MASS OUT-DOOR CULTURE OF THE MICRO-ALGA *Chaetoceros gracilis*

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Abstract

The mass culture of the micro-alga, *Chaetoceros gracilis*, a live-food for feeding the larval stages of Black Tiger shrimp, *Penaeus monodon,* was carried out in the in-door shrimp hatchery of Nigerian Institute for Oceanography and Marine Research. Guillard modified f/$_2$ medium was used in the production of starter culture and maintenance of the pure algal culture in an indoor laboratory. Mass cultures of *C. gracilis* were thereafter developed in 1.5 m^3 out-door plastic tanks using Guillard modified f/$_2$ medium and Liao and Huang modified medium. The culture reached the stationary phase within 120 hours when exposed to sunlight. Three phases of cell growth of *C. gracilis* were observed during the culture periods which were lag phase followed by exponential and stationary phases. Average cell density of the out-door culture of the micro-alga was 780,000 cells/mL and the culture remained in this phase for 120-144 hours with a brown colouration. The highest cell density was 1,039,145 cells/mL recorded in the month of October while the lowest was 248,154.7 cells/mL recorded in the month of July. High cell density of the species occurred during the months of January to May and in October. These months were characterized by low humidity and higher temperature. Water temperature and salinity of the mass culture medium ranged from 25-29^{o}C and 28-30 ppt respectively. The culture was sustained by semi-continuous culture throughout the study-period which lasted for ten months. The use of enrichment media enhanced sustained continuous culture for all year round alga production. The results obtained in this study indicated that mass culture and all year round production of *C. gracilis* is feasible in Nigeria.

Keywords: *Chaetoceros gracilis*, mass culture, marine algae, Guillard modified f/$_2$ growth medium, Liao and Huang modified growth medium.

Introduction

Marine algae are widely used as food in the hatchery production of commercially valuable fish and shellfish (Liang, 1991). Micro-algae are phytoplankton that are less than 20 microns and their use in aquaculture consists of culturing pure strains of the selected alga (Lavens and Sorgeloos, 1996, Muller-Feuga, 2004). Over 40 different species of micro-algae have been isolated in different parts of the world and some have been cultured as pure strains in intensive systems (Nwankwo, 1988, Rines and Theriot, 2003, Nwankwo and Onyema, 2004, Sen *et al*., 2005, and Richtel, 2007). These include the diatoms *Skeletonema costatum, Thalassiosira pseudonana, Chaetoceros gracilis, C. calcitrans,* the flagellates *Isochrysis galbana, Tetraselmis suecica, Monochrysis lutheri* and the chlorococcalean *Chlorella spp*.

Chaetoceros species are one of the useful diatoms suitable for feeding the larval stages of marine animals due to its small size (Sen *et al* 2005). Pioneering work to grow micro-algae in dense laboratory culture was done by Warburg (1919) in Berlin, Germany. Ketchum and Redfield (1938) described a method for maintaining continuous culture of marine diatoms in large supplies, which is still used in aquaculture as a means of rapid production of phytoplankton. According to the authors, a volume of the alga culture containing the number of algal cells equivalent to the daily increase in algal cells is withdrawn each day and replaced by an equal volume of fresh sea-water enriched with nutrients.

For algae culture, direct use of natural sea-water is seldom acceptable without the addition of further nutrients and trace metals. The algae density is usually too low for culture maintenance and hence enrichment

This study was accepted on 11th January 2011.

of nutrients, trace metals and vitamins is usually required (Anderson, 2005). The preparation of natural sea-water with the enrichment solution of nutrients, trace metals and vitamins has been reviewed by Anderson (2005). The pioneering work on the hatchery propagation of the penaeid shrimp *P. monodon* by Nigerian Institute for Oceanography and Marine Research, Lagos, incorporated the culture of micro-algae, *Chaetoceros gracilis* for feeding the early larval stages. This study was carried out to determine the feasibility of mass culture of *C. gracilis* in Nigeria.

Materials and methods

The mass culture of *C. gracilis* was carried out at the shrimp hatchery and laboratory of Nigerian Institute for Oceanography and Marine Research (NIOMR), Victoria Island, Lagos, from January to October 2009. The algal stock of *C. gracilis* used in this study was obtained from Phytoplankton Culture Laboratory, Prince of Chumpon Fishery College, Chumpon, Thailand in 2008.

Sourcing and treatment of seawater

Sea-water was collected from the Atlantic Ocean using water tankers and transferred to the sedimentation tank in the shrimp hatchery for settlement of solid particles. The water was treated with 30 ppm chlorine and aerated for three days to ensure removal of chlorine residue before use. The treated-water was filtered twice using a candle filter and 0.5 micron filter bag and stored in 5m^3 GEEPEE plastic tanks.

Establishment of in-door alga culture

Monocultures of *C. gracilis* were carried out in 1,000 ml conical flasks in the in-door shrimp laboratory. The medium for *C. gracilis* culture (Table 1) was prepared by adding 1 mL each of the working stock solution of sodium nitrate, sodium di-hydrogen phosphate, sodium silicate, trace metals solution (Table 2) and 0.5 mL of vitamin solution (Table 3) to 1 liter of filtered sea water. Inoculation process at the flask level was carried out near spirit lamp to avoid bacterial contamination. All apparatus used for the in-door culture were autoclaved.

White fluorescence bulbs were used as a source of light. Room temperatures ranged from 25-28^{0}C. The maintenance of the culture medium was carried out by using a semi-continuous alga culture technique as described by Ketchum and Redfield (1938). Treated seawater was filtered twice using 0.5 micron filter bag and autoclaved before usage.

Establishment of out-door alga culture

The mass culture of *C. gracilis* was carried out in the out-door algae culture unit using 0.5 m^3 and 1.5 m^3 circular plastic tanks. The tanks were filled with treated and filtered sea water before inoculation. The sea-water was enriched with Guillard (1975) f/$_2$ growth medium (Table 1) and Liao and Huang (1970) enrichment medium (Table 4).

Algal inoculants were then introduced at 10% volume of the tanks the next day for mass culture. Water samples of the mass-culture were collected after eight days and transferred to a new medium to create a new culture line of production. The experimental period was eight days and the culture was repeated monthly from January to October. Productions were closely synchronized with the various procedures as shown in Table 5.

Table 1: Nutrient composition of Guillard (1975) f/$_2$ growth medium.

Component	Stock Solution (per litre of distilled water)	Quantity Used
$NaNO_3$ (g) (Sodium nitrate)	75	1 mL
$NaH_2PO_4 .H_2O$ (g) (Sodium di-hydrogen phosphate monohydrate)	5	1 mL
$Na2SiO_3 . 9H_2O$ (g) (Sodium silicate nanohydrate)	30	1 mL
Trace metal solution	Table 2	1 mL
Vitamins' solution	Table 3	0.5 mL
Sea-water	–	950 mL

Table 2: Nutrient composition of trace metals solution.

Component	Stock Solution (per litre of distilled water)	Quantity Used
$FeCl_3 \cdot 6H_20$ (g) (Ferric hydrochloride hexa-hydrate)	–	3.15 g
$Na_2EDTA. 2H_2O$ (g) (Sodium ethylene di-tetraamine di-hydrate)	–	4.36 g
$MnCl_2.4H_2O$ (g) (Manganese Chloride tetra-hydrate)	180.0	1 mL
$ZnSO_4.6H_20$ (g) (Zinc sulphate hexa-hydrate)	22.0	1 mL
$CoCl_2.6H_2O$ (g) (Cobalt chloride hexahydrate)	10.0	1 mL
$CuSO_4.5H_2O$ (Copper II sulphate penta-hydrate)	9.8	1 mL
$NaMoO_4.2H_2O$ (Sodium molybolate di-hydrate)	6.3	1 mL
Distilled water	make up to 1 litre	

Table 3: Nutrient composition of vitamins solution.

Component	Stock Solution (per litre of distilled water)	Quantity Used
Thiamin Hydrochloride (Vitamin B_1)	–	200 mg
Biotin (Vitamin H)	0.1	1 mL
Cyanocobalamin (Vitamin B_{12})	1.0	1 mL
Distilled water	–	950 ml

Table 4: Nutrient composition of Liao and Huang (1970) Modified Medium.

Component	Quantity used
KNO_3 (Potassium nitrate)	100.000 mg
$Na_2HPO_4.H_2O$ (Sodium hydrogen phosphate monohydrate)	10.000 mg
$FeCl_3.6H_2O$ (Ferric hydrochloride hexa-hydrate)	3.000 mg
$Na_2SiO_3.9H_2O$ (Sodium silicate nanohydrate)	1.000 mg
Sea-water	Make up to 1 liter

Table 5: Procedures for mass algae culture using Guillard $f/_2$ and Liao and Huang modified media.

Culture Type	Container		Medium Type	Remarks
	Type	Water Volume		
Primary Starter Culture (in-door)	Conical flask	1 litre	Guillard $f/_2$ medium (1975)	20 mL of established culture stock was used to inoculate 1L in the primary production flask
Secondary (Intermediate Scale Culture (0ut-door)	Plastic tank	0.5 m^3	Guillard $f/_2$ medium (1975)	50 mL of primary culture was used to inoculate the secondary culture tank.
Mass Culture (out-door)	Circular Plastic tank	1.5m^3	Liao and Huang modified medium (1970) Tungkang Marine Laboratory.	150 L of secondary culture was used to inoculate the mass outdoor culture tank.

Monitoring algae density

Cell density was monitored twice daily, morning and afternoon using Sedgwik Rafter (S52 micrometer) cell counting chamber under a Trinocular Olympus microscope equipped with photo scope digital camera (DCM35, USB 2.0). During photography, light setting and exposure times were kept constant where possible. Details of the pictures taken were in 2,078 x 1,583 pixels. The cells were counted in the out-door culture system at the start of the experiment and everyday for seven days. The estimated number of algae cells were recorded.

Water temperature, salinity and pH of the culture medium were measured. During the study period, the optimal photoperiod varied from 14-16 hours of light period to 10-12 hours of dark period.

Meteorological data

Meteorological parameters including air temperature (^oC), rainfall (mm), and relative humidity (%) were obtained from Nigeria Meteorological Agency, (Marine section) stationed at NIOMR, Lagos.

Results

Data obtained from the out-door mass culture of *C. gracilis* indicated that the mean cell density ranged from 23,488.08 cells/mL at the start of the experiment to a maximum of 1,554,376 cells/mL at the end of the experiment on the 8th day. The mean monthly cell density ranged from 877,526.3 to 1,039,145 cells/mL with the highest value recorded in October. The average total cell density of the outdoor culture was 780,000 cells/mL.

The out-door mass culture reached the stationary phase within 120 hours under sunlight and remained in the phase till 144 hours with a brown colouration. The peak period of *C. gracilis* was observed on the 6th and 7th day after inoculation in the out-door 0.5 m^3 culture tanks with an average density of 520,000 cells/mL. Peak abundance of the micro alga in the outdoor culture occurred from January to June. Cells of *C. gracilis* viewed under the microscope are shown in Plates 1 and 2.

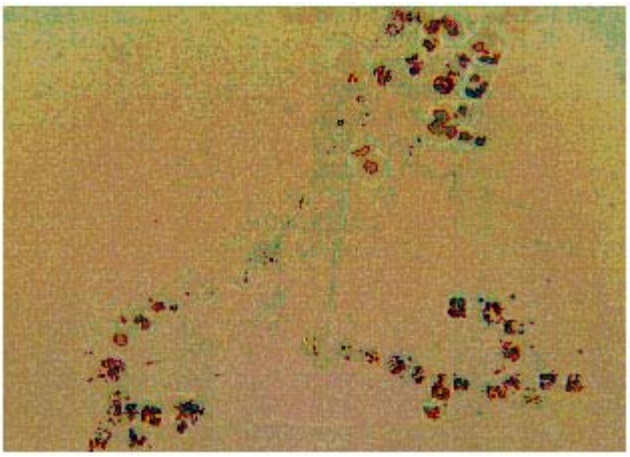

Plate 1: Filamentous cells of *Chaetoceros gracilis.*

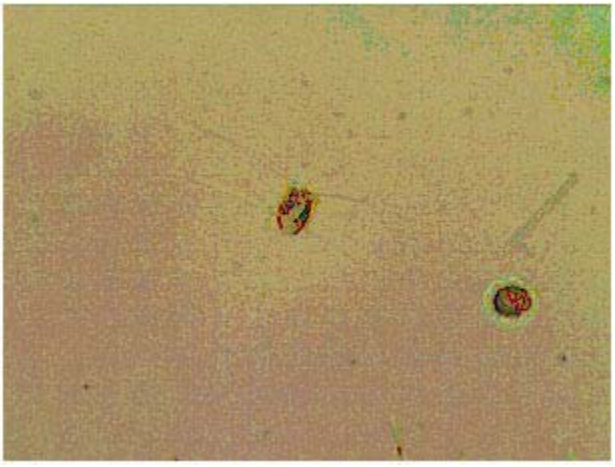

Plate 2: Single cells of *Chaetoceros gracilis.*

Three phases of cell growth of *C. gracilis* were observed during the culture period. These were lag phases followed by exponential and stationary phases. The exponential phase was recorded from the second to the fourth day of inoculation. During the fifth day it reached the stationary phase. However, the growth curves for the months of July and August did not follow the above pattern. The growth curve of the micro-alga in the out-door culture is presented in Figure 1.

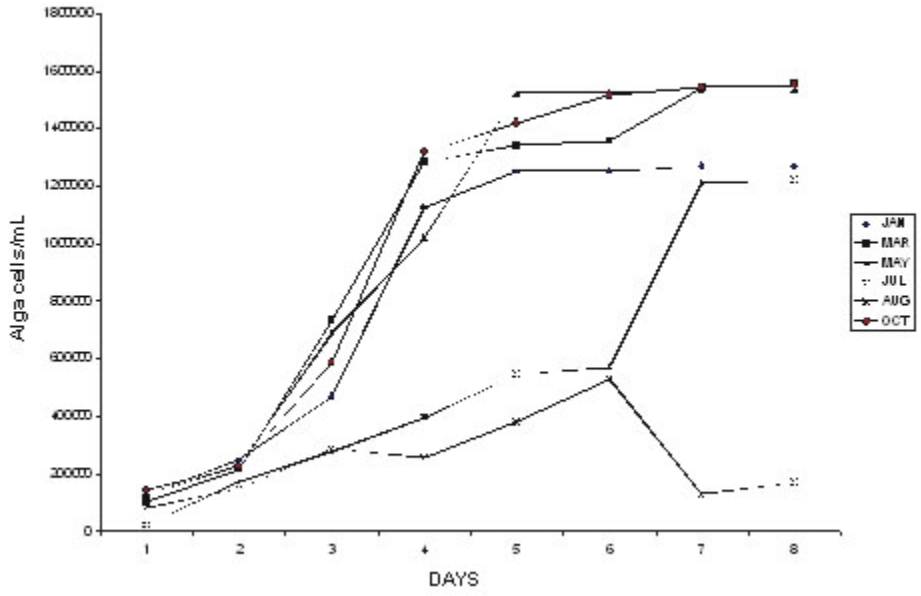

Figure 1: Growth curve of *Chaetoceros gracilis* (outdoor mass culture).

Microscopic observations of the out-door mass culture showed contamination with *Parameciu*m, *Scenedesmus* and bacteria species in some culture productions. These productions were discontinued and totally discarded.

During the study-period, water temperature ranged between 25 to 29°C, salinity 28 to 30 ppt. and pH, 7.0 to 7.6. Mean air temperature ranged between 26 and 30°C, relative humidity, 76-89% and rainfall, 18.2 to 455.7 mm. The details are presented in Figure 2. The months of January to May showed low relative humidity.

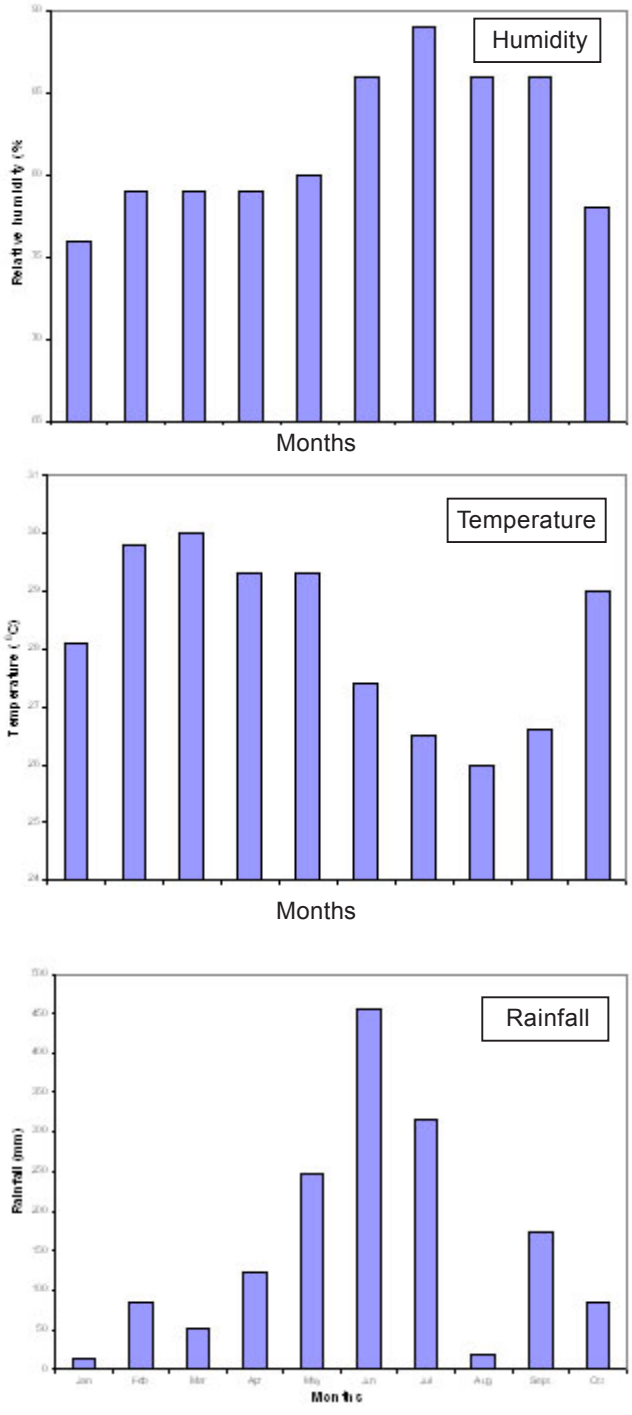

Figure 2: Mean monthly values for rainfall, temperature and relative humidity.

Discussion

High cell densities of pure culture of *C. gracilis* (1,554,376 cells/mL) were produced in the out-door culture unit of NIOMR Shrimp Hatchery during this study. This may be attributed to the use of enrichment media. Similar results have been reported by Uddin and Zafar (2006) for *Skeletonema* species which is also a micro-algae used as live food in aquaculture. They reported that using the Gulliard $f/_2$ medium influenced the high density of *Skeletonema costatum* compared to the low density recorded using commercial fertilizer medium. Takabayeshi *et al* (2006) also reported that nutrient availability in a batch culture of *S. costatum* enhanced the growth rate.

The exponential phase observed from the second to the fourth day of inoculation can be characterized by rapid cell division. An average cell density of 930,000 cells/mL recorded by Uddin and Zafar (2006) for the outdoor mass culture of *S. costatum* was similar to the average cell density of *C. gracilis* (780,000 cells/mL) recorded in this study. The growth curve of *C. gracilis* (Figures 1) was similar to results obtained by Uddin and Zafar (2006). The authors obtained the stationary phase on the fifth day which was similar to what was obtained in the present study.

Generally, small-celled algae are known to dominate in warmer sub-tropical waters which are characterized with low humidity and high temperature (Parsons and Takabayashi 1973). Uddin and Zafar (2006) reported that light enhances photosynthetic activity and multiplication of phytoplankton cells. Similar observation was made in this study. The intensity of light and exposure time played vital roles in the growth and development of *C. gracilis*. This can be attributed to the low relative humidity recorded during algal growth peak periods in the months of January to May and October. The lower the relative humidity, the higher the light intensity and growth of the alga and *vice versa.*

The peak abundance of *C. gracilis* in the out-door culture in the months of March, May and October can also be attributed to high water temperature recorded during these periods (Figure 3). Rate of cell division in micro-algae culture has been reported to increase with increasing temperature (Eppley, 1972; Goldman and Carpenter, 1974 and Goldman and Ryther, 1976). Takabayeshi *et al* (2006) also emphasized the importance of high temperature to the growth of *S. costatum*. Low relative humidity and relatively high temperature according to Margalef (1967), Guillard and Kilhan (1977), Baarud and Nygaard (1978), Smayda (1980) and Shevchenko *et al* (2004) are also known to support higher algae growth. Similar observations were made in this study.

Conclusion

The results of the present study have shown that, mass culture of marine micro-alga *C. gracilis* is feasible in Nigeria. High cell density of the species occurred during the months of January to May and in October. These months were characterized by low humidity and higher temperature. The use of enrichment media enhanced sustained continuous culture for all year round alga production.

Acknoledgement

The authors are grateful to Samuel Essang, Akin Oni and Peter Diyelegha for their diligent and dedicated work at the hatchery during their night duties especially in ensuring steady electricity supply.

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