view of *Nannochloropsis* sp. in 2 L conical flask

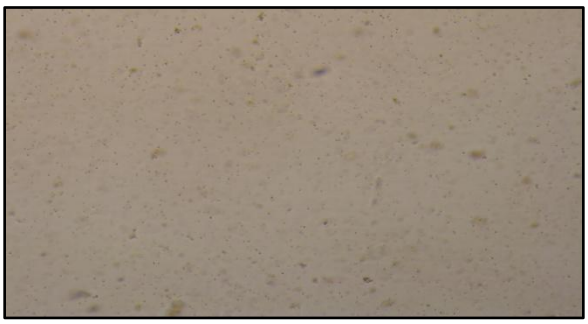

Figure 3: Microscopic view of *Nannochlorum* sp. in 2 L conical flask

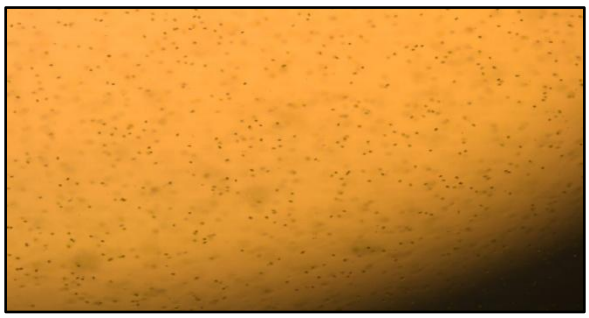

Figure 4: Microscopic view of *Tetraselmis* sp. in 2 L conical flask

CONCLUSION

Microalgae remain an important component of the aquaculture production chain, particularly for hatchery white shrimp feed, despite expensive culture installation and high production cost. From this experiment, it can be concluded that the feasibility of achieving better production of *Tetraselmis* sp. in indoor condition is comparatively higher ($6.91\times10^6 \pm 1.0\times10^6$cells/ml) than the other two studied species of microalgae. Further research should be conducted using another experimental setup with different environmental parameters & culture condition to obtain the highest production feasibility of another

two species. More research on other potential microalgal strains is recommended. This is mainly driven by the concern over sustainability and productivity of local farms.

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REFERENCES

Winter School Course Manual on "Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish".30.12.2008 - 19.1.2009. CMFRI (Central Marine Fisheries Research Institute) Indian Council of Agricultural Research.

Chew KW, Chia SR, Show PL, Yap YJ, Ling TC, Chang JS. (2018). Effects of water culture medium, cultivation systems and growth modes for microalgae cultivation: A review. Journal of the Taiwan Institute of Chemical Engineers. (1–13).

Gallardo PP, Pedroza-Islas R, Garcia-Galano T, Pascual T, Rosal C, Sanchez A, Gaxiola G. (2002). Replacement of live food with microbound diet in feeding *Litopaneussetiferus*(Burkenroad) larvae. Aquaculture Research. 33:681-691.

Conceicao LEC, Yufera M, Makridis P, Morais S, Dinis MT. (2010). Live feeds for early stages of fish rearing. Aquaculture Research. 41(5):613-640.

Hemaiswarya S, Raja R, Kumar RR, Ganesan V, Anbazhagan C (2011). Microalgae: a sustainable feed source for aquaculture. World Journal of Microbiology and Biotechnology 27:1737-1746.

Sanchez DR, Fox JM, Gatlin III R, Lawrence AL (2012). Dietary effect of fish oil and soybean lecithin on growth and survival of juvenile *Litopenaeusvannamei*in the presence or absence of phytoplankton in an indoor system. Aquaculture Research, 45:1367-1379.

Mezriui N, Oudra B, Oufdou K, Hassani L, Loudiki M and Darley J (1994). Effect of microalgae growing on wastewater batch culture on *Escherichia coli* and *Vibrio cholera* survival. Water Science & Technology, 30:295–302.

Regunathan C and Wesley SG (2004). Control of vibrio spp. in shrimp hatcheries using the green algae *Tetraselmis suecica*. Asian Fisheries Science, 17:147–158.

Walsh DT, Withstandley CA, Kraus RA and Petrovits EJ. (1987). Mass culture of selected marine microalgae for the nursery production of bivalve seed. Journal of Shellfish Research, 6:71-77.

Ma Z and Qin JG (2012) Replacement of fresh algae with commercial formulas to enrich rotifers in larval rearing of yellowtail kingfish *Seriolal alandi* (Valenciennes, 1833). Aquaculture Research, 45:949-960.

Guillard RR (1975). Culture of phytoplankton for feeding marine invertebrates. In Culture of marine invertebrate animals. (29-60).

Lim LC (1991). An overview of live feeds production systems in Singapore. In W. Fulks& K.L. Main (Eds), Rotifer and Microalgae Culture Systems, Proceedings of U.S. – Asia Workshop (pp. 203-220). Redmond, Washington: Argent Laboratories.

Lee SJ, Go S, Jeong GT, Kim SK. (2011). Oil production from five marine microalgae for the production of biodiesel. Biotechnology and Bioprocess Engineering, 16(3):561–566.

Kumar K, Mishra SK, Shrivastav A, Park MS, Yang J-W. (2015). Recent trends in the mass cultivation of algae in raceway ponds. Renewable and Sustainable Energy Review, 51:875–885.

Banerjee S, Hew WE, Khatoon H, Shariff M, Yusoff FM. (2011). Growth and proximate composition of tropical marine *Chaetoceros calcitrans* and *Nannochloropsis oculata* cultured outdoors and under laboratory conditions. African Journal of Biotechnology, 10(8):1375-1383.

Cho SH, JI UC, Hur SB, Bae J, Park IS, Song, YC. (2007).Optimum temperature and salinity conditions for growth of green algae *Chlorella ellipsoidea* and *Nannochloris oculata*.Fisheries Science, 73: 1050–1056.

Boopathy BA, Jayakumar T, Chinnasamy S, Rajaram GM, Mohan N, Nagaraj S, Rengasamy R, Manubolu M, Sheu JR, Chang CC. (2020). Biomass and lipid production potential of an indian marine algal isolate *Tetraselmis striata* BBRR1. Energies, 13:341.