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Comparative Analysis on the Growth Response of Diatom *Skeletonema costatum* in Different Culture Media

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Nine culture media, such as Walnes, PM, SEAFDEC, TMRL, Suto, Miquels, Guillard f, f/2 and f/4 have been used to find out suitable media for successful culture of *Skeletonema costatum* which are widely used as larval food of prawns. Among the tested media, Walnes has given best multiplication (14.5 lakhs cells ml^{-1}) of this species under laboratory conditions. The study revealed that the culture has to be harvested on 3rd to 4th day in media Walnes, Guillard f, TMRL, PM, SEAFDEC and Miquels whereas algae in the media of Suto, Guillard f/2 and Guillard f/4 can be harvested on 2nd to 3rd day of culture after inoculation. The availability of nutrients such as nitrate, phosphate, iron and silicate is essential for the production of this species, and their appropriate proportions are required in the culture media to escalate the maximum cell multiplication. To keep the starter culture in good condition, *Skeletonema* culture is needed to be reinoculated on every 3rd day of its culture.

(***Key words:*** *Skeletonema costatum, Microalgae, Culture media, Stock culture*)

Microalgae being the predominant component in the aquatic food chain have got immense value as aquaculture live feed, and as a result the production of unicellular algae has gained importance in several countries due to their wide use as food in the hatchery seed production of commercially important shell and fin fishes. Therefore, the microalgae are indispensable food source in commercial rearing of many cultivated species including all growth stages of bivalve molluscs, larval stages of some crustacean species and very early growth stages of some fin fishes. Microalgae are used for the mass production of zooplankton, which in turn serve as food of the larval stages of fish species. Moreover, the algae are also directly introduced into the larval rearing tanks of marine fishes as they play a vital role in stabilizing the water quality, nutrition of the larvae and microbial control. Among the microalgal species *S. costatum* belonging to the class Bacillariophyceae is the first widely used prawn larval food for *Penaeus monodon* and other prawn species in research and in commercial hatcheries (Fujinaga, 1967, Liao, 1970). Even though *S. costatum* has several advantages over the other microalgal species, the maintenance of its stock culture is always met with difficulties especially during summer season as it could not thrive under high temperature (Mock and Murphy, 1971). However this species has been extensively used for the larval rearing of shrimp (Hudinaga, 1942, Cook and Murphy, 1969). Thus, the development of stock culture of Skeletonema culture is inevitable as it holds great potential in future for breeding of marine prawns in Andaman and Nicobar Islands. In this context an experiment has been carried out to find out suitable culture media for its stock culture maintenance in Andaman waters.

MATERIALS AND METHODS

Microalgae *S. costatum* were isolated from the water samples collected form Dundus point of Bay island by micropipette method (Sournia, 1978) and maintained in 50 ml test tube in algal culture laboratory under controlled conditions of temperature, i.e. 23 to 25° C, pH 7.8 to 8.2, salinity 30 to 32 ppt and illumination provided for 12 h. These cultures were then transferred to 250 ml conical flask under the same environmental conditions of the inoculum. In order to find out befitting media for the successful maintenance of stock culture of *S.costatum*, nine different culture media Walnes (Walney, 1974), PM, SEAFDEC (Pantastico, 1977), TMRL (Tung Kong Marine Research Laboratory, Tahiti), Suto (Suto, 1959), Miquels (Miquel, 1892) Guillard f, f/2 and f/4 (Guillard and Ryther, 1962) were freshly prepared prior to the culture experiments.

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Three haufkin flasks containing one litre of sterilized seawater enriched with culture media in standard proportions were carried out to study the variability, growth rate and differentiation of the culture media along with control (without addition of nutrients). For each media and control, three replicates were maintained and to each culture flask an initial volume of 5 ml algal inoculum having cell density of 15x10^5 cells ml^{-1} was added and maintained under the controlled condition of temperature, i.e. 23 to 25°C, pH 7.8 to 8.2, salinity 32 to 34 ppt, and illumination for 12 hours (2000 lux). The flasks were swirled 3 times daily to enhance gas exchange and to avoid settlement of cells. During the experiment daily one ml of samples were taken from each flask and kept in stoppered test tubes with a few drops of Liguol's iodine solution which helped to kill and stain the cells for counting. Before counting, the samples were vigorously shaken to break up dividing cell and number of cells were counted daily at 10 hrs with the aid of an improved neubauer ruling haemocytometer as per the methodology followed by Smith *et al.* (1993). The mean value of triplicates of each media was accounted for the expression of algal growth. The relative growth rate (k) of algae in terms of cell division was calculated as suggested by Hoogenhout and Amesz (1965).

RESULTS AND DISCUSSION

During the first and second day of the experiment, the tested algae underwent the lag phase which did not show much variation among the tested media and the growth of *S. costatum* was slow during these days in all tested media. This might have been due to deactivation of enzyme or decreased level of metabolic level of inoculum. Cell size may have increased without cell division or some diffusible factors produced by the cells themselves may have been necessary for carbon fixation or introduction of inoculum into a medium containing high concentration of some particular substance. But on the second day onwards all media influenced the growth of algae to start the exponential phase. The media which showed maximum multiplication were 14.5 lakh cells ml^{-1} in Walnes media, 12.2 lakh cells ml^{-1} in Guillard f, 11.00 lakh cell ml^{-1} in TMRL, 10.8 lakh cells ml^{-1} in PM, 9.65 lakh cells ml^{-1} in SEAFDEC, and 8.8 lakh cells ml^{-1} in Miquels medium on 4th day, whereas the peak cell density in Suto, Guillard f/2 and Guillard f/4 was 11.625, 11.15, 9.5 lakh cells ml^{-1}, respectively on 3rd day (Table 1). Thereafter, it showed sudden decline in cell density which might have been due to depletion of particular materials or change in the rate of supply of carbondioxide or oxygen or change in pH. This was due to preferential absorption or with a balance formed between growth rate and limiting factor or depletion of nutrients to a level incapable of sustaining growth or build-up of metabolites to toxic level. In the experiment it was observed that cell size gradually decreased during consecutive cell division and the size of the cell became less than 7μ which accelerated the formation of auxospores, and after this the formation of course chain was repeated. The onset of decay of a Skeletonema culture was observed later in coarser chain lines and at this stage the cultures were neither suitable as food for larvae nor as inoculum for subsequent culture.

Out of the tested media, Walnes medium showed maximum cell density and the relative growth constant k (number of doubling per day) was found to be 4.393 doublings per day on fourth day, whereas other media showed comparatively less doublings on the same day (Table 2). The higher multiplication of *S. costatum* in the Walnes medium may be due to the availability of higher level of nitrate, phosphate, iron, silicate and vitamins,

Table 1. *Multiplication of* S. costatum *in different culture media*

Day	Cell density (lakh cells/ml) in different culture media									
	Walnes	Guillard f	Suto	Guillard f/2	Guillard f/4	PM	TMRL	SEAFDEC	Miquels	Control
1	0.75	0.675	0.6	0.5	0.2	0.625	0.5	0.45	0.35	0.25
2	2.05	1.875	1.7	1.4	0.8	1.45	1.25	1.05	0.95	0.875
3	8.5	7	11.625	11.15	9.5	6.3	5.95	5.65	5.25	1.2
4	14.5	12.2	10.05	10.275	7.5	10.8	11	9.65	8.8	1.95
5	11.75	10.075	4.2	3.55	3.1	9.3	9.55	7.325	7.325	1.3
6	4.575	2.725	3	2.8	2.05	2.95	3	2.9	2.75	0.8
7	1.325	1.175	1.1	1.1	0.5	0.95	1	0.875	0.6	0.375

***Table 2.** Relative growth rate of* S.costatum *(division/day) in different culture media*

Day	Culture media									
	Walnes	Guillard f	Suto	Guillard f/2	Guillard f/4	PM	TMRL	SEAFDEC	Miquels	Control
1	0.909	0.818	0.727	0.606	0.242	0.757	0.606	0.545	0.424	0.303
2	1.242	1.136	1.030	0.848	0.485	0.879	0.757	0.636	0.576	0.530
3	3.431	2.828	4.696	4.504	3.838	2.545	2.404	2.282	2.121	0.485
4	4.393	3.696	3.045	3.113	2.272	3.272	3.333	2.924	2.666	0.591
5	2.848	2.442	1.018	0.860	0.751	2.254	2.315	1.775	1.775	0.315
6	0.924	0.550	0.606	0.566	0.414	0.596	0.606	0.586	0.555	0.162
7	0.229	0.203	0.190	0.190	0.087	0.164	0.173	0.152	0.104	0.065

Cyanocobalamine (B12) and Thiamine Hydrochloride (B1). The relative growth in terms of doubling per day also showed low values during the initial days of culture and the rate of growth was maximum in the exponential phase and thereafter showed a declining trend. The study also elucidated that multiplication was comparatively high in media containing silicates which indicates that an adequate level of silicate is essential for the growth of *S. costatum.* Though the Guillard f, f/2 and f/4 media are similar in composition except the quantity of nutrient added, on the reduction of quantity of the nutrient in f/2 and f/4, they showed noticeable decrease in cell density than the Guillard f. This revealed that nutrients in appropriate proportions were required in the culture media to accelerate the multiplication of cells. Thus, the higher cell density obtained in Guillard f medium is attributed to optimum quantity of minerals, trace metals and vitamins in this medium. Walney (1974), Suba Rao (1981), Wikfors (1986) and Laing (1991) also reported that the nutrients such as trace metals, nitrate and phosphate had an influence on the growth of microalgae. Smith *et al.*(1993) reported that Guillard medium is the most widely used for seawater enrichment and it would support the growth of virtually all marine microalgal species. According to the cell quota concept, the growth rate of algae decreases and growth ceases (Droop, 1975) and biochemical composition decreases (Fabregas *et al.*, 1985, Fernandez-Reiriz *et al.*, 1989) when the nutrient is in shortest supply relative to metabolic needs of the algal population, and the population enters the stationary phase of growth cycle.

In the present study though the control also showed an initial multiplication of cells, the cell density and growth rate were less compared to other media and the intensity of multiplication was less during the subsequent days of culture. This indicates that supplementation of additional nutrients, which are required for their rapid growth and multiplication, is essential for the culture of microalgae. Smith *et al.* (1993) also reported that in the natural seawater, the nutrients are available only in very limited quantity to sustain good growth under culture condition. The present study thus suggests that the natural seawater needs to be enriched with suitable media to cater to the maximum multiplication under controlled condition, and Walnes media can be considered as the suitable media for stock culture maintenance, while the culture needs to be reinoculated at every third day of culture for the maintenance of healthy starter culture of *S. costatum.*

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