

## Physiological observations on a diatom *Skeletonema costatum* (Greville) Cleve

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### Abstract

A chain-forming diatom *Skeletonema costatum* (Greville) Cleve collected from Yatsushiro Sea, Japan was cultured to determine the optimum level of some physico-chemical factors for their growth under laboratory conditions. Filtered and sterilized aged sea water enriched by adding nutrient solution (Provasoli 1968) was used as the culture medium. The plankton could tolerate a wide range of salinities (3-55 ppt). Optimum growth was observed at salinities of 20-35 ppt, temperatures of 20-25°C, light intensities of 80-120  $\mu\text{E m}^{-2} \text{sec}^{-1}$  and pH between 7.5 and 8.0. Growth did not occur at salinities below 3 ppt and at temperatures above 30°C. From the present study, it is concluded that *S. costatum* was extremely euryhaline and tolerable to very low salinities.

**Key words :** Diatom, *Skeletonema costatum*, Physico-chemical factors, Euryhaline

### Introduction

Mass culture of marine microalgae has received much attention recently due to their potential use as live feed in the culture of zooplankton and rearing larval forms of commercially important crustaceans, molluscs, and fishes. Diatoms have for many years been recognized as an extremely important source of food for planktonic animals. It provides one of the few practical means of feeding aquatic filter feeders and, without doubt, is the most important food for the pelagic copepods and indirectly for the fish larvae.

*Skeletonema costatum* is widely distributed, euryhaline and abundant in estuaries, particularly in the spring. In both fresh and marine waters, this species is ingested by lower animals (protozoa, insect larvae, copepods and rotifers) which in turn are eaten by higher animals such as fish. In many countries this diatom is considered as one of the best algae for feeding prawn larvae. This species is also very important due to its potential use as valuable assay organism for examining water quality. Sanchez *et al.* (1995) suggested that *S. costatum* can serve as a good biological source of proteins and fatty acids.

*S. costatum*, in some situations, can have a negative effect, forms heavy blooms when gets suitable environment due to eutrophication and causes economic losses to aquaculture. This microalga is a major component of most plankton blooms, observed in eutrophic waters, particularly associated with toxic microorganisms in USA, Romania, France, Norway, Uruguay, China, Japan and Hongkong.

The abundance of phytoplankton in nature is regulated by a multitude of environmental factors such as nutrients, light, temperature, salinity, and grazing. Iwasaki (1979) mentioned that knowledge of the physiological characteristics of phytoplankton is indispensable for understanding their growth mechanism.

Ecological and physiological parameters may vary for different strains of same species of algae. Honjo (1993) reported five different optimum salinities for five different strains of *Heterosigma akashiwo* (Hada) Hada. There are some studies on the occurrence, morphology and autecology of *S. costatum* (Medlin 1991, Blanchemain et al. 1994). There is no published report on the effects of physico-chemical factors on the growth of *S. costatum* in Yatsushiro Sea, Japan. The purpose of this study was to determine the effects of temperature, salinity, light intensity and pH on the growth of Yatsushiro Sea's strain of *S. costatum*.

## Materials and methods

*Skeletonema costatum* used in this study was collected in 1991 from Yatsushiro Sea, Japan. An axenic culture was established by using the micropipette washing method. Stock cultures were grown in Provasoli's ES medium (Provasoli 1968) at  $25 \pm 1^\circ\text{C}$ , light intensity  $60 \mu\text{E m}^{-2} \text{sec}^{-1}$  and photoperiod 12:12-h, L:D cycle.

Growth was determined at temperatures from 15-40°C in 5°C intervals, salinities from 0-65 parts per thousand (ppt), pH from 6.5 to 9.5 in 0.5 intervals and with irradiance adjusted to 10, 20, 40, 80, 120 and 200  $\mu\text{E m}^{-2} \text{sec}^{-1}$ . Two salinity series (one spanning 0-5 ppt in 1 ppt intervals to determine minimum salinity for growth, and the other 5-65 ppt in 5 ppt intervals) were established by evaporating filtered Kagoshima Bay water (34.6 ppt) to get higher salinity media and by diluting with deionized water to get lower salinity media. The pH of the medium was adjusted by addition of 1 N HCl or 1 N NaOH.

Culture media were autoclaved for 15 min at 121°C, and aged for several days prior to inoculation. Before starting the experiment the algae were acclimated to the experimental condition for at least two generations. Cells of mid logarithmic growth phase were used for inoculation to ensure that the cells were nutritionally replete. Sterilized micropipette were used to transfer the inocula. Individual growth medium in the culture tubes was gently shaken once a day for accelerating growth and to avoid settlement of algal cells. All growth studies were done in triplicate. The cell concentration was determined by direct counting by using a Sedgewick-Rafter chamber. Counts were made immediately after inoculation and then each other day up to 10 days. For reducing errors due

to possible synchronous divisions counts were made at the same time each day. The average number of cell divisions per day (K) for the 6-day growth period was calculated from:

$$K = \ln \frac{C_t}{C_0} \left( \frac{1}{t \ln 2} \right)$$

where,  $C_t$  and  $C_0$  are cell concentrations at times  $t$  and  $0$ , respectively (Guillard 1973).

Division rates under different conditions were subjected to analysis of variance (ANOVA) (Statview S.E. + Graphics, Abacus. Concepts, Inc.). Significant differences among the means were determined using Duncan's multiple range test (DMRT) (Gomez and Gomez 1984).

## Results

Growth of *Skeletonema costatum* at different salinities and at constant temperature (25°C), irradiance (60  $\mu\text{E m}^{-2} \text{sec}^{-1}$ ) and pH (8.2) is shown in Figs. 1-3. The plankton could tolerate a wide range of salinities (3-55 ppt). It grew well at the salinity range of 20-35 ppt. The maximum cell density  $11.46 \times 10^5$  cells  $\text{ml}^{-1}$  was found at 20 ppt on the 6th day (Fig. 2). No lag phase was exhibited at salinities from 10-45 ppt with the exponential growth from the 2nd to 6th day. The alga failed to grow below 3 ppt but cells were able to survive up to 8 days at 2 ppt. At 0 and 1 ppt no living cells were found after 2 days and 5 days, respectively (Fig. 1). Cultures at higher ranges of salinity (50-55 ppt) exhibited a short lag phase (2 days). The exponential growth was found from the 4th to 8th day with maximum growth on the 8th day at higher salinities (Fig. 2). Neither increase nor decrease in cell numbers occurred in the media at 60 ppt by the 4th day and thereafter the number of cell started to decline. No growth was observed at 65 ppt and the rate of survivability was transient.

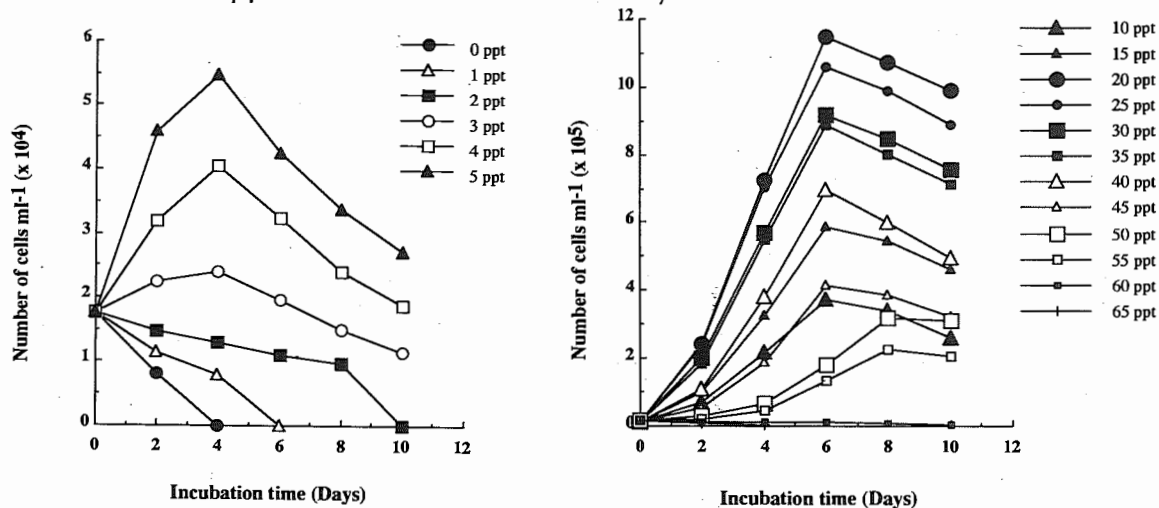


Fig. 1. Growth curves of *S. costatum* in media of low salinities (0-5 ppt).

Fig. 2. Growth curves of *S. costatum* in media of medium (10-45 ppt) and high salinities (55-65).

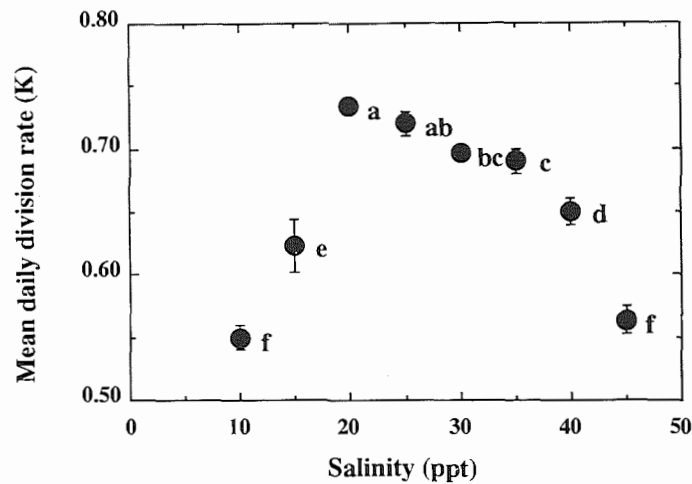


Fig. 3. Mean daily division rate of *S. costatum* at different salinities. Each point and vertical line represent mean  $\pm$  SD for three replicates. Means with different letters are significantly different (Duncan's multiple range test,  $p < 0.05$ ).

Analysis of variance (ANOVA) showed that the difference in mean daily division rate at various salinities were highly significant. The highest mean daily division rate (0.73 divisions day<sup>-1</sup>) was observed at 20 ppt which was not significantly higher than at 25 ppt (Fig. 3). The division rate of the plankton in relation to different salinities showed that the growth tends to increase from 10 to 20 ppt and showed a slow declining trend up to 40 ppt then a rapid declining trend from 40 ppt (Fig. 3).

The relationship between growth and temperature at constant salinity (30 ppt), irradiance (60  $\mu\text{E m}^{-2} \text{sec}^{-1}$ ) and pH (8.2) is presented in Figs. 4 and 5. The plankton was cultured at different temperatures from 15 to 40°C in 5°C intervals. The maximum cell density 9.80  $\times 10^5$  cells ml<sup>-1</sup> was at 20°C on the 6th day. No lag phase was exhibited at temperatures from 20 to 25°C with the exponential growth from the 2nd to 6th day. The alga failed to grow at 35°C but cells were able to survive up to 6 days. At 40°C, no living cells were found on the second day after inoculation.

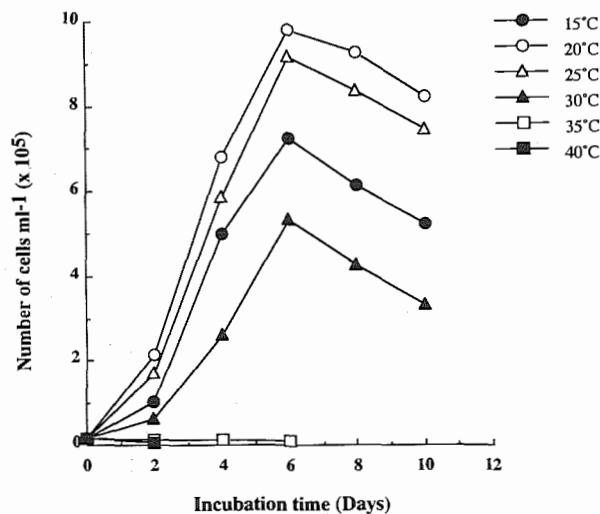


Fig. 4. Growth curves of *S. costatum* at different temperatures.

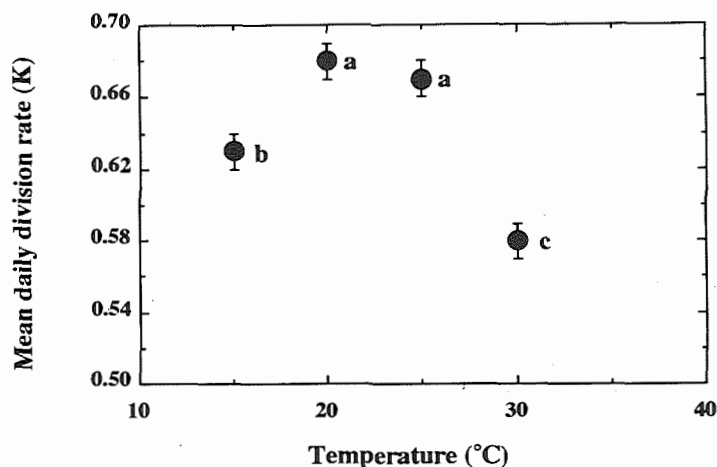


Fig. 5. Mean daily division rate of *S. costatum* at different temperatures. Each point and vertical line represent mean  $\pm$  SD for three replicates. Means with different letters are significantly different (Duncan's multiple range test,  $p < 0.05$ ).

The division rate of the plankton in relation to different temperature levels shows that the growth tends to increase from 15° to 20°C and then shows a declining trend (Fig. 5). The highest division rate 0.68 divisions day<sup>-1</sup> was observed at 20°C which was not significantly higher than at 25°C. The mean division rate decreased significantly with increasing temperature of above 25°C.

Growth of *S. costatum* at different pH's with the fixed temperature (25°C), salinity (30 ppt) and light intensity (60  $\mu\text{E m}^{-2} \text{sec}^{-1}$ ) is shown in Figs. 6 and 7. During the course of the experiment, the final pH of the culture media changed slightly (within 0.05 pH units) from the initial pH. The maximum cell density (10.37  $\times 10^5$  cells ml<sup>-1</sup>) was recorded at pH 7.5 on the 6th day. The rapid growth was found at pH 7.0-8.5 without passing any lag phase. The highest division rate (0.73  $\pm$  0.01 divisions day<sup>-1</sup>) was observed at pH 7.5 which is not significantly higher than at pH 8.0 (0.72  $\pm$  0.01 divisions day<sup>-1</sup>) (Fig. 7). Again pH 7.0 and 8.5 were equally effective. Slower growth was found at pH 9.0 and 9.5 with maximum cell yields of 5.77  $\times 10^5$  and 4.89  $\times 10^5$  cells ml<sup>-1</sup>, respectively on 6th day of culture. Poor growth was found at pH 6.5 with 2 days of lag phase.

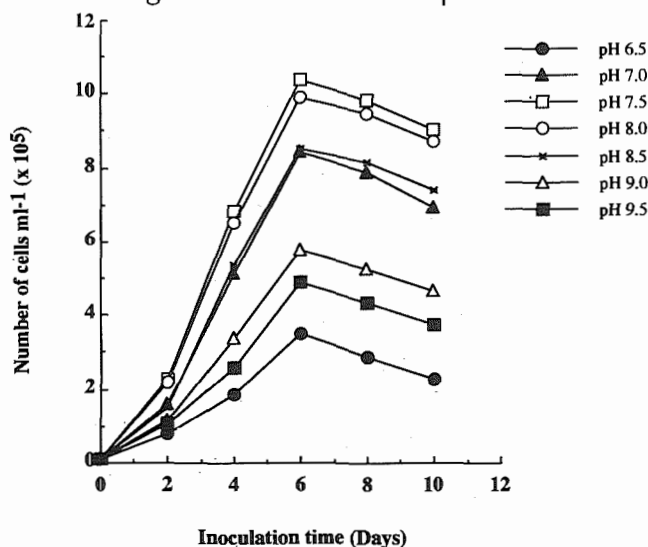


Fig. 6. Growth curves of *S. costatum* at different pH.

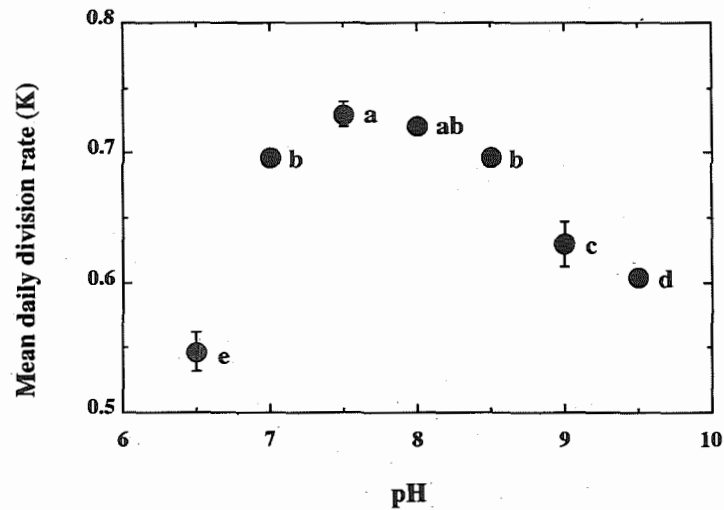


Fig. 7. Mean daily division rate of *S. costatum* at different pH. Each point and vertical line represent mean  $\pm$  SD for three replicates. Means with different letters are significantly different (Duncan's multiple range test,  $p < 0.05$ ).

*S. costatum* was cultured at different light intensities, from 10-200  $\mu\text{E m}^{-2} \text{sec}^{-1}$  for 10 days. The plankton grew well with light intensities more than 40  $\mu\text{E m}^{-2} \text{sec}^{-1}$  (Fig. 8). The optimum light intensity for its growth was 120  $\mu\text{E m}^{-2} \text{sec}^{-1}$ . The growth was comparatively slower and poorer at 10  $\mu\text{E m}^{-2} \text{sec}^{-1}$  and the growth curve tends to increase up to the 4th day and then entered into the stationary phase (Fig. 8).

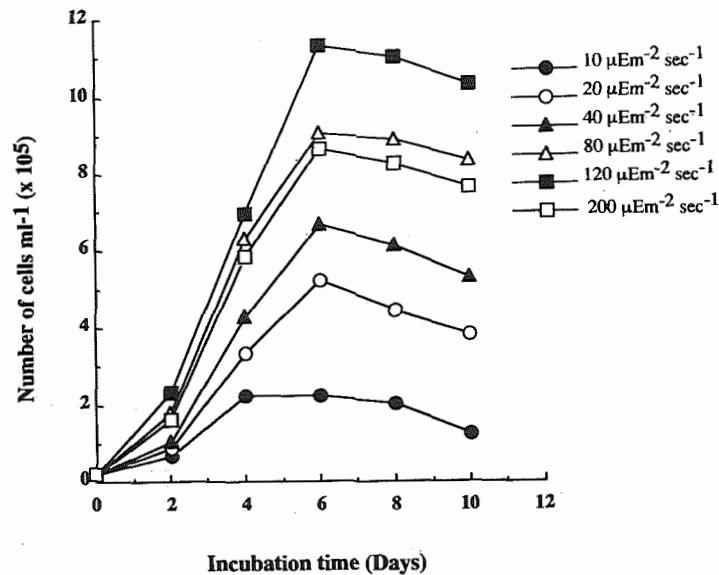
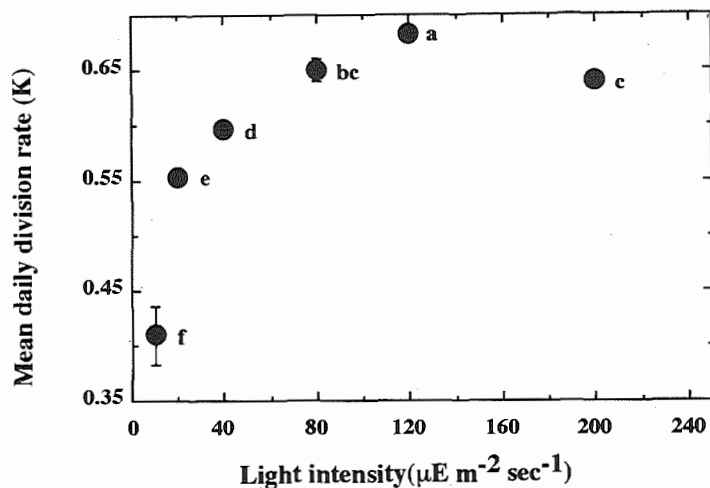


Fig. 8. Growth curves of *S. costatum* at different light intensities.



**Fig. 9.** Mean daily division rate of *S. costatum* at different light intensities. Each point and vertical line represent mean  $\pm$  SD for three replicates. Means with different letters are significantly different (Duncan's multiple range test,  $p < 0.05$ ).

Analysis of variance (ANOVA) showed that the difference in mean daily division rate at various light intensities were highly significant. The highest division rate ( $0.73 \text{ divisions day}^{-1}$ ) was observed at  $120 \mu\text{E m}^{-2} \text{sec}^{-1}$  which was significantly higher than all other light intensities (Fig. 9). No significant difference in division rate was observed between  $80$  and  $200 \mu\text{E m}^{-2} \text{sec}^{-1}$ .

## Discussion

In the experiments it was found that within a range of 10-45 ppt salinity 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 microalgal culture.

The salinity tolerance of phytoplankton varies with species and strains. Table 1 summarizes the data reported by several investigators on the range of salinity tolerance and the optimum salinity for growth of some marine diatoms. Shimura *et al.* (1979) reported that the optimum salinity for the growth of Harima Nada's strain of *S. costatum* was 25 ppt which was within the optimum salinity range (20-35 ppt) in cultures of the present study. *S. costatum* of Yatsushiro Sea could tolerate a wide range of salinities, ranging down to 3 ppt or up to 55 ppt and it does not agree with that reported by Shimura *et al.* (1979) for Harima Nada's strain (4.4-40 ppt). Other diatoms were found to be less euryhaline than our strain of *S. costatum* (Table 1). On the otherhand, red-tide producing dinoflagellates and phytoflagellates are generally highly sensitive to lower ( $< 15$  ppt) salinities (Khan *et al.* 1995, 1996). White (1978) reported that a red-tide producing dinoflagellate *Gonyaulax excavata* (Braarud) Balech did not grow

below 10 ppt. Similar observations were found for raphidophycean flagellates *Fibrocapsa japonica* (Toriumi and Takano) (Khan et al. 1996) and *Chattonella antiqua* (Hada) Ono (Khan et al. 1995).

**Table 1.** Range of salinity tolerance and optimum salinity for the growth of some marine diatoms

Diatoms	Salinity tolerance (ppt)	Optimum salinity (ppt)	Author
<i>Skeletonema costatum</i>	3.0-55	20-35	Present study
<i>S. costatum</i>	4.4-40	25	Shimura et al. (1979)
<i>Cerataulina pelagica</i>	6.0-45	20	Takano (1963)
<i>Chaetoceros radians</i>	6.0-48	15	Takano (1963)
<i>Cyclotella criptica</i>	3.4-51	11.2	Liu & Hellebust (1976)
<i>Cyclotella nana</i>	5.0-32	16	Guillard & Ryther (1962)

In the present study, optimal growth of *S. costatum* occurred under a narrow temperature range (20-25°C), which agrees fairly well with those previously reported for *Chaetoceros armatum* T. West (Lewin and Mackas 1972) and *Gymnodinium catenatum* Graham (Ellegaard et al. 1993). Although many phytoplankton species are very resistant to temperature change (Tomas 1978, Watanabe et al. 1982), *S. costatum* is very sensitive to high temperature. The temperature tolerance range observed in the present study does not agree with those reported by Admiraal (1977) for three estuarine benthic diatoms *Nitzschia* c. f. *dissipata* (Kützting) Grunow, *Amphiprora* c. f. *paludosa* W. Smith and *Nitzschia sigma* (Kützting) W. Smith. All of them were found to be more tolerant to high temperatures than *S. costatum* and optimum temperature of these three species were 25°C or higher. In the present study, the plankton failed to grow at 35°C, although cells were able to survive up to 6 days and were found to be either severely damaged or died rapidly at 40°C, in agreement with previous reports of Saks et al. (1974) who found that temperature above 36°C stress many marine and estuarine algae and tend to inhibit growth. The temperature tolerance range of *S. costatum* was different from the description of Tomas (1978) for some coastal phytoplankton species, who found them to tolerate a wide range of temperatures and reported to be eurythermal.

Though most marine and estuarine phytoplankton studied in laboratory conditions have a similar pH tolerance, an optimum pH at around 8.0 and a decrease in growth rate at more acidic and alkaline pH values (Kain and Fogg 1958), but it has sometimes been observed that growth of some species was enhanced in acid media. Some strains of *Chlorella kessleri* and *Chlorella saccharophila* could tolerate pH as low as 3.0 (Kessler 1980). Ikemori and Nishida (1967) suggested that microscopic algae showed decreasing growth rates with increasing pH values. Goldman et al. (1982), while studying the effect of pH in intensive continuous microalgal cultures using pH range from 7.6-10.6, observed that although *Chlorella vulgaris* Beij grew up to pH 10.6, it was



adversely affected by alkaline pH. In our study, the optimum pH for the growth of *S. costatum* was found to be between 7.5-8.0. Adequate growth was also found at pH 7.0 and 8.5. Even at pH 9.0 and 9.5, growth rates were 0.63 and 0.60 divisions day<sup>-1</sup>, respectively. Nishijima and Hata (1986) reported that a raphidophycean flagellate *C. antiqua* grew well at pH 8.0-8.2, could not grow at pH 6.5 and adversely affected at pH 7.0-7.5. From these results, it is concluded that *S. costatum* can tolerate broader ranges of pH.

Algae being mostly photoautotrophic require light for their growth and the effect of light depends on the quality and intensity of light. The optimum light intensity for the growth of the plankton (*S. costatum*) was 120  $\mu\text{E m}^{-2} \text{sec}^{-1}$ . Cell division was limited at low light intensity (Fig. 9). Similar results were found by Lewin and Mackas (1972) for *Asterionella socialis* Lewin and Norris.

From the present experiment, it is concluded that *S. costatum* from Yatsushiro Sea, Japan is extremely euryhaline and tolerable to very low salinities. Optimum growth occurred under a narrow range of temperature and it was strongly inhibited at high temperatures. Nutrients are one of the most important environmental factors that influence growth of any alga. The nutrient dynamics in Yatsushiro Sea, Japan and their effects on growth of *S. costatum* and other phytoplankton are needed to be studied.

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