EFFECT OF SALINITY ON THE CULTURE OF STANDARD MICROALGAE SPECIES USED IN AQUACULTURE.

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Abstract: Three standard microalgae species viz. *Nannochloropsis oculata, Tetraselmis chui* and *Chaetoceros muelle*ri , commercially used in aquaculture in many countries, were cultured in small scale batch culture for 28 days to determine the effect of salinities (05 ppt to 30 ppt) on the growth under laboratory conditions at Brackishwater Station of Bangladesh Fisheries Research Institute (BFRI). The BFRI microalgae culture nutrient medium, which is the modification of Guillard's f/2 medium (Gillard & Ryther, 1962), was used as the culture medium. *Nannochloropsis oculata* showed maximum cell growth (345 X 10^4 cells /ml) at 20 ppt salinity. *Tetraselmis chui* showed better growth at salinities ranging from 25 ppt to 30 ppt but failed to grow at salinities from 5 to 10 ppt. The maximum cell density (458 X 10^4 cells / ml) of *Tetraselmis chui* was observed at 30 ppt on the 24^{th} day. *Chaetoceros muelleri* did not tolerate a salinity of 5 ppt but grew well at the salinity range of 20-30 ppt and the highest cell density (395 X 10^4 cell/ ml) of this alga was found at 25 ppt.

Key Words: Growth; *Nannochloropsis oculata*; *Tetraselmis chui*; *Chaetoceros muelleri;* and *Salanity*.

Introduction

Microalgae are essential food sources in the rearing of all stages of marine bivalve mollusks (clam, oyster, scallop), the larval stages of some marine gastropods (abalon,conch),larvae of marine fish (cod, halibut) and shrimp (*penaeus*), some fish species (tilapia,milkfish) and zooplankton (rotifer, copepods, cladocerans, brine shrimp). The zooplankton are fed in turn to the late larval stages of various species of fish and crustaceans (prawn, shrimp, crab, lobstars) (Dhart and Sorgellos, 1995).

Chaetoceros, Nannochloropsis, Isochrysis,Chlorella and *Tetraselmis* are the genera of algae most commonly cultured for aquacultural purpose (Persoone and Claus 1980,Ukeles 1980,Laing and Millican 1986 and Liao *et al*. 1983). *Nannochloropsis oculata* is widely used as marine rotifer food. It is easily cultured and is high in vitamin B12 (needed for rotifer growth and reproduction) and the highly unsaturated fatty acid EPA needed by larval and juvenile marine fishes. This microalgae species can be useful in establishing the rotifer, *Brachionus plicatilis* culture protocol (Wilkerson, 1998). *Chaetoceros mulleri* is high in HUFA's and its overall nutritional value is also high (Okauchi, 1991). *Tetraselmis chui* may also prove useful as a direct food in culturing organisms that are too small to accept rotifers (Wilkerson, 1998)..

The tolerance of marine microalgae to changes in salinity is considered to be extremely broad. Most grow best at a salinity that is a bit lower than that of their native habitat. Different species have different nutrient, illumination, temperature, salinity and pH requirements for optimum growth (Chen and Long, 1991). Tolerate and optimum salinities have been investigated by a number of authors for a variety of commercially important species (Ukeles, 1976; Kaplan *et a.,l* 1986; Fabreges *et a.,l* 1984;Laing and Utting, 1980; Duerr and Mitsui, 1982).

Considering the need for a thorough investigation with the suitable salinity for microalgae culture for larval rearing in brackishwater hatcheries in Bangladesh, the present study was designed with the objective to determine the effect of salinity on the growth performance of the three microalgae viz. *Nannochloropsis oculata, Tetraselmis chui* and *Chaetoceros mulleri*..

Materials and Method

The batch culture of the microalgae species in these trials was done in a temperature controlled Microalgal Laboratory at the Brackishwater Station of Bangladesh Fisheries Research Institute (BFRI). Temperature was maintained at about 25° C. The culture vessels, 1.5-L cola bottles available from local sources were used to culture the microalgae followed by Hoff and Snell (1989) as the shape of the cola bottle is cylindrical and the bottom is round to provide good circulation. Cleaning of the culture vessels was performed according to the cleaning procedure for small-scale culture recommended by Hoff and Snell (1989) as it is essential for successful culture. The cola bottles were provided with an aeration tube entering and an exit tube coming through the plastic screw cap of the bottle. The brackishwater of different salinities was filtered through 1-micron cartridge filter bag to remove particulate materials and treated with 30 ppm chlorine at a rate of 0.1gm/l and dechlorinated by using 0.175 g/l of sodium thiosulphate. To avoid the excess sodium

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thiosulphate in prepared brackishwater media for inoculation of algal cells, the rate of application and period (30 minutes) of dechlorination was maintained properly. Salinity range tested was 05- 30 ppt in 5 ppt intervals. The pH of prepared brackishwater media was adjusted at about 8. Growth study was done in triplicate observations. The bottles were set up approximately 15 cm from the two 'Daylight' fluorescent tube light sources (a new fluorescent bulb is about 2500 lux). BFRI medium was used as culture nutrient medium (the compositions of BFRI medium is shown in Table1).

Table.1. Composition of BFRI microalgae culture nutrient medium

No.	Ingredient	Amount of ingredient (g or ml) to add to 1-L distilled water.	Comments
1.	Ammonium sulphate	100g	1 ml of BFRI microalgae culture medium to 1-L of seawater or brackishwater to be used to culture microalgae.
2.	Urea	230g	
3.	TSP	20 g	
4.	Borax	10g	
5.	Sodium metasilicate	20 g	Application rate of sodium metasilicate: 1 ml to 1-L seawater or brackishwater for Diatoms only.
6.	5 trace metals solutions *	1 ml of each	This media contain 36.88 % nitrogen.
7.	3 vitamins solutions **	1 ml of each	

*Trace metals solutions: Make up of five separate solutions of trace metals, dissolving the amounts below into 100 ml of distilled water;

Ingredients	Amount (g)
Copper sulphate	1.0
Zinc sulphate	2.2
Sodium molibdate	0.6
Manganese chloride	18.0
Cobalt chloride	1.0

**Vitamin solutions: make up 3 separate solutions of vitamin, dissolving the amounts below in to 100 ml of distilled water.

Ingredients	Amount (g)
Thiamin HCL (Vitamin B10	10.0
Cyanocobalamin (Vitamin B12)	0.05
Vitamin H (Biotin)	0.05

The BFRI microalgae culture nutrient medium was prepared at the Microalgal Laboratory, Brackishwater station of BFRI. The trace metals and vitamins ingredients were similar to the standard microalgal culture media, Guillard's modified f/2 medium (Gillard & Ryther, 1962) but the amount was not the same. The application rate of BFRI culture medium was 1ml/L of seawater. The algal cells were inoculated into the bottles with equal initial concentration (44 x 10^4 cells /ml) for all species of microalgae. Aeration by aquarium aerators was moderate. The total culture period was twenty-eight days. Counts of microalgae cells were made every four days up to the 28th day. The cell concentration was determined by using an improved Neubauer haemocytometer by applying the methodology used by Hoff and Snell (1989). All the data were analyzed statistically using Analysis of Variance (ANOVA) and the mean values were compared using Duncun's New Multiple Range Test (Zaman *et al.*, 1982).

Results

A summery of salinity effects on cell growth (cells x 10^4/ml) of three microalgae species is presented in Table-2. At the 16th day of culture the peak cell growth of *Nannochloropsis oculata* and *Chaetoceros muelleri* were attained in all salinity treatments during the study period. When a one-way ANOVA was run comparing the maximum cell densities attained at various salinities for *Nannochloropsis oculata,*, there was significant ($P<0.05$) difference between the means and this species showed maximum cell growth (345 X

10^4 cells /ml) at 20 ppt salinity. One-way ANOVA showed that the difference in maximum cell density of and *Chaetoceros muelleri* at various salinities was highly significant (P<0.05). Significantly highest (395 X 10^4 cell/ ml) cell density of this alga was found at 25 ppt which was not significantly higher than 30 ppt. Peak growth of *Tetraselmis chui* was observed at 20 days in 15 ppt & 20 ppt but in 25 ppt and 30 ppt it was found at 24 days. *Tetraselmis chui* showed significantly (P<0.05) highest cell density (458 X 10^4 cells /ml) at 30ppt salinity at 24 days during the culture period. The 5 ppt salinity treatment for both *Chaetoceros muelleri* and *Tetraselmis chui* had a reduced density by the fourth days of culture after inoculation and crashed by12 and 16 days of culture respectively indicated a lethal reaction to the low salinity.

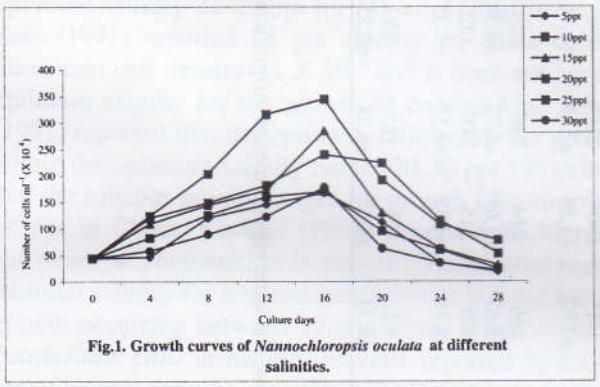

Fig.1. Growth curves of *Nannochloropsis oculata* at different salinities.

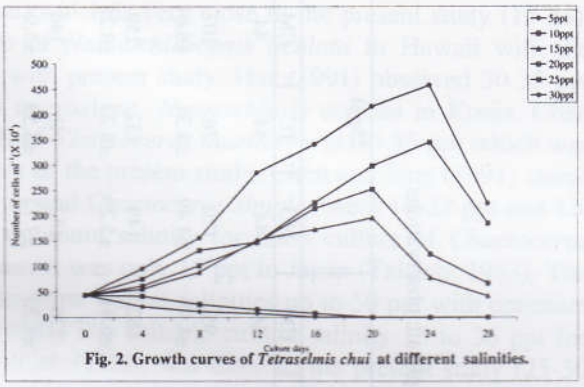

Fig. 2. Growth curves of *Tetraselmis chui* at different salinities.

Maximum cell growth of *Nannochloropsis oculata* was found at 20 ppt on the 16th day of culture (Fig.1.). The plankton could tolerate a wide range (5-30 ppt) of salinities of brackishwater. It grew well at the salinity range of 15-25 ppt. No lag phase was exhibited at salinities from 10-30 ppt with the exponential growth from the 4th to 16th day. At 5-10 ppt salinity the maximum cell growth of *Nannochloropsis oculata* was more or less similar.

The maximum cell density of *Tetraselmis chui* was observed at 30 ppt on the 24th day (Fig.2.).This plankton species grew well at the salinity range of 25-30 ppt. No lag phase was exhibited at salinities from 25-30 ppt with the exponential growth from the 4th to 24th day. The exponential growth was found from the 8th to 20th day with maximum growth on the 20th day at the salinity range 15-20 ppt. The alga failed to grow below 15 ppt but cells were able to survive up to 16 days at 5-10 ppt. At 5 and 10 ppt no living cells were found after 16 days.

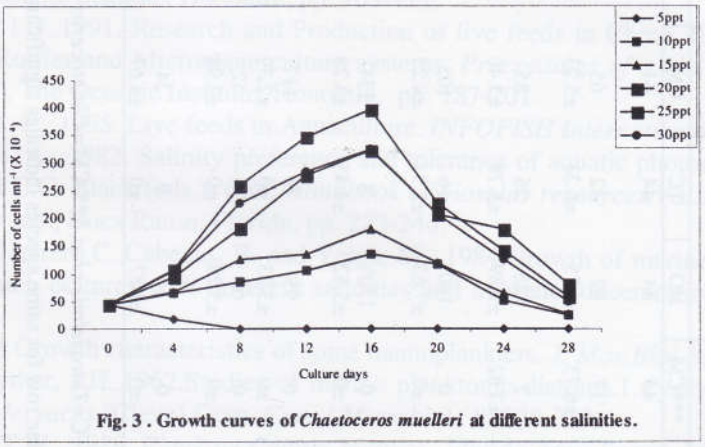

Fig. 3 . Growth curves of *Chaetoceros muelleri* at different salinities.

The maximum cell density of *Chaetoceros muelleri* was observed at 25 ppt on the 16th day (Fig.2.). This species also grew well at the salinity range of 20-30 ppt . The exponential growth was found from the 4th to 16th day with maximum growth on the 16th day at the salinity range 20-30 ppt. *Chaetoceros muelleri* failed to grow at 5 ppt but cells were able to survive up to 16 days and thereafter no living cells were found.

Table. 2 Effect of salinity on the growth (cells x 10^4/ml) of three standard microalgae species. Values are means ± SD from triplicate observations

Culture days	05ppt			10ppt			15ppt			20ppt			25ppt			30ppt		
	*NO	**TC	***CM	NO	TC	CM	NO	TC	CM	NO	TC	CM	NO	TC	CM	NO	TC	CM
0	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44
4	46 ±6.51	22 ±7.34	17 ±6.73	82 ±4.75	42 ±6.23	65 ±5.23	109 ±8.12	56 ±9.83	67 ±6.68	124 ±5.52	62 ±5.52	95 ±4.98	120 ±4.49	76 ±8.31	107 ±6.61	58 ±9.18	96 ±6.83	105 ±5.87
8	120 ±9.16	12 ±5.91	2 ±7.82	125 ±9.89	26 ±5.78	85 ±3.46	144 ±7.73	89 ±12.23	126 ±7.89	204 ±7.16	102 ±5.78	182 ±6.91	149 ±5.29	132 ±6.23	259 ±4.28	89 ±4.39	156 ±4.78	228 ±4.96
12	145 ±8.21	8 ±4.73	0 ±0	160 ±7.63	16 ±5.34	105 ±4.23	172 ±6.52	152 ±6.76	135 ±6.81	316 ±5.28	147 ±6.87	276 ±8.23	182 ±7.72	148 ±8.59	346 ±5.94	122 ±5.28	285 ±6.74	284 ±6.61
16	167^{c} ±4.35	1^{c} ±5.94	0 ±0	170^{c} ±5.52	8 ±4.45	134^{c} ±3.28	285^{a} ±6.79	$172b^{c}$ ±8.17	179^{b} ±6.12	345^{a} ±5.23	214^{b} ±7.91	321^{a} ±7.92	240^{ab} ±4.56	225^{b} ±7.24	395^{a} ±6.76	178^{c} ±6.16	341^{a} ±6.17	325^{a} ±5.65
20	112 ±11.18	0 ±0	0 ±0	95 ±3.23	0 ±0	117 ±2.39	130 ±7.12	195^{c} ±4.36	120 ±9.58	192 ±8.27	259^{b} ±12.16	206 ±5.67	224 ±3.68	298^{b} ±6.72	228 ±4.38	62 ±4.97	416^{a} ±4.36	208 ±4.96
24	35 ±8.29	0 ±0	0 ±0	60 ±5.28	0 ±0	52 ±2.98	62 ±9.81	78^{d} ±7.71	66 ±5.35	108 ±4.29	125^{c} ±9.91	179 ±9.78	115 ±4.35	345^{b} ±8.52	142 ±5.35	35 ±4.39	458^{a} ±7.41	125 ±3.28
28	19 ±3.29	0 ±0	0 ±0	24 ±4.87	0 ±0	24 ±4.67	31 ±7.23	42 ±5.87	28 ±4.89	78 ±6.34	67 ±5.81	82 ±6.34	53 ±6.37	185 ±6.38	58 ±5.23	21 ±5.45	224 ±5.87	49 ±5.37

*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.

Discussion:

In the experiments it was found that in the culture of these three microalgae species at different range of salinities the exponential growth began without passing any lag phase and that might be due to the inoculation of the culture at its exponential phase of growth. According to Spencer (1954) the length of the lag phase is least when the inoculum is in its exponential phase of growth. Ammini (1984) and Gopinathan (1984) have observed similar results in microalgae culture.Marine unicellular algae are generally considered to be tolerant of and adoptive to a wide range of salinities (McLachlan, 1961). The salinity tolerance and the optimum salinity for growth of marine microlagae varies with species and strains. Lim (1991) observed the optimum salinity 22-25 ppt for *Nannochloropsis oculata*, which is very close to the present study (15-25). Sato (1991) reported 32 ppt salinity for mass culture of *Nannochloropsis oculata* in Hawaii with the maximum cell density 38 X 10^6 and it does not agree with present study. Hur (1991) observed 30-35 ppt optimum salinity for the growth of another blue green microalgae *Nannochloris oculata* in Korea. Chen (1991) reported that the optimum salinity for the growth of *Chaetoceros muelleri* was 20-35 ppt which was within the optimum salinity range (20-30 ppt) in cultures of the present study. Chen and long (1991) stated that the suitable salinity ranges for growth *Chaetoceros sp* and *Chaetoceros simplex* were 18-22 ppt and 13-18 ppt in China. Konkeo (1991) observed 25-30 ppt optimum salinity for mass culture of *Chaetoceros calcitrans* in Thailand but in case of *Chaetoceros radicuns* it was only 15 ppt in Japan (Takano, 1963). The minimal salinity for *Chaetoceros gracilis* is 6 ppt,but can grow well at salinities up to 50 ppt with optimum growth occurring between 17 and 25ppt (Liao *et al.*, 1983). The suitable culture salinity 15 to 36 ppt for ***Tetraselmis chui*** in natural seawater reported by Liao *et al.* (1986) was close to the present study (25-30 ppt). Optimal salinity range for growth in medium prepared from artificial seawater was found to be 25-30 ppt for *Tetraselmis suecia* (Laing and Utting, 1980). Lim (1991) found 26-31 ppt optimal salinity for the culture of *Tetraselmis tatrathele* in Singapore. Chen (1991) reported suitable salinity 30-40 ppt for *Tetraselmis subcordiformis* culture in China.

Conclusion

Nannochloropsis oculata was the most euryhaline species of microalgae tested here and can tolerate a wide range (5-30 ppt) of salinities. This species is one of the most important species in aquaculture so the findings from this study have positive implications for brackishwater hatcheries in Bangladesh.

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