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Growth performances of three microalgal species in filtered brackishwater with different inorganic media

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Abstract
The growth of three microalgae species, *viz., Nannochloropsis oculata, Tetraselmis chui* and *Chaetoceros muelleri,* which are commonly used in aquaculture, was investigated using three different inorganic nutrient media: (i) Modified Guillard's f/2 medium (ii) Rix Mix medium and (iii) BFRI medium. Each microalgae species was cultured for 24 days in small- scale with initial inoculation density of $17x10^4$ cell /ml in the three media with triplicates. *N. oculata* cultured in modified Guillard's f/2 medium showed superior growth with a mean peak density of 221 ± 4.24 x 10^4 cell/ ml, to Rix Mix medium ($141\pm 10.54x10^4$ cell/ml) and BFRI medium (47 ± 4.94 x 10^4 cell/ml) on the 16^{th} day of culture at stationary phase. Considering the increase in cell density for 20 days of culture in Rix Mix medium, *C. muelleri* was significantly ($P<0.05$) highest than in other two media. *N. oculata* cultured in BFRI medium resulted in the poorest growth with a mean peak increase in density of 84 ± 9.19 x 10^4 cell/ml in 12 days of culture. However, with an increase in cell density, growth of *T. chui* (182 ± 6.26 x 10^4 cell/ml) was significantly ($P<0.05$) higher in BFRI medium than in modified Guillard's f/2 medium. The results of the present study suggest that *N. oculata* and *C. muelleri* can be grown very well in both the modified Guillard's f/2 medium and Rix Mix medium. Better growth of *T. chui* can be obtained while culturing either in BFRI and Rix Mix medium. These three nutrient media used in the present study may be useful for microalgae species culture for establishing green-water culture for suitable target zooplankton, and fish and crustacean larvae in marine and brackishwater hatcheries.

Key Words: Microalgae, Brackishwater, Nutrient media

Introduction

Microalgae are of great importance to the commercial culture of bivalves (larvae, juvenile and adults), crustaceans (mostly the early larval stages), zooplankton and to a lesser degree to finfish (larvae and /or adults). The algae are used to feed during mass culture of zooplankton such as rotifers, copepodes and brine shrimp. These zooplankton are the live food for late larval stages of crustaceans and fish. *N. oculata* can be useful in establishing the rotifer, *Brachionus plicatilis* culture protocol because of its high levels of vitamin B_{12} and eicosapentaenoic acid (EPA) content (Okauchi 1991). *C. muelleri* is high in HUFAs and its overall nutritional value is also high (Okauchi 1991). *T. chui,*

though nutritionally inferior to *N. oculata*, may also prove useful as a direct food in culturing organisms that are too small to accept rotifers (Wilkerson 1998).

Growth of phytoplankton in nature is mainly controlled by various environmental factors such as temperature, salinity, irradiance, stratification, water turbulence, etc. (Tomas 1978, Uye and Takamatsu 1990). However, nutrients are also very important environmental factors that influence the growth of any alga (Okaichi *et al.* 1989). Since the objective of growing microalgae in controlled condition is to obtain the highest density in the shortest possible time, utilization of natural seawater or freshwater without enrichment is not expected to yield significant results. Nutrient media used in the microalgae culture include Conway, Modified F or TMRL medium depending on the species cultured (Kongkeo 1991). Microalgae grow best in media with quite different primary and trace nutrient composition than natural seawater (Hoff and Snell 1989). Marine or brackishwater microalgal species require a culture medium with a chemical composition similar to that of seawater. Besides carbon, the requirement of principal nutrients for phytoplankton are nitrogen and phosphorus, in an approximate ratio of 6:1 by weight, respectively. Trace minerals and vitamins (especially B_{12} and thiamin and biotin) can also be added. These are necessary in most axenic cultures (Fulks and Main 1991) with addition of silicate for diatom. The need for microalgae to employ either as direct food for larvae and rotifers or in green water culture is important to consider in light of the results of trials to test growth of some well known microalgae species against varying microalgal culture media.

The effect of the three culture nutrient media, *viz.*, modified Guillard's f/2 medium, Rix Mix medium and BFRI medium on the growth of the three species of microalgae, *viz.*, *Nannochloropsis oculata*, *Tetraselmis chui* and *Chaetoceros muelleri*, which are commonly used in aquaculture, have not yet been studied in prevailing local condition. Considering the need for a thorough investigation with the suitable culture nutrient media for microalgae culture, the present study has been designed with the objective to determine the effect of three selected inorganic nutrient media on the growth performance of above three microalgae.

Materials and method

The culture of the microalgae in these trials was done in a temperature controlled (about 25 °C) microalgal laboratory at the Brackishwater Station of Bangladesh Fisheries Research Institute (BFRI), Paikgacha, Khulna. The culture was maintained in 1.5-liter mineral water bottles with sufficient aeration in axenic condition. The brackishwater of 25 ppt was filtered through 1.0μm cartridge filter to remove particulate and treated with 30 ppm chlorine at the rate of 0.1g/l and dechlorinated by using 0.175 g/l of sodium thiosulphate. As excess of sodium thiosulphate may reduce trace metal availability (Hoff and Snell 1989), the rate of application and period of dechlorination for 30 minutes were maintained properly. Media types tested were modified Guillard's f/2 medium, Rix Mix medium and BFRI medium. The standard nutrient medium used for algal culture was the Medium-F presented by Guillard and Ryther (1962). The Guillard's F medium is

suitable for the growth of most algae and is being used extensively (Fox 1983). The modified Guillard's f/2 medium used in this study was modification from Guillard's F medium for microalgal culture. The Rix Mix medium used by the private laboratory in North Queensland, Australia for stocks and medium size working culture (Braley 2001). The BFRI medium was prepared at the laboratory, which contain similar trace metals and vitamin solution of modified Guillard's f/2 medium. The composition of Rix Mix, BFRI medium and modified Guillard's f/2 medium is shown in Table1. The application rate of BFRI medium and modified Guillard's f/2 medium was 1 ml/L brackishwater and for Rix Mix it was 2 ml/L brackishwater. The application rate of sodium metasilicate was 1 ml to 1-L brackishwater for diatom only. The algal cells were inoculated into the bottles with equal initial concentration ($17x10^4$ cell/ml) for all species of microalgae. Aeration was moderate and the source was aquarium aerators. The bottles were set up approximately 15 cm from the two 'daylight' fluorescent tube light sources with a light intensity of about 2500 lux / m^2/ s.

Table 1. Composition of Rix Mix, BFRI medium and Modified Guillard's f/2 medium

Ingredients	Amount of ingredient(g or ml)		
	Rix Mix	BFRI medium	Modified Guillard's f/2 medium
Thrive *	99g/1-L dw	-	
Ammonium sulphate	27g/1-L dw	10 g/100 ml distilled water (dw)	
Urea	-	23 g/100 ml dw	-
TSP	-	2g/100 ml dw	-
Borax	-	1g/100 ml dw	-
Sodium nitrate	-	-	75g/1000 ml dw
Sodium dihydrogen orthophosphate	-	-	5g/1000 ml dw
Citric acid	-	-	16.8 g/1000 ml dw
Iron (ferric) citrate	-	-	3g/1000 ml dw
Sodium metasilicate	20 g/L dw	20 g/L dw	20 g/L dw
Copper sulphate	-	1g/100 ml dw	1g/100 ml dw
Zinc sulphate	-	2.2 g/100 ml dw	2.2 g/100 ml dw
Sodium molibdate	-	0.6g/100 ml dw	0.6g/100 ml dw
Manganese chloride	-	18g/100 ml dw	18g/100 ml dw
Cobalt chloride	-	1g/100 ml dw	1g/100 ml dw
Multi-Vitamin B	1- tab crushed/1-dw	-	-
Vitamin B_1	-	10g/100ml dw	10g/100ml dw
Vitamin B_{12}	-	0.05g/100 ml dw	0.05g/100 ml dw
Vitamin Biotin	-	0.05g/100 ml dw	0.05g/100 ml dw

*Thrive is common garden fertilizer in Australia having following chemical constituents:

Nitrogen (N) as Nitrate	3.0%	Sulphur (S) as sulphates	0.22%
Nitrogen (N) as ammonia form	2.6%	Copper (Cu) as copper sulphate	0.005%
Nitrogen as urea	21.4%	Zinc (Zn) as zinc sulphate	0.02%
Total Nitrogen (N)	27.0%	Boron (B) as sodium borate	0.005%
Phosphorus (P) as water soluble	5.5%	Manganese (Mn) as manganese sulphate	0.04%
Potassium (K) as potassium nitrate	9.0%	Iron (Fe) as chelated iron	0.18%
Magnesium (Mg) as magnesium sulphate	0.25%	Molybdenum (Mo) as sodium molybdate	0.002%
		Maximum Biuret	0.4%

There were three replications for each treatment i.e. for each nutrient medium. The total culture period was twenty-four days. Counts of algal cells were made at every four days interval using an improved Neubauer Haemocytometer by applying the methodology used by Hoff and Snell (1991). All the data were analyzed statistically using Analysis of Variance (ANOVA) and the significance test among mean values of treatments was done using Duncun's New Multiple Range Test (Zaman *et al.* 1982).

Results and discussion

Nannochloropsis oculata showed the best growth performance in modified Guillard's f/2 medium among the three media tested in this study (Fig. 1). The peak density of *N. oculata* in modified Guillard's f/2 medium was 221±4.42 x 10^4 cell/ml at the 16th day of culture before stationary phase. Lim (1991) found a peak density of 20-25 x 10^6 cells/ml using Walne's medium in 3 L polyethylene bag. In Rix Mix medium the growth increased to the highest density of 185±9.26 x 10^4 cell/ml at the 12th day of culture. BFRI medium showed the poorest growth performance for this species.

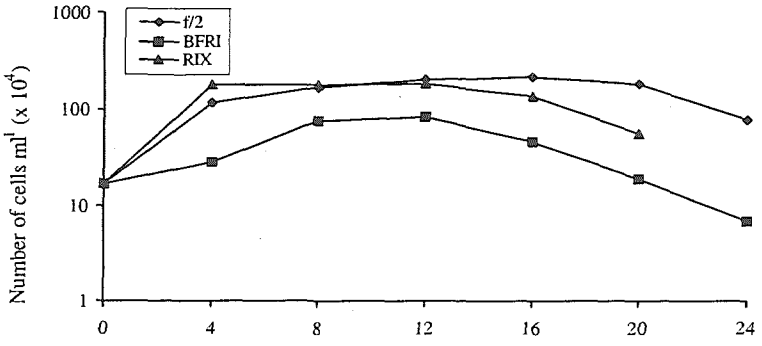

Fig.1. Logarithmic growth curves of *Nannochloropsis oculata* in three culture media

Fig. 1. Logarithmic growth curves of *Nannochloropsis oculata* in three culture media.

Chaetoceros muelleri showed unconventional growth pattern in f/2 and Rix Mix media, but followed the typical growth pattern of microalgae in BFRI medium (Fig. 2). The reason of this is less understood. *C. muelleri* attained the highest density of 321± 5.41x10^4 cells/ml in Rix Mix medium and about same density (319±12.02 x 10^4 cells/ml) at the 24th day of culture in modified Guillard's f/2 medium. The maximum cell density of *C. muelleri* (135±6.95 x 10^4 cells/ml) in BFRI medium was observed at the 12th day of culture. Chen (1991) observed a maximum 200-250 x 10^4 cells/ml of *C. muelleri* in cemented ponds and tanks in large scale using $NaNO_3$:60g/ton,KH_2PO4:4 g/ton, Vit B:100 mg/ton and Vit B_{12}: 0.5 mg/ton.

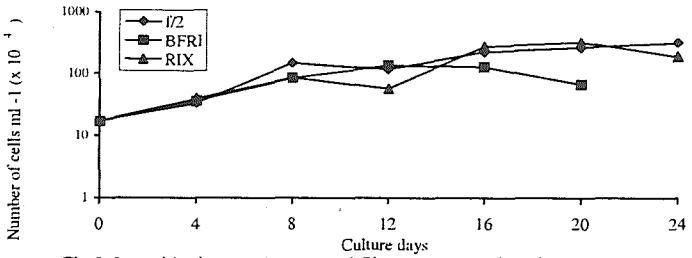

Fig.2. Logarithmic growth curves of *Chaetoceros muelleri* in three different culture media

Fig. 2. Logarithmic growth curves of *Chaetoceros muelleri* in three culture media.

Tetraselmis chui was grown reasonably well in both BFRI and Rix Mix medium compared to modified Guillard's f/2 medium (Fig. 3). As water quality deteriorates and algal cells starve with the increase in density, death and breaking of cells occur after exponential growth phase of microalgae (Hoff and Snell 1991, Fox 1983). *T. chui* is less sensitive to environmental stress (Liao *et al.* 1991), which could be responsible for the extended exponential growth phase of this species in all the media. The maximum cell density of 182±6.26x10^{4}cell/ml was with BFRI medium at the final day (24th day) of culture. The highest density of the species with Rix Mix medium was 169±4.48x10^4 cells/ml at the final day (24th day), which was close to the maximum value with BFRI medium at the same day. *T. chui* gave reduced growth in modified Guillard's f/2 medium during the entire culture period, reaching a maximum cell density of only 78 ± 4.24x10^4 cells/ml at the final day (24th day) of culture. Different microalgal culture test media resulted in varying growth rates of cultured microalgae species (Table 2). Considering the increase in density at the 16th day of culture, in modified Guillard's f/2 medium, the cell growth of *C. muelleri* (222±4.24x10^4 cell/ml) was significantly ($P<0.05$) higher than the other two species. After 16 days, *N. oculata* and *T. chui* were reduced in their growth, but *C. muelleri* increased at the highest density up to the last day of culture.

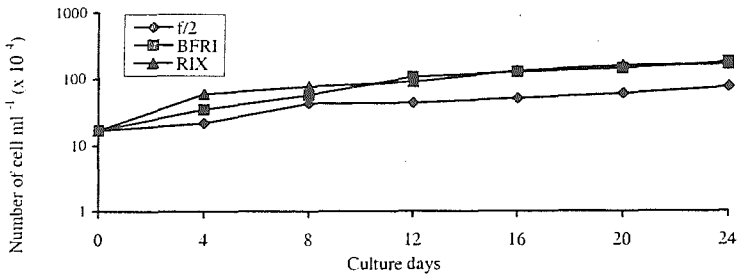

Fig.3. Logarithmic growth curves of *Tetraselmis chui* in three different culture media

Fig. 3. Logarithmic growth curves of *Tetraselmis chui* in three culture media.

Table 2. The effect of different microalgae culture test media on the growth (cells x 10^4/ml) of three microalgae species. Values are means ± SD from triplicate observations

Culture days	Modified Guillard's f/2 media			BFRI medium			RIX MIX medium		
	*N O	**CM	***TC	NO	CM	TC	NO	CM	TC
0	17	17	17	17	17	17	17	17	17
4	119±7.07	33±9.19	22±2.82	29±5.65	36±10.06	35±9.89	180±7.28	39±8.20	60±4.59
8	170±1.31	147±12.84	43±6.36	76±4.94	85±4.24	58±7.27	178±4.56	86±5.19	77±6.71
12	206±9.19^a	117±10.60^a	44±8.48^b	84±9.19^b	135±6.95^a	108±10.12^a	185±9.28^a	57±8.31^b	91±4.81^a
16	221±4.42^a	222±4.24^b	52±7.77^b	47±4.94^c	128±11.11^c	129±5.62^a	141±10.54^b	273±9.61^a	134±9.27^a
20	189±11.55^a	262±6.36^b	61±2.12^b	19±4.98^c	67±9.01^c	147±8.18^a	57±6.81^b	321±5.41^a	161±7.61^a
24	79±7.08	319±12.02	78±4.24^b	7±1.41	0±0	182±6.26^a	0±0	191±7.79	169±4.48^a

*NO= *Nannochloropsis oculata*, **TC= *Tetraselmis chui*,***CM = *Chaetoceros muelleri*

Note: Different superscript in the same row for same species indicates significant variation and same superscript in the same row for same species means insignificant at 5 % level of significant.

In BFRI medium, considering the increase in cell density at the 12th day, growth of *C. muelleri* (135±6.95 x10^4 cell/ml) was significantly ($P<0.05$) higher than other species, but at the 24th day *T. chui* showed significantly ($P<0.05$) higher density (182±6.26 x 10^4 cells/ml) than other two species. *N. oculata* and *C. muelleri* concentration decreased after 12 days and reached at minimum level at the last day of culture. At the 12th day of culture in Rix Mix medium, *N. oculata* concentration was significantly ($P<0.05$) higher (185±9.28 x 10^4 cells/ml) than other two species. After 12 days this species gradually decreased but *T. chui* increased up to the end and *C. muelleri* reached the highest density (321±5.41 x 10^4 cell/ml) at the 20th day and then decreased.

The growth of *N. oculata* reached a higher density of 221±4.42 x 10^4 cells/ml in modified Guillard's f/2 media at the 16th day than that of (141±10.54 x 10^4 cell/ml) in Rix Mix medium. Modified Guillard's f/2 medium promoted significantly ($P<0.05$) higher growth than other media. Hur (1991) observed the highest cell density of 197.5 x 10^5 of *N. oculata* cultured in F/2 medium, which is much higher than the density obtained in present study and this might be due to the changes in chemical composition of nutrient medium. *N. oculata* grew reasonably well in modified Guillard's f/2 and Rix Mix media among the three media tested in this study.

The highest cell density of *N. oculata* was attained between 12 and 16 days of culture period in three culture media. On the other hand, the cell number of *T. chui* increased up to the 24th day (final) day of culture and this difference might be due to the difference in exponential growth phase of these two microalgal species. *C. muelleri* showed difference in maximum cell density at different days of culture.

In the above view, it may be concluded that, *N. oculata* and *C. muelleri* might be grown very well in both the modified Guillard's f/2 and Rix Mix media. Better growth of *T. chui* might be obtained while growing in BFRI and Rix Mix media. These three microalgal culture media used in the present study may be useful for culture of different algal species to supply as live food for suitable target zooplankton and larvae of fish, crustaceans, etc. in marine and brackishwater hatcheries.

References

Braley, R.D, 2001. Manual for the operation of the hatchery at Brackishwater station, Paikgacha, Khulna. ARMP Report. Winrock International Institute for Agricultural Development. Arkansas. USA. pp. 108.

Chen, J.F., 1991. Commercial Production of Microalgae and Rotifer in china. *In:* Rotifer and Microalgae culture systems (W. Fulks and K.L. Main eds.). The Oceanic Institute, Honolulu. pp. 105-111.

Fox, J. M., 1983. Intensive algal culture Techniques. *In:* CRC Handbook of Mariculture. Vol-1 .Crustacean Aquaculture (J.P. McVey ed.). CRC press , Florida. 15 pp.

Fulks, W. and K.L. Main (eds.), 1991. Rotifer and Microalgae Culture Systems. Proc. of a US – Asia Workshop, Honolulu, Hawaii, 28-31 January 1991, 3-12, The Oceanic Institute, Honolulu. 364 pp.

Guillard, R.R.L. and J.H. Ryther, 1962. Studies of marine planktonic diatoms. 1. Cyclotella nana Hustedt and Detonula confervaces (Cleve) Gran. *Can. J. Microbiol*., 8: 229-239.

Hoff, F.H. and T.W. Snell, 1989. Plankton Culture Manual. Florida Aqua Farms, Inc. 126 pp.

Hur, B.S., 1991. The selection of optimum phytoplankton species for rotifer culture during cold and warm seasons and their nutritional value for marine finfish larvae. *In:* Rotifer and Microalgae culture systems (W. Fulks and K.L. Main eds.). The Oceanic Institute, Honolulu. pp. 163-173.

Kongkeo, H., 1991. An overview of live feed production systems design in Thailand. *In:* Rotifer and Microalgae culture systems (W. Fulks and K.L. Main eds.). The Oceanic Institute, Honolulu. pp 175-186.

Liao, I.C., K.H. Su and J.H. Lin, 1983. Larval foods for Penaeid prawn. *In*: CRC Handbook of Mariculture. Vol-1. Crustacean Aquaculture (J.P. McVey ed.). CRC press , Florida. pp. 43-69.

Lim, L.C., 1991. An overview of live feeds production systems in Singapoor. *In:* Rotifer and Microalgae culture systems (W. Fulks and K.L. Main eds.). The Oceanic Institute, Honolulu. pp. 187-201.

Okaichi, T., S. Montani, J. Hiragi and A. Husui, 1989. The role of iron in the outbreaks of *Chattonella* red tide. *In*: Red tides: biology, environmental science and toxicology (T. Okaichi, D.M. Andersons and T. Nemoto eds.). Elsever, New York. pp. 353-356.

Okauchi, M., 1991. The status of phytoplankton production in Japan . *In:* Rotifer and Microalgae culture systems (W. Fulks and K.L. Main eds.). The Oceanic Institute, Honolulu. pp. 257-273.

Tomas, C.R., 1978. *Olisthodiscus luteus* (Chrysophyceae). 1. Effect of salinity and temperature on growth, motility and survival. *Phycol.*,14: 309-313.

Uye , S. and K. Takamatsu, 1990. Feeding interactions between planktonic copepodes and red-tide flagellates from Japanese coastal waters. *Marine Ecology progress Series*, 59: 97-107.

Wilkerson, Joyce D, 1998. Clownfish: a guide to their captive care, breeding and natural history. Microcosm Ltd, Shelburne, VT 05482. 181pp

Zaman, S.M.H, K. Rahim and M. Howlader, 1982. Simple lessons from biometry, Bangladesh Rice Research Institute, Joydebpur, Dacca, Bangladesh. pp. 85-92.

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