

# Live Feed Production in Different Nutritive Condition as Diet for *Penaeus monodon* in Shrimp Hatchery, Bangladesh

SHEIKH AFTAB UDDIN<sup>1</sup> AND MOHAMMAD ZAFAR

Institute of Marine Sciences, Chittagong University, Chittagong-4331, Bangladesh

<sup>1</sup>Corresponding author's e-mail: [aftabims@yahoo.com](mailto:aftabims@yahoo.com)

## ABSTRACT

The growth of *Skeletonema costatum* under different artificial nutrient media was studied using different culture vessels. *S. costatum* was collected from the Cox's Bazar coast around the Bay of Bengal. Different growth stages i.e. lag phase, exponential phase, prestationary phase, stationary and death phase were observed during the culture period. The number of cell increased during the active division period and decreased after the beginning of the prestationary phase. The average densities of *S. costatum* in primary culture and secondary culture were  $0.55 \times 10^6$  cells mL<sup>-1</sup> and  $0.93 \times 10^6$  cells mL<sup>-1</sup> accordingly. In mass culture of *S. costatum* two types of medium were used. Highest cell densities of *S. costatum* in cemented tank culture were recorded  $1.23 \times 10^6$  cells mL<sup>-1</sup> and  $0.78 \times 10^6$  cells mL<sup>-1</sup> in their respective f/4 media and commercial fertilizer media. In the cemented tank culture commercial fertilizer media was selected to be best for the mass culture of *S. costatum* in respect of culture stability and product quality.

**Key Words:** Phytoplankton; *Skeletonema costatum*; Mass culture; Nutrient; Bay of bengal

## INTRODUCTION

Phytoplanktons are widely used as food in the culture of commercially important fish and shell fish hatchery. Phytoplanktons constitute the sole food supply in the hatchery for rearing fish, shell fish and oysters. In general, shrimp larvae feed on phytoplankton, detritus, polychaetes and small crustaceans and their food preference changes with age. They start feeding at protozoa stage. Protozoa and early mysis stages prefer phytoplankton (James & Fox, 1983). At mysis and early post larvae food preference changes to zooplankton such as rotifer or brine shrimp. Many authors have emphasized that the importance of live food items in the diet of *Penaeid* shrimps (Liljestrom & Romaine, 1987; Reymond & Lagardere, 1990). The success of any shrimp hatchery depends on the availability of suitable nutrient rich phytoplankton (Whyte, 1987). At present many species of phytoplankton are used in rearing of penaeid shrimp larvae. *Skeletonema costatum* has been widely used both in extensive and intensive hatchery systems of *Penaeus japonicus* (Hudinaga, 1942 & 1969) and *Penaeus monodon* (Su *et al.*, 1990). Feeding *S. costatum* to the penaeid shrimp in the zoea stage resulted in a survival rate of 30% to the mysis stage, compared to a previous survival rate of only 1%. The fact that *Skeletonema costatum* was found to be a suitable food for shrimp zoea stages was of critical importance (Liao *et al.*, 1983). In Bangladesh *S. costatum* is almost 62% dominant in the coastal area specially in Cox's Bazar coast line (Hoque *et al.*, 1999). In view of the importance of mass culture of *S. costatum* in the development of shrimp hatcheries and shrimp farming in Bangladesh, the present work was taken

to provide information and techniques on production efficiency, culture stability and product quality.

## MATERIALS AND METHODS

The present study was carried out from December 2001 to May 2002 in the Prime Shrimp Hatchery Limited, Cox's Bazar and Institute of Marine sciences, Chittagong University, Bangladesh.

For primary culture one ton of sea water was collected in a cemented tank during high tide from the Cox's Bazar coast. The nutrients was added with sea water and left under light till diatom bloom. *S. costatum* was isolated and purified by serial dilution and micropipette method (Allen & Nelson, 1952; Droop, 1954). Test tubes containing sea water enriched with f/2 medium were inoculated with 1 mL of diluted solution. The clones were kept to develop for 10 to 15 days. That was stock for mass culture. The stock culture of *S. costatum* was maintained in 20 mL sterilized test tubes with enriched sea water and inoculated with 0.1 mL of stock culture and then incubated in light with a photoperiod of 12 h for seven to ten days.

Secondary and Mass culture of *Skeletonema costatum* were done in 200 liter FRP tank and cemented tanks. Table I describes the procedure of secondary and mass culture of *Skeletonema costatum*. Cell counts was observed by microscope and concurrently salinity (ppt), temperature (°C) and pH were recorded everyday. All test tubes with media were autoclaved for sterilization. Stock culture was maintained for one month, and then transferred to create a new culture line. Inoculation process at the flask level was carried near spirit lamp to avoid the bacterial contamination.

**Table I. Procedure of Secondary and Mass culture of *Skeletonema costatum***

Culture type	Container	Volume	Medium	Inoculant source	Inoculant volume	Incubation period (day)	Activity
Laboratory or Secondary	Conical flask	200 ml	f/2	Stock culture	20 ml	7-10	Selected good culture were transferred to 2 liter flasks from the 4 <sup>th</sup> day after the inoculation
	Flask	2 liter	f/2	200ml flask	200 ml	5-7	Good culture transferred to 20 liter carboys
	Carboy	20 liter	f/2	2 liter flask	2 liter	4-5	Transferred to 200 liter FRP tank
Outdoor or Mass culture	FRP tank	200 liter	f/4	20 liter flask	20 liter	3-4	Transferred to 1 ton and 10 ton cemented tank
	FRP tank	1 ton	f/4	200 liter tank	100 liter	3	Transferred to 10 ton and 20 ton capacity cemented tank
	Cemented tank 10 ton	10 ton	f/4	1 ton tank	1 ton	3	Transferred to 20 tons cemented tank
	Cemented tank 20 ton	20 tons	f/4	10 ton tank	2 ton	3	Transferred to larval rearing tank

Ten % inoculant's was inoculated at all culture levels to get desirable density. From the cemented tank *Skeletonema costatum* was harvested with a common nylon cloth bag or plankton net 150 µ mesh size.

**Preparation of culture medium.** Reagent grade chemicals were used for stock cultures, while technical and agricultural grade nutrients were used for mass culture. The media for *S. costatum* culture were prepared by adding 1 mL of the working stock solution of nitrate, phosphate, silicate, trace metals and vitamins to 1 liter of filtered sea water (Table II & III). Washed and clean flasks (20 mL; 200 mL; 2 L) were filled with media (except vitamins) and autoclaved at 121°C and 15 psi for 15 min. The flasks were allowed to cool down to room temperature and vitamins were added next day just before inoculation. The clean buckets, canes, cemented tanks were filled with filtered sea water and enriched with nutrients on the next day before inoculation. Primary solutions (Table II) of trace metals and vitamin were stocked for one month in refrigerator. Working solutions (Table III) of nitrate, phosphate and silicate were prepared every ten days. The commercial fertilizer media were prepared by Potassium Nitrate-100 g, sodium phosphate-10 g, sodium silicate gel-1 mL, ferric Chloride-4 g and commercial EDTA-10 g. All were added into one ton of filtered sea water.

**RESULTS AND DISCUSSION**

*Skeletonema costatum* is eurythermal (Liao *et al.*, 1983). It grows at water temperature ranging from 3 to 34°C with an optimum temperature of 25° to 27°C. *Skeletonema* growth well in salinities ranging from 15 to 34 ppt with optimal growth being attained at 25 to 29 ppt. The growth rate of *Skeletonema* increases with light intensity progresses through 500 to 10,000 lux and declines at intensities exceeding 10,000 lux (Liao & Huang, 1973).

In this study an illumination of 1000-10,000 lux was maintained for good *Skeletonema costatum* growth. It was observed that light enhance the photosynthetic activity and multiplication of phytoplankton cells. The intensity of light and exposure time plays a vital role in *Skeletonema costatum* growth and development. Optimal light period

varied 10 h of dark per 14 h of light in summer and 12 h of dark per 12 h of light in winter season. Temperature ranged between 25 - 28°C and salinity was maintained 27 ppt to 30 ppt. The pH was maintained from 7.0 to 7.6. The growth of *Skeletonema costatum* attained to peaks on the seventh days for primary culture. The average concentration of *S. costatum* was observed 0.55 x 10<sup>6</sup> cells mL<sup>-1</sup>. Secondary flask culture was the intermediate phase of the pure culture of diatom. The peak period of *Skeletonema costatum* was observed on seventh and sixth days of inoculation in 200 mL flask and 2 L flasks, respectively (Fig. 1). The average density 0.72 x 10<sup>6</sup> cells mL<sup>-1</sup> was observed in 200 mL flask and 0.83 x 10<sup>6</sup> cells mL<sup>-1</sup> in 2 L flask In 20 L carboys, growth of *Skeletonema costatum* reached to the peak within fifth days and the cell concentration was 0.93 x 10<sup>6</sup> cells mL<sup>-1</sup> During the fourth day the average cell density of 0.98 x 10<sup>6</sup> cells mL<sup>-1</sup> was recorded in 200 L FRP tank.

For mass culture of *Skeletonema costatum* two types of culture media (f/4 & commercial fertilizer media) were used. Five phases of growth in the mass culture of *Skeletonema costatum* were observed. In the starting phase, changed the population density of the studied species was not so remarkable. The exponential or growth phase in second and third day of inoculation was characterized by rapid cell division. During the fourth day it was reached the declining phase of relative growth level, then moved the stationary and death phase.

The 10 ton cemented tank culture, enriched with f/4 media *Skeletonema costatum* attained to the peak on third day and the average cell density was recorded 1.2 x 10<sup>6</sup> cells

**Table II. Primary Stock Solutions of *Skeletonema costatum* culture**

S. No.	Nutrients/	(Quantity/1 liter distilled water)
1. (Trace Metals)	CuSO <sub>4</sub>	10 g
	ZnSO <sub>4</sub>	22 g
	CoCl <sub>2</sub> .6H <sub>2</sub> O	10 g
	MnCl <sub>2</sub> .4H <sub>2</sub> O	180 g
	Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	6 g
2. (Vitamins)	Thiamine Hydrochloride	20 g
	Biotin	0.1 g
	Cyanocobalamine	0.1 g

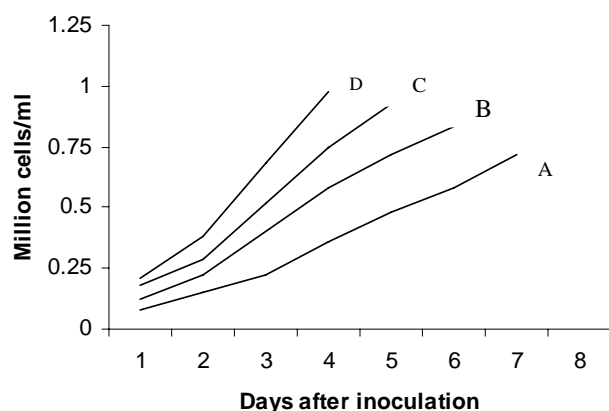
**Table III. Working Stock Solutions of *Skeletonema costatum* culture**

S. No.	Nutrient	Quantity/1 liter of distilled water	Dosage (1 liter of sea water)		
			f	f/2	f/4
1.(Nitrate & Phosphate)	NaNO <sub>3</sub> (sodium nitrate)	75 g	2 ml	1 ml	0.5 ml
	NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O(sodium phosphate)	5 g			
2.(Silicate)	Sodium silicate gel	38 ml	2 ml	1 ml	0.5 ml
3.(Trace metal)	EDTA	4.30 g	2 ml	1 ml	0.5 ml
	Ferric Chloride	3.15 g			
	And 1 ml of P.S.S.	1 ml of P.S.S.			
4.(Vitamins)	Vitamins	5 ml of P.S.S.	2 ml	1 ml	0.5 ml

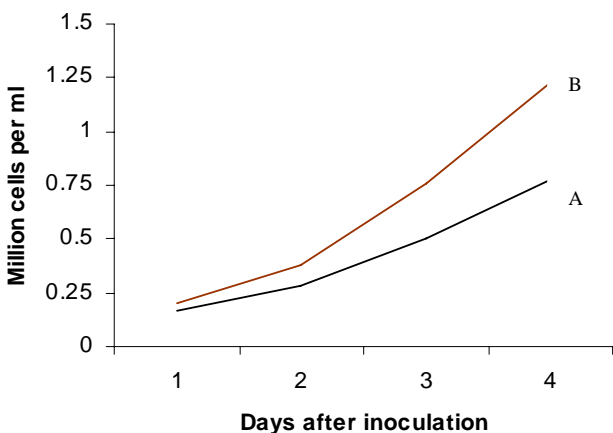
mL<sup>-1</sup>. In the commercial culture media cell concentration was recorded 0.75 x 10<sup>6</sup> cells mL<sup>-1</sup> and attained to the peaks during third day. In 20 ton cemented tank, the cell concentration were reached 1.23 x 10<sup>6</sup> cells mL<sup>-1</sup> and 0.78 x 10<sup>6</sup> cells mL<sup>-1</sup> in f/4 media and commercial fertilizer media, respectively after three days of inoculation.

In this study it was clear that using the f/4 medium influenced the high density of *Skeletonema costatum* but it

**Fig. 1. *Skeletonema* growth in Indoor culture system. (A. 200 ml Flask, B. 2 liter flask, C. 20 liter flask, D. 200 liter carboy)**



**Fig. 2. Growth of *Skeletonema costatum* in 20 ton cemented tank. (A. Commercial fertilizer media, B. f/4 medium)**



was expensive. Whereas the commercial fertilizer medium was less expensive, but cell density was lower. In comparison of two culture medium the commercial fertilizer medium was better related to production process and product quality.

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