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Original Research Article

Culture of the phytoplankton *Skeletonema costatum*, Cleve, 1873

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A B S T R A C T

Algal culture collections are specific repositories for living organisms and play a crucial role in phycology and microbiology as they are a source of high quality research material. Moreover, culture collections also serve purposes in education, ex-situ conservation and industry, e.g. production of biofuels or dietary supplements. Present study conducted from December 2009 to November 2010. Marine diatom, *Skeletonema costatum* were collected from Vellar estuary and cultured at CAS in Marine Biology, Annamalai University in India. One species of marine diatom, *Skeletonema costatum* (Bacillariophyceae) were commercially cultured in laboratory conditions.

Introduction

Diatoms are indisputably the major component of many food webs, and so estimating their seasonal abundance, fluctuations and growth rate have been, and will be, an important component of marine science studies. Aquatic environments are subject to high temporal variability, with frequent reorganization of relative abundance and species composition of phytoplankton, as a result of interaction between physical, chemical and biological variables (Reynolds *et al.*, 2000). Like all other marine organisms, diatom eco-physiology is influenced by water temperature, salinity, light intensity and nutrient concentrations (Tomas, 1996; Thomas and Sommerfeld, 1998; Thessen *et al.*, 2005).

Temperature is an important factor controlling the algal growth in natural environments (Lund, 1949; Talling, 1955) and growth response to temperature may be essential in regulating the predominance of phytoplankton species. It is well known that salinity is an important abiotic factor affecting phytoplankton growth. Wide ranges of salinity and temperature may explain frequent appearance of phytoplankton throughout the year in the ocean (Hoshiai *et al.*, 2003).

Skeletonema costatum commonly dominates the diatom abundance in coastal waters (Pratt, 1965, Durbin and Durbin 1981, Reid *et al.*, 1985, Marshall and Ranasinghe 1989, Ramaiah and Furuya 2002, Balkis, 2003).

The intercalary valves of the chains *Skeletonema* are all alike and different from the separation valves (Crawford, 1979). *Skeletonema* is increasingly used as live feed in the larviculture of penaeids, polychaetes, lobsters, marine ornamental prawns, barnacles, mussels, oysters, scallops and pearl spats (Kitto and Regunathan, 1997). Dried cells are an excellent substrate bed for edible mushroom culture. For copepods, live *Skeletonema* cells form an excellent feed (Paffenhöfer, 1970). In the present study, *S. costatum* was chosen because it is an important coastal organism that can tolerate a wide variety of light regimes and temperatures, and it is an ideal laboratory organism that grows readily in various media. It is also a worst-case selection in terms of non-toxic bloom management because it grows rapidly and attains high population densities.

Materials and Methods

Culture species

The marine centric diatom, *S. costatum* was collected from Vellar estuary. It was isolated, axenic culture developed and stock culture maintained in Guillard's F/2 medium at MoES-ICMAM "Marine Ecotoxicology (PD/11th plan/ME/CAS/54/2007)" Project Algal Culture Laboratory, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai.

Isolation of Phytoplankton

The phytoplankton samples were diluted and filtered with seawater. The sample was again diluted and one mL was transferred to a Sedgewick Rafter counting chamber. Single cell of the diatoms was picked up from the counting chamber by using micropipette under an inverted microscope (Olympus, CH2Oi BIMF, Noida). Subculture of the isolated species was done with autoclaved seawater which was filtered through 0.45 mm Millipore and enriched with F/2 medium at the room temperature of 25°C and the water temperature of 20°C with salinity 30 ppt. Most species grow best at a salinity that is slightly lower than that of their native habitat, which is obtained by diluting sea water with tap water. Light (4500 ± 400 Lux) was provided and maintained 12:12 h light:dark cycle. The isolation process was carried out until getting the mono strain of the *S. costatum*, for getting the bacteria free strain, various doses of antibiotics were used and absolutely bacteria free mono strain was found above a dose of 200 units-penicillin/ mL, 200 µg-Streptomycin/mL solution were found to be lethal for *S. costatum* and cells were grown in axenic condition. The mono species of *S. costatum* was inoculated for a mass culture. The experiments were conducted in 5 liter conical flasks with 50 ml and 20 liters of glass tank with 200 ml of 4–5 days old exponentially grown algal cultures (Fig. 1). The cell density was estimated at every 24 hours intervals in all the experiments.

Morphology and identification of *S. costatum*

S. costatum was observed under microscope to their morphological characteristics (Olympus, CH2Oi BIMF, Noida and Fluorescence microscope) (Fig. 3). For identification of the species following literature was used viz., Venkataraman (1939), Subrahmanyan (1946), Prescott (1954). Growth characteristic experiments were done to find out the best growth condition for mass culture.

Chlorophyll 'a'

Chlorophyll 'a' analyses were performed by the modified method of Strickland and Parsons (1972). In 2 ml of algal culture, 5

ml of acetone was added and vortexed for one minute and kept at 4ºC under dark in refrigerator for 24 hrs. Then the samples were centrifuged at 5000 rpm for 10 min. supernatant was read at 630, 645 and 660 nm in UV-Vis. Spectrophotometer (Perkin-Elmer Lamda 25). Raw acetone used as blank.

Results and Discussion

Systematic position of *Skeletonema costatum* (Greville) Cleve, 1873

Empire	**:**	*Eukaryota*
Kingdom	**:**	*Chromista*
Division	**:**	*Ochrophyta*
Class	**:**	*Mediophyceae*
Order	**:**	*Thalassiosirales*
Family	**:**	*Skeletonemaceae*
Genus	**:**	*Skeletonema*
Species	**:**	*S. costatum*

Description of the species

Cells were cylindrical with rounded ends. Cells form long straight chains, held together by fine marginal processes, parallel with longitudinal axis. Spines are straight and slender and unite with the spines of the next cell to form a junction hence, two chromatophores per cell. Nucleus is central. Chain in girdle view, uppermost cell with two chloroplasts was observed (note that the linking structures may be much shorter and less distinct). The *S. costatum* valves small, lens shaped with rounded ends and form long and slender chains with the help of marginal spines.

In the present study, the growth of *S. costatum* was observed, the average cell density $21X10^3$/ml in log phase and the average cell density $328X10^3$/ml at the time harvesting in stationary phase. The chlorophyll 'a' concentration (19.823µg/l) was analyzed with the help of UV-Vis Spectrophotometer (Fig. 2).

In the present study, *Skeletinema costatum* were cultured successfully in both in-door and out-door systems with maximum biomass. Cell growth and biomass of *S. costatum* was clearly indicating that, their production efficiency and high growth rate with cost effective and environment friendly nature. Cells were found to multiply and increase day by day for entire culture period.

The maximum growth of *S. costatum* was found to be in the stationary phase of 4–6 days. During the stationary phase the average cell density was $328X10^3$/ml. On the 9^{th} day onwards *S. costatum* appeared as dark brown colored. Maximum density was obtained on 9^{th} day of the culture whereas the minimum was obtained at 1^{st} day. *S. costatum* is chain forming one and it may occupy more space in the culture tank, the biomass (wet weight) of *S. costatum* was comparatively high. Similarly, *S. costatum* showed best performance in terms of growth in out-door culture, it was high enough to yield a large biomass in culture within a reasonable period of time. Since the *S. costatum* has chain forming nature which could increase the total biomass (Rekha *et al.,* 2012).

Salinity is an important factor, which profoundly influences the abundance, distribution, growth and composition of phytoplankton in the marine environment. There are several species of diatom, which showed higher rate of growth in different salinities. Diatoms from estuaries have the widest adaptability to any change salinity of the external medium (Williams, 1965). Growth rates of estuarine clones are not very much affected in media of wide salinities and the clones isolated from sea do not survive in lower salinities.

Skeletonema is characterized by "cylindrical cells with a peripheral ring of tubular processes, fultoportula, that runs perpendicular to the valve face and link to those of sibling valves to form permanent colonies of variable length" (Zingone *et al.,* 2005). The production and management of *Skeletonema costatum* cultures can be achieved in a number of ways without adversely affecting the quality of the algal cells produced. In culture condition the phytoplankton growth has been reported to increase with increasing intensity at light up to a point declined thereafter. This trend has been widely reported in many phytoplankton species.

The optimum requirement of light different in the range between 2500 and 6000 Lux for different diatom species viz, *S. costatum, Asterionella socialis* and *Navicula* (Hoff and Snell, 1989). Small portions of cellular carbon lost to the medium during dark periods following period of photo assimilation (Hellebust, 1974). Nutrition is essential component for phytoplankton and its groups. The growth depends on the availability of certain nutrients like nitrogen phosphorus and silicon. These nutrients were found to limit the reproduction in the marine phytoplankton (Carpenter *et al.,* 1971).

Environmental shifts that result in improved conditions can induce a suite of molecular and physiological responses that lead to higher growth rates (Schaecter, 1968). Time lags in the response of phytoplankton to improved growth conditions have been observed in the field (Macisaac *et al.,* 1985). Identification of genus *Skeletonema* is according to the key characteristics: cylindrical cells with a ring of long processes emerging from the edge of the valve face. However, exact identification of species in the genus *Skeletonema* is very difficult due to varying expression (Castillo *et al.,* 1995). In the present work, we have obtained cultures of N-deficient *S. costatum* cells by using nitrite as the limiting N source in the Medium (Juan *et al.,* 1978). The results show that *Skeletonema costatum* grows exponentially at 2.0 div. d^{-1} under the present light and temperature conditions, which is near the optimum growth rate (Sakshaug and Andresen, 1986).

The *S. costatum* cultures produced a distribution of cells dominated by single cell and 2 cell-long chains, with some 3, 4 or 5 cell-long chains also present (Gerard *et al.,* 1988). An altered light regime may limit phytoplankton growth and productivity. In response to this stress, diatom cells undergo physiological changes to protect themselves (production of photoprotectant pigments), or accumulate 'non-protective' components, which are the result of a stress-induced metabolic impairment. *Skeletonema costatum* a marine chain forming centric diatom species is common in the coastal waters of India (Perumal *et al.,* 1999). *S. costatum* using bi-algal cultures under axenic conditions, and our findings indicated that the growth of either species could be suppressed by the unidentified allelochemicals of the other, depending on cell densities (Yamasaki *et al.,* 2007).

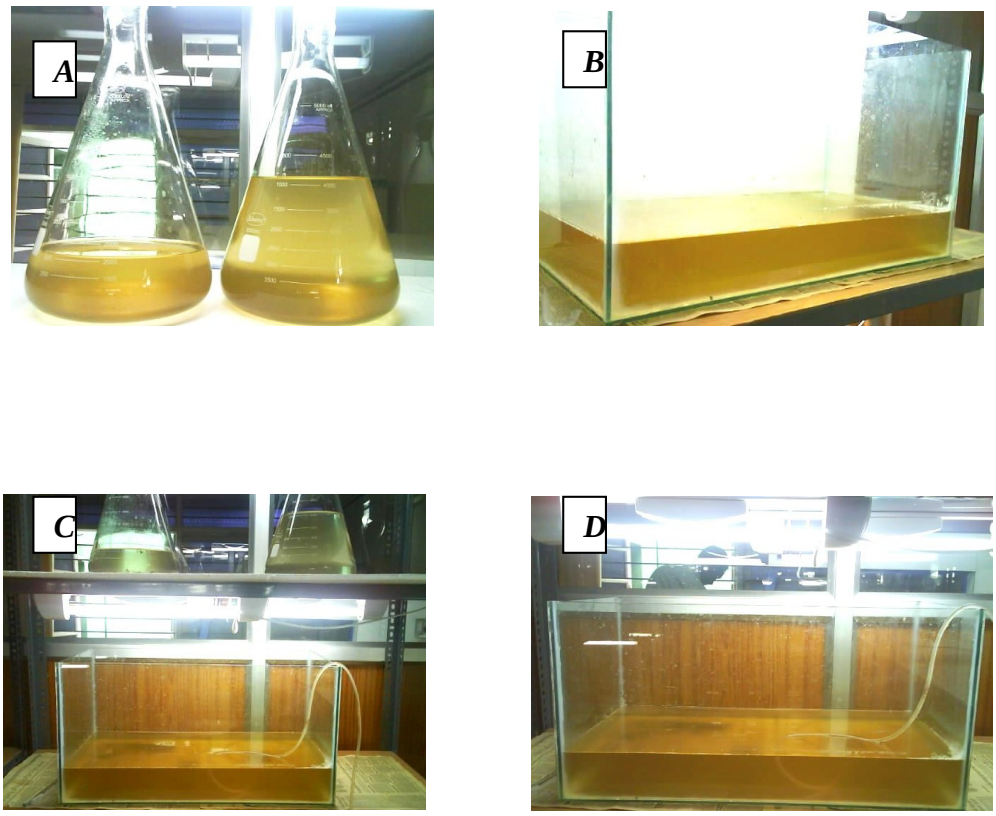

Figure.1A. Showed *S. costatum* culturing in 5 liter conical flask. B, C and D showed *S. costatum* culturing in Glass tanks

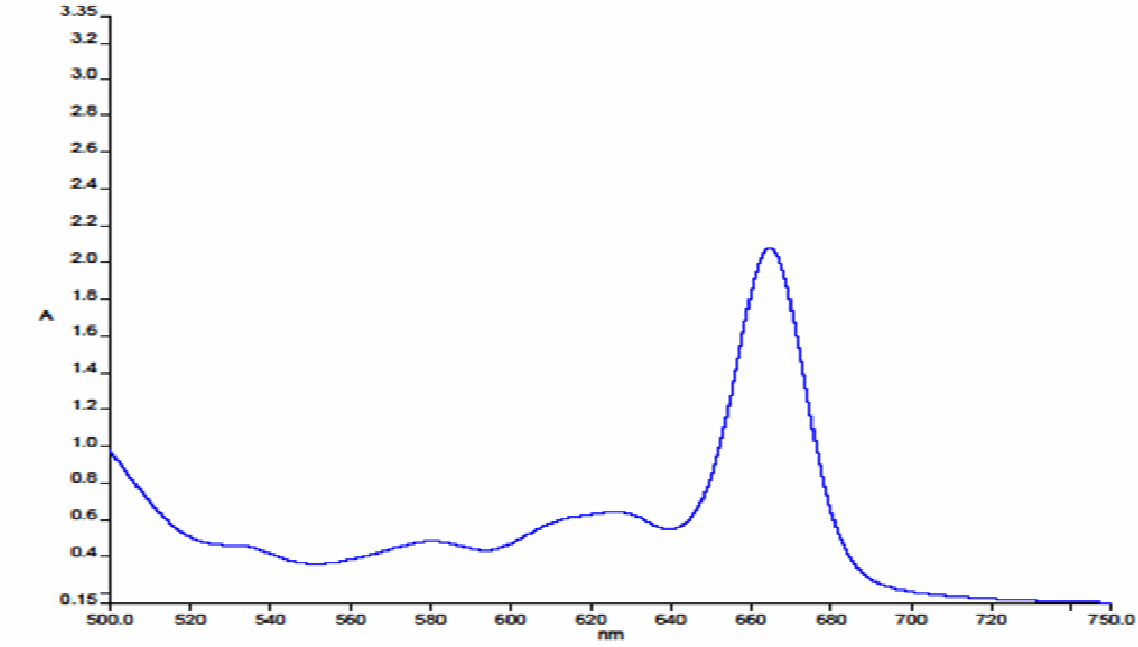

Figure.2 Concentration of chlorophyll 'a' (UV-Vis Spectrophotometer)

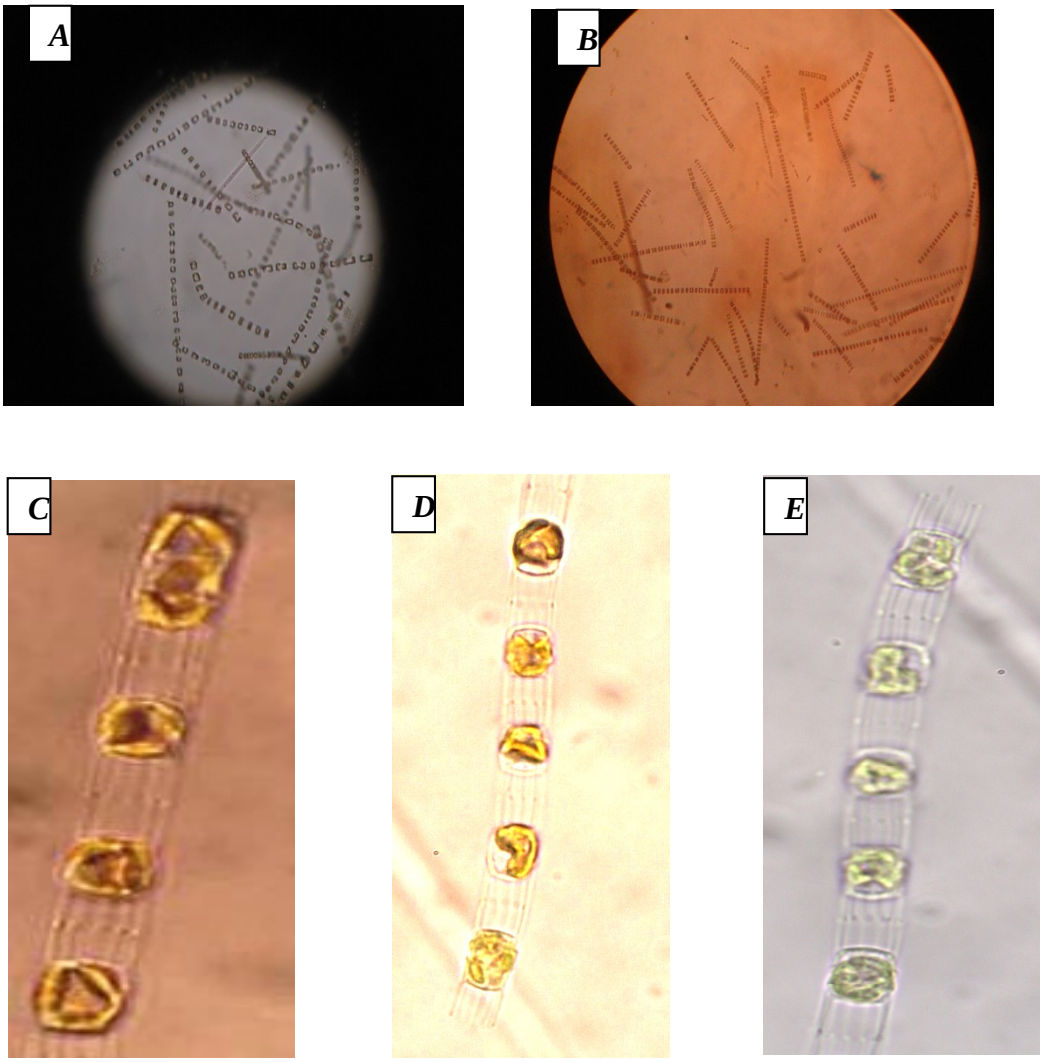

Figure.3 Microscopic view of *Skeletonema costatum*, A and B showed Light microscopic (10x) view of *S. costatum* C, D and E showed Florescence microscopic (40x) view of *S. costatum.*

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