THE EFFECTS OF NUTRIENTS ON *THALASSIOSIRA FLUVIATILIS* (HUST.) IN DARK AND LIGHT CONDITIONS

ABSTRACT

The effects of nutrients on growth of *Thalassiosira fluviatilis* were found out by incubating the culture for a period of one week in complete darkness and exposing it to light of 3000 lux intensity continuously. Complete darkness did not favour much growth though the nutrients are supplied as in F/2 medium level. When the culture exposed to light after dark period, maximum growth was observed at full enrichment level. Nitrogen and phosphorous had been found to be very effective than other nutrients in enhancing or declining the growth of *T. fluviatilis*.

LONG periods of darkness are a feature of natural environment, yet comparatively little is known of their influence on the light activities of algal metabolism (Yentsch and Reicherf, 1963). The algae incubated in continuous light for a longer duration when subjected to darkness for a short period capable of increasing in its growth. The present study is an attempt to explore how long can an autotrophic microalgae retain its viability in the dark when the nutrients are supplied in a different compositions and its growth response to periods of light exposure.

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Materials and methods

Unialgal culture of *Thalassiosira fluviatilis* grown exponentially at 30‰ salinity (Temperature 29±2°C ; pH 7±0.5) under 3000 lux of continuous light was inoculated in sterilized seawater and kept for four days in dim light. A series of media omitting nitrate (NO_3), phosphate (PO_4), silicate (SiO_3), trace metals (TM) and vitamins (V) from Guillard medium of F/2 (Guillard, 1967) and the medium containing only NO_3, PO_4, SiO_3, TM and V as in F/2 level were prepared and sterilized separately. F/2 medium and sterilized seawater were used as two controls. The nutrient deficient cultures were then inoculated with series of media in 150 ml flask containing 50 ml media and incubated for 7 days in continuous dark. The experiment was set up in duplicate. Every day aliquots were taken from these culture for growth estimation and the same culture was exposed to continuous light of 3,000 lux intensity for a week. Cell counts were taken from these flasks every day with Sedgewick Rafter Counter and growth rate was estimated using the formula

$$K = \frac{\log_2 (N_1 - N_0)}{t_1 - t_0}$$

where N_1 and N_0 are the cell concentrations at the end and beginning of a period of time T days (Guillard, 1973).

Results

The final yield of cell number and divisions/day after a week in the dark and after 7 days in the light are shown in Table 1 a, b. The culture showed very low growth when incubated in the dark, particularly when sea-

water alone used as medium (Control I). The addition of NO_3 and PO_4 individually did not promote much growth in the dark whereas the full enriched medium (F/2,control II) favour maximum growth among all the culture conditions (K = 0.016). With the omission of nutrients from the F/2, nitrogen deficiency supported growth of 0.01 divisions/day and the generation time was decreased with omission of PO_4, SiO_3 and V. TM deficiency did not decrease the growth rate like other nutrients.

TABLE 1 a. *Effects of nutrients under continuous darkness on growth rate (Final yield Cell number* X 10^3/ml ; *division rate (K) of* Thalassiosira fluviatilis

Nutrients as in F/2	Final yield X 10^3 cells/ml	K
Sea water (SW) Control I	8.75	0.004
SW + NO_3 ..	11.95	0.010
SW + PO_4 ..	11.85	0.009
SW + SiO_3 ..	10.20	0.006
SW + TM ..	9.50	0.005
SW + V ..	11.25	0.008
Full Enrichment		
F/2 (Control II) ..	17.00	0.16
F/2 — NO_3 ..	12.3	0.010
F/2 — PO_4	12.6	0.011
F/2 — SiO_3 ..	12.70	0.011
F/2 — TM ..	15.25	0.014
F/2 — V ..	13.00	0.012

Cell counts taken from the cultures exposed to continuous light for a week after seven days in the darkness showed that non-enriched medium supports growth rate of only 0.042 divisions/day. Nitrate enrichment promoted higher growth than any other nutrients when supplied individually. Vitamins enrichment alone supported higher cell division next to nitrate and phosphate. Silicate had little effect in increasing the growth followed by TM. The omission of nutrients had the effect in the order of NO_3 — PO_4 — V — TM and SiO_3 in bringing down the growth rate to a lower level. NO_3 deficiency had higher effect in reducing the growth to 0.065 divisions/day. Omission of vitamins also caused increase in generation time, though the other nutrients are present as in F/2. Lack of SiO_3 and TM in the medium did not reduced the growth to a large extent (K = 0.79 and 0.78).

TABLE 1 b. *Effects of nutrients on growth rate of* T. fluviatilis *under continuous light for one week after incubation in continuous dark for seven days.*

Nutrients as in F/2	Final yield X 10^3 cells/ml	K (divisions)
SW ..	67.2	0.042
SW + NO_3 ..	190.8	0.061
SW + PO_4 ..	140.0	0.056
SW + SiO_3 ..	109.7	0.051
SW + TM ..	95.8	0.049
SW + V ..	113.6	0.052
F/2 ..	750.0	0.087
F/2 — NO_3 ..	230.4	0.065
F/2 — PO_4 ..	290.1	0.070
F/2 — SiO_3 ..	500.0	0.079
F/2 — TM ..	440.0	0.077
F/2 — V ..	400.0	0.075

Initial Inoculum 7.15 × 10 cells/ml.

DISCUSSION

When the culture was exposed to light for a period of week after seven days in the darkness, seawater without enrichment enhance minimum growth rate of *T. fluviatilis*. *Rhizosolenia fragilissima* also showed no growth when seawater alone was supplied. (Ignatiades and Smayda, 1970). In the present study nitrate and phosphate enrichment individually yields maximum growth rate of 0.061 and 0.56 respectively. Smayda (1973) found that nitrate enrichment alone favours luxurious growth in *Skeletonema costatum*. Similarly in *Biddulphia sinensis* and *S. costatum* higher

growth rate was recorded when nitrate and phosphate supplied individually (Subramanian, 1979). Full enriched medium (F/2) supported eleven times increase in the growth of *T. fluviatilis* over the non-enriched medium. This was much higher than the value obtained in *B. sinensis* and *S. costatum*, where only 3-4 fold increase over control (seawater) was observed (Subramanian, 1979). Smayda (1973) recorded 10 to 24 fold increase in growth of *S. costatum* and 10 to 24 fold increase in growth of *S. costatum* and *Cyclotella nana* due to enrichment (Smayda, 1973). In the present study nitrogen deficiency caused reduced growth rate representing 0.065 divisions/day. Phosphorous deficiency also lowered the growth rate of *T. fluviatilis*. Similar observations were made by Subramanian (1979) when exclusion of phosphate and nitrate resulted in reducing growth rate of *S. costatum* and *B. sinensis*. Low cell number of *S. costatum* was recorded when nitrate and phosphate were omitted from the medium (Curl, 1962). In the present observation, growth was not much limited in the medium devoid of vitamin or silicate or trace elements, but the division rate was comparatively lower than that of full enrichment. No remarkable reduction in the growth of *B. sinensis* and *S. costatum* were observed when SiO_3, TM and V were omitted from the medium (Subramanian, 1979).

The present study suggests that nutrients particularly nitrate and phosphate seem to be responsible for either increasing or decreasing the growth of *T. fluviatilis* under both dark and light conditions. In the natural aqautic environment under certain circumstances phytoplankton are forced to undergo unfavourable situation for their growth especially during monsoon period when turbidity of water is high and the light penetration to deeper layers is almost nil. Under those conditions, *T. fluviatilis* may sustain its survival potential if nitrate and phosphate are available in adequate concentrations.

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