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On the diel dynamic of chlorophyll a content in *Sceletonema costatum* (Grev.) Cl. as a function of light and temperature variation

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Introduction. Knowledge on the physiology of phytoplankton species is important for better understanding of dynamic of phytoplankton communities and the bloom phenomenon in particular. Beside chemical and physical environmental factors the specific biological features seem to be of equal significance for the dominance of a given phytoplankton species on a space-time scale. The chlorophyll a content per cell and its diel dynamics has been considered an important physiological characteristic as the chlorophyll synthesis lies behind the photoadaptation potential of phytoplankton (Shlyk, 1965; Yorgensen, 1969). The ability of some species to change their chlorophyll a content during one photocycle could be of ecological advantage for an optimum light utilization under turbulent conditions (Platt, 1981). The variability of chlorophyll a per cell could be crucial in modeling primary productivity too.

The diatom *Sceletonema costatum* is one of the most common winter-spring blooming species in Black Sea (Petrova-Karadjova, 1973; Nesterova, 1987; Bodeanu, 1984). The recent alteration in the annual reproductive cycle with the occurrence of summer blooms of this species has been attributed to the increased eutrophication of the basin (Senicheva, 1980; Mihnea, 1985; Nesterova, 1987; Moncheva, 1991). Hulbert and Guillard (after Round, 1982) however have considered the existing of two different ecotypes - cryofile and thermofile - that could be responsible for the two bloom outbursts of this species during the annual cycle.

While there are a lot of field and laboratory investigations on the response of *Sceletonema costatum* (division rate, nutrient uptake etc.) to different environmental conditions (Conway, 1977; Mihnea, 1980; Horrigan, McCarthy, 1981; Moncheva, 1991a, b) the variability of chlorophyll a content is a less exploited area (Glooshenko et al., 1972; Yorgensen, 1977; Hitchcock, 1980).

The subject of the present paper is to study the pattern of the diel dynamic of the specific chlorophyll *a* content of autochtonous (Black Sea) culture of *Sceletonema costatum* as a function of temperature and light variation and its possible relation to the blooms of this diatom in Black Sea.

Materials and Methods. An autochtonous Black Sea culture inoculum was studied, maintained in a Stosh (1964) nutrient medium under 12h light:12h dark (12L:12D) photoperiod of cool-white illumination. Although day-length at 42-44° N ranges from 8h (March) to 15h (June-July) a constant 12h photoperiod was selected for the laboratory experiments so that variations in chlorophyll *a* content would be attributable to changes in the examined factors.

Several sets of experiments were conducted: the influence of temperature was examined under light saturation intensity for this species (3000 lx, as reported by J o d e r, 1978) in a temperature range between 12-26° C. The co-influence of light and temperature was studied at temperature 12° C and 22° C and at low light intensity (LI) - 3000lx and at high Light intensity (HI) - 6000lx (equal to the average light intensity during winter-spring and summer).

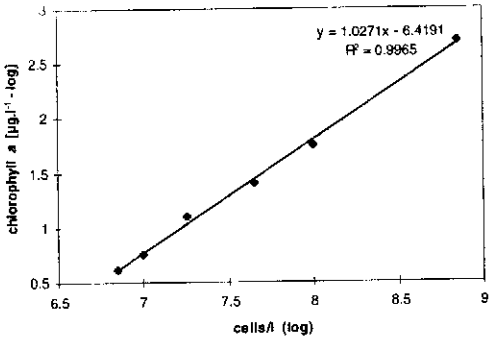

Fig.1. Log-plot of chlorophyll a concentration (mg/l) versus cell density (cell/l) of Sceletonema costatum for all experimental media

Cultures were transferred to experimental conditions a week before experiments were initiated. Experimental bottles were inoculated with log-phase stock culture to yield an initial density of about $0.8x10^6$ cells/l. The chlorophyll a content was measured following SCOR-UNESCO (1966) method and the cell counts - under an inverted microscope (U t e r m o l, 1958) at a 6h frequency from the beginning of the experiment. The measurements during three diel cycles of 3 treatments were averaged. For all experiments the culture cell density was within the range for which the relationship between chlorophyll *a* and cell density was linear, e.g. $6x10^6$ - $1x10^9$ cells/l (Fig.1). Analysis of random samples by acidification showed that phaeophytin was virtually absent during the experiments.

All the results were processed by a special computer program in he Department of Physics and Biophysics.

Results and discussion. As depicted on Fig.2. at light intensity close to the saturation intensity (3000lx) the chlorophyll a content per cell increases with a maximum (0.93 pg per cell) at temperature 22-24° C and decreases thereafter.

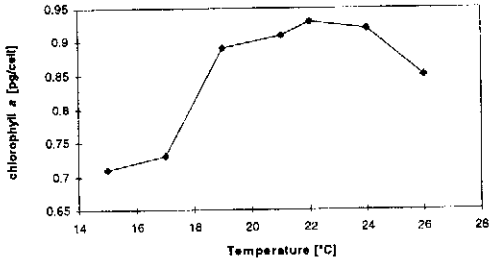

Fig.2. Influence of temperature [C°] on chlorophyll a content [pg/cell] of Sceletonema costatum at light saturation intensity

At both experimental temperatures (12° and 22°C) and light intensities (2000 lx and 6000 lx) a change in the chlorophyll a content is evident during the diurnal cycle. The general trend is an increase during the photophase and a decrease during the scotophase (Fig.3, 4). A departure from the general trend is registered only at a temperature 12°C and LI (Fig.3), probably due to

the specific circadian cycle of this species (P l a t t, 1981).

The analysis of the two curves on Fig.3 reveal that at temperature 12°C and LI, the specific chlorophyll a content per cell during the 24h period increases about 1.6 times (from 0.5 to 0.81pg per cell), the shift being evident during the first 12h.

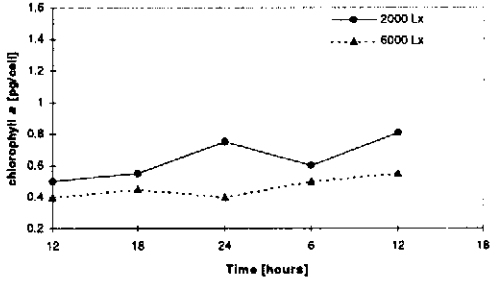

Fig.3. Diel dynamic of chlorophyll a content [pg/cell] of Sceletonema costatum at t° 12°C under two light intensities: 1- 2000lx; 2- 6000lx.

The experimental curves at temperature 22°C although following a similar pattern of changes differ both by the extent and the rate of the diurnal variability. At LI the increase in the chlorophyll a content per cell is better expressed - about 3 times in the photophase (from 0.4 to 1.4 pg per cell) in the first 6h and about two fold during the diel cycle (0.4 - 1.05 pg per cell) (Fig.4). At HI the increase is less well expressed (0.4 - 0.7 pg per cell) during the photophase and after a decrease during the scotophase to almost the initial value, increases again to 0.7 pg per cell (Fig.4).

The analysis of the co-influence of the two factors (temperature and light) on the diurnal dynamic of chlorophyll a content per cell reveal a more significant shift at the higher temperature (22°C) and the LI (2000 lx).

Similar results have been reported for Rhodos and Pacific ocean inoculum of the same species under similar experimental conditions (G l o o s h e n k o, 1972; H i t c h c o c k, 1980).

Obviously temperature has a dual effect on chlorophyll a synthesis of *Sceletonema costatum* culture. First the increase in the temperature results in an increase in the chlorophyll a content per cell, and second the difference between the two light intensities was greater as temperature increases. This response most likely reflects the Q_{10} (temperature coefficient of E p p l e y, 1972) effect of temperature on enzyme mediated biochemical processes regulating growth.

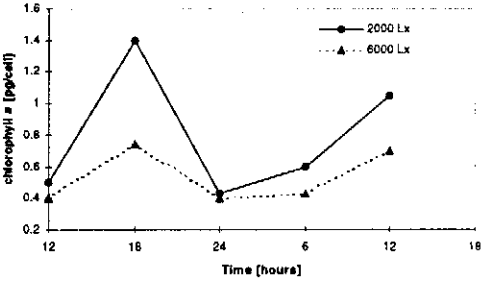

Fig.4. Diel dynamic of chlorophyll a content [pg/cell] of Sceletonema costatum at t° 22°C under two light intensities: 1- 2000lx; 2- 6000lx.

As synthesis and turnover of chlorophyll a are mediated by enzymes (S h l y k, 1965) temperature influences the rate at which cells alter their chlorophyll a, as well as the specific chlorophyll a content per cell, e.g. these two processes, which are of basic importance for the photoadaptation potential of phytoplankton are temperature dependent. The results of the co-influence of temperature and light experiments support the statement that the rate of adaptation to light intensity and its variability could very seasonally (H a r r i s, 1973; M a r a, 1978). The greater variations at LI in comparison to the HI suggests that during winter-spring the temperature mediated adaptation response to light could be less affected by daily variations than during the summer, e.g. the rate at which Sceletonema costatum shifts its

chlorophyll a synthesis copes with the rate of variability of temperature and light as environmental factors, the rapid change at LI giving an advantage of the species to effectively utilize the light in a turbulent media. The latter suggestion is well supported by the pattern of variability of another enzyme mediated process - the specific growth rate. As reported by H i t c h c o c k (1980) at low temperature the fluctuations in light intensity have a limited effect on the growth rate of Sceletonema costatum as the low temperature impose a low growth rate and low saturation intensity, while the most rapid response to a change in light is at 20°C, near its temperature optimum. Our laboratory results reveal a temperature optimum at 22-24° C and μ changing from 2.23 to 3.27 div/day in a temperature range 12-22° C, for which Q_{10} is 1.74 (M o n c h e v a, 1991). According to S m a y d a (1973) μ of 2.0 div./day at 10° C nearly equaled the maximum reported for *Sceletonema costatum* for this temperature.

On the other hand G l o o s h e n k o (1968) found no evidence of phasing in the diel division of *Sceletonema costatum* at all, which is considered a growth strategy suitable for unpredictable , widely varying environments (C h i s h o l m et al., 1980), like those subjected to a high anthropogenic pressure (M o n c h e v a, 1991). As our field data reveal this diatom manifests two maxima during the year ("blooms" registered both in winter-spring and summer), an indicator of the increasing eutrophication in Black Sea (M o n c h e v a, 1991a, b).

Principally, the laboratory results hardly allow a direct correlation to field behavior, but if we assume that during a bloom the environment provides the necessary conditions for a species to exhibit its biological potential (in the sense O d u m, 1975 interprets the term), than our results suggest that the established flexibility in chlorophyll a synthesis of *Sceletonema costatum* together with its high reproduction rate and division strategy could be considered as biological features lying behind the "ecological advantage" of this species to overdominate and succeed others in the phytoplankton community.

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Върху денонощната динамика на хлорофил *a* при морската диатомея *Sceletonema costatum* (Grev.) CL. под влияние на промени в температурния и светлинен режим

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(Резюме)

Изследвано е влиянието на температурата и светлината върху динамиката на съдържанието на хлорофил а на клетка в денонощен аспект при морската диатомея *Sceletonema costatum* (Grev.) CL. (автохтонен черноморски изолат) в лабораторни условия.

Установено е, че с увеличаване на температурата нараства и съдържанието на хлорофил а, като максимумът му е при температура 22-24° С. Основният тренд в денонощната промяна под влияние на експерименталните условия е увеличение през светлинната фаза и намаляване през тъмнинната фаза. Денонощната динамика е по-ясно изразена при съвместното въздействие на висока температура (22-24°С) и ниска осветеност (2000 lx).

Дискутирано е значението на тази физиологична особеност на *Sceletonema costatum* за проявата на два цъфтежни максимума на вида (зимно-пролетен и летен) в естествени условия.

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