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APPLICATION OF CHLOROPHYLL FLUORESCENCE IN STUDIES OF PHYTOPLANKTON IN THE MEDITERRANEAN SEA

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ABSTRACT

By measuring fluorescence and high-temperature thermoluminescence of chlorophyll, we studied ecological state of natural phytoplankton in several areas of Tyrrhenian Sea and in the Kotor Bay of the Adriatic Sea. We found that algal biomass and the activity of photosystem II were non-uniformly distributed over water depth and area, as well as in time. The level of lipid peroxides was elevated in the Kotor Bay at the surface due to intense sunlight, which correlated with a decrease in water salinity. The work was conducted in the frames of the Agreement on scientific cooperation between the Department Biophysics of the Faculty of Biology of the Moscow State University, (Moscow, Russia) and the Department of Marine Biology (Kotor, Yugoslavia).

Key words: phytoplankton; chlorophyll fluorescence; thermoluminescence.

PRIMJENA HLOROFILNE FLUORESCENCIJE U IZUČAVANJU FITOPLANKTONA MEDITERANA

REZIME

Mjerenjem fluorescencije i visoko-temperaturne termoluminescencije hlorofila izučavali smo ekološku situaciju prirodnog fitoplanktona u nekoliko područja Tirenskog mora, te u Kotorskom zalivu kao dijelu Jadranskog mora. Utvrdili smo da biomasa algi i aktivnost fotosistema II nije uniformno raspoređena kako prostorno tako i vremenski. Nivo lipidnih peroksida je bio povišen u Kotorskom zalivu u površinskom sloju kao rezultat intenzivne sunčeve svjetlosti, a takođe je u korelaciji sa smanjenim salinitetom. Rad je ostvaren u okviru Ugovora o naučnoj saradnji između Katedre za biofiziku Biološkog fakulteta Moskovskog državnog Univerziteta i Instituta za biologiju mora iz Kotora.

Ključne riječi: fitoplankton, hlorofilna fluorescencija, termoluminescencija

INTRODUCTION

Recently, methods for measuring chlorophyll fluorescence, which are characterized by a high sensitivity and allow rapid non-invasive assessment of several characteristics of phytoplankton became widely used (Matorin *et al.* 1992; Falkowski & Raven, 1997; Fadeev *et al.* 1999). Fluorescence yield at a low intensity of exciting light (Fo) was shown to correlate with the concentration of chlorophyll and biomass of microalgae. This characteristic is mainly determined by the total concentration of light-harvesting pigments in algal cells (Ostrowska, 2000) and is a characteristic of phytoplankton abundance.

Measurement of variable fluorescence of chlorophyll (Fv) can be used to estimate the photosynthetic activity of algae. Photosynthetic activity of phytoplankton depends on the primary processes of light energy transduction into the energy of chemical bonds, which take place in photosystem II reaction centers (PSII RC), which are involved in water splitting and O_2 evolution. The value of Fv is generally determined as the difference between the maximum fluorescence yield, Fm, measured with closed (photochemically inactive) reaction centers, in which all light energy absorbed by pigments of photosynthetic apparatus is emitted in the form of fluorescence and is dissipated into heat, and the fluorescence yield Fo with open PSII RC, in which most part of the absorbed energy is used in photosynthetic reactions, and energy loss is at its lowest (Matorin *et al.* 1996; Falkowski & Raven, 1997). Thus, variable fluorescence (Fv=Fm-Fo) is a measure of the energy, which has been photochemically converted by PSII. The Fv/Fm ratio was shown to reflect the efficiency of photochemical conversion of light energy in PSII (further referred to as photochemical activity) algae (Long et al. 1994) and to correlate with photosynthetic rate measured from the rates of O_2 evolution or CO_2 fixation (Schreiber *et al.* 1995).

At the Department of Biophysics of the Faculty of Biology of the Moscow State University we designed a complex of luminescence methods for diagnostics of the physiological state of microalgal cells, which comprises a single-beam on-board fluorometer, a submersible pump-and-probe fluorometer, microfluorometer, and a device for measuring delayed fluorescence and thermoluminescence of chlorophyll.

Here we present the data of studies of *in situ* ecological state of the natural phytoplankton in some areas of the Adriatic and Tyrrhenian Sea with the use of the developed method for measuring chlorophyll luminescence.

METHOD

Natural populations of phytoplankton were studied in the Kotor Bay of the Adriatic Sea (in cooperation with scientists of the Institute of Marine Biology, Kotor) and along a section of the Tyrrhenian Sea between Corsica Island and the Strait of Messina during a cruise of the R/V Moscow University.

Fluorescence was measured with a laboratory single-beam fluorometer (Matorin *et al* 1992), and with a portable submersible pump-and-probe fluorometer. Pump-and-probe fluorometer consisted from a submersible probe, on-board power supply unit connected to the probe, and a notebook IBM-compatible computer, which controlled the measurement procedure by a user-defined program (Matorin *at al.* 1996). The fluorometer allows measuring of the vertical distribution of such production characteristics of phytoplankton as its abundance (F_O) and photochemical activity (F_V/F_M), as well as underwater irradiance and water temperature. The intensity of the high-temperature thermoluminescence (TL) for the detection of lipid peroxidation products in cells of photosynthesizing organisms was measured with a device described previously (Matorin *et al.* 1989,1992).

RESULTS AND DISCUSSION

The study of probes obtained by means of submersible fluorometer makes it possible to record environmentally induced changes in the activity of PS II directly in natural phytoplankton cenoses *in situ*. The measurement of underwater irradiance and temperature by special sensors in these probes allows determining the effect of these factors on the activity of algae. Our experiments carried out in Mediterranean Sea showed pronounced spatial-and-time heterogeneity in distribution of PS II activity, as measured by the Fv/Fm ratio in phytoplankton populations. Often, the highest values of Fv/Fm ratio do not correlate with the highest phytoplankton concentrations. On the other hand, in several water bodies, the Fv/Fm ratio was shown to correlate with the availability of mineral nutrients for phytoplankton. For example, in oligotrophic low-productive regions of the Mediterranean Sea, which are characterized by a low level of mineral nutrients in the photic layer, values of the Fv/Fm ratio varied from 0.3 to 0.6. In mesotrophic waters the Fv/Fm ratio increased to 0.5-0.6, whereas phytoplankton activity in eutrophic waters in the Kotor Bay of the Adriatic Sea attained high values of Fv/Fm, which is peculiar to algae grown under optimum conditions (Vuksanović 1983). Similar dependence of the PS II activity on the water body trophicity was described in Falkowski & Raven (1997).

The dependence of the Fv/Fm ratio on the concentration of mineral nutrients was evident also in recorded depth profiles of chlorophyll fluorescence (Fig. 1). In open regions of Mediterranean Sea, the activity of PS II, judged by the Fv/Fm ratio, was at its lowest in the surface layers and increased with the depth. The highest level of the Fv/Fm ratio was found at the depth of 40- 60 m, where mineral salts intruded with depth waters, whereas light intensity was sufficient to support photosynthesis. In shore waters, significant activity was found in surface layers. An elevated level of the Fv/Fm ratio of phytoplankton was found during the investigation of the upwelling zones, where cold depth waters enriched with mineral salts go up, in the Tyrrhenian Sea Favorable conditions lead to a higher efficiency of PS II functioning, accompanied by stimulated phytoplankton growth. Previously, we mentioned that changes in the

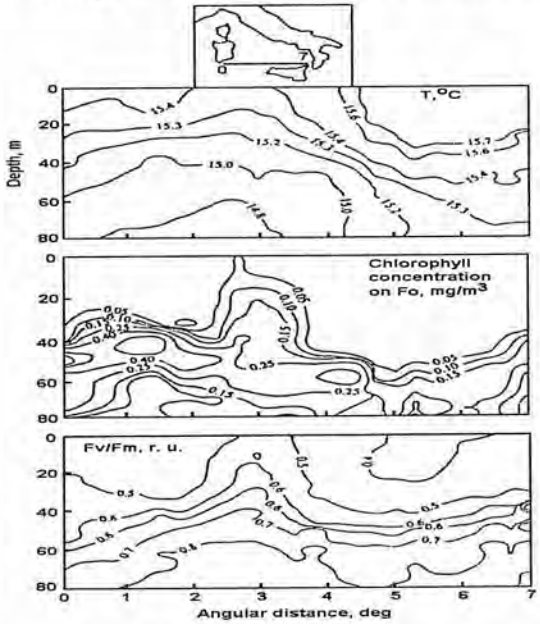

Figure. 1. Depth profiles of water temperature (T, °C), phytoplankton concentration (chlorophyll concentration- Chl μg\l) and photosynthetic activity (Fv/Fm) of phytoplankton on sections through the Tyrrhenian Sea.

activity of PS II and, respectively, in the Fv/Fm ratio of algal cultures affected both photosynthetic productivity of natural phytoplankton and also determined the rate of cell growth.

The effect of light on the activity of PS II manifested itself in a suppression of the Fv/Fm ratio in surface water during the day. This corresponds to diurnal changes in the primary production, which exhibits midday depression described by many authors (Long *et al.* 1994). The extent of the Fv/Fm suppression was at its highest in surface layers and rapidly decreased as the underwater irradiance decreased with depth. The decrease in the Fv/Fm ration during the day depended on light intensity, vertical mixing, and physiological state of phytoplankton. Waters with different trophicity differed considerably in diurnal changes in the Fv/Fm ratio. Under conditions of adequate mineral nutrition (for example, in shore regions, in the Kotor Bay, (Fig. 2) the Fv/Fm ratio in the surface layer retained its high level throughout the whole day. At a lower mineral supply in mesotrophic waters and in the absence of mixing, the Fv/Fm ratio decreased during the day due to photoinhibition. The suppression of Fv/Fm was mainly due to a decrease in the Fm level. Under conditions of lower light intensity in the evening, the Fv/Fm ratio returned to its initial value. In oligotrophic waters, depleted of mineral nutrients, the Fv/Fm ratio was significantly reduced during the day. In this case, the inhibition of the Fv/Fm ratio was largely due to an increase in the intensity of Fo fluorescence during the day, and the Fv/Fm ratio was restored only in the morning, accompanied by a decrease in the Fo intensity. Comparison with experiments on nitrogen starving algal cultures allowed to conclude that this process is due to the consumption of mineral nutrients by algae (Matorin *et al.* 2000).

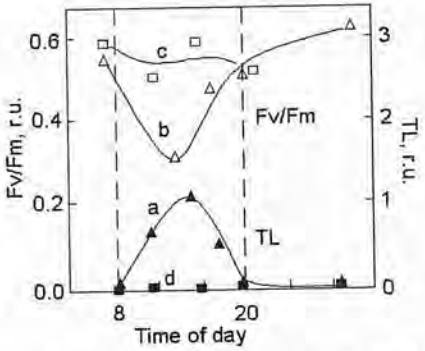

Fig.2. Diel dynamics of chlorophyll fluorescence (Fv/Fm- photosynthetic activity, b,c) and intensity of the high-temperature thermoluminescence (TL,a,d) in phytoplankton of Kotor Bay in surface water during a sunny day (c,d) - before rains, and (a,b) - on salinity decrease after rains.

The role of the physiological state of phytoplankton in the development of photoinhibition was clearly seen when the salinity at a Kotor Bay station near a river mouth decreased after rains. During rainy period, a decrease in water salinity in the Kotor Bay led to drastic enhancement of the midday depression of PS II activity (Fig. 2).

It is under high light intensities due to damage of photosynthetic membranes and uncoupling of light and dark stages of photosynthesis that the excess of chlorophyll excitation energy and electrons in electron transfer chain is created. This leads to generation of active oxygen species and to lipid peroxidation. In the course of lipid peroxidation in photosynthetic membranes, lipid hydroperoxides are formed, accumulation of which is an indicator of a damage of cell membranes. Decomposition of hydroperoxides is associated with the formation of electron-excited carbonyl products. These products can efficiently transfer excitation energy to chlorophyll, which results in the generation of a slowly decaying chemiluminescence of chlorophyll. This phenomenon was used to develop a luminescence method for the detection of lipid peroxidation products in cells of photosynthesizing organisms (Matorin *et al.* 1988,1989). At high temperatures, hydroperoxides are decomposed, which is accompanied by an increase in the intensity of chlorophyll chemiluminescence with the maximum intensity at 120°C. The intensity of the high-temperature thermoluminescence (TL) is directly proportional to the amount of accumulated lipid peroxidation products (Matorin *et al.* 1989,). By measuring the intensity of chlorophyll TL, one can study lipid peroxidation processes in cells of microalgae and phytoplankton under natural conditions (Matorin *et al.* 1988; Venediktov *et al.* 1989). The TL emission was examined in phytoplankton samples collected in different waters. The intensity of high-temperature TL generally increased at mid-day time in phytoplankton sampled in oligotrophic waters and showed little changes throughout the day in the waters with high concentration of mineral nutrients (Kotor Bay). Under unfavorable conditions and when algae had a low activity of PS II, the amount of lipid peroxides also increased in surface water under effect of intense sun light (Fig. 2) on a decrease in water salinity after rains at a Kotor Bay near a river mouth. On the basis of these observations, a method was suggested for the assessment of phytoplankton sensitivity to photooxidative stress by measuring the high-temperature TL.

CONCLUSION

Investigation of natural phytoplankton with the use of the developed instruments showed that the processes of regulation of PS II activity in response to changing environmental factors could be studied in natural phytoplanktonic cenoses. The development of photooxidative stress can be studied by fluorescence methods in surface water during sunny time of the day (midday depression). The efficiency of PS II reaction centers affects both the photosynthetic productivity of natural phytoplankton and cell growth rate. The appearance in a water body of algal cells with active PS II reaction centers is a forecasting sign of algal bloom. The data described above provide theoretical background for the use of biophysical luminescence methods in oceanology to study the state and productivity of phytoplanktonic cenoses. The application of these methods allows estimating both photosynthetic productivity and parameters of the primary processes of photosynthesis and, thus, to assess the state of phytoplankton cells in a water body. This work was conducted in frames of the Agreement on Scientific Cooperation between the Department of Biophysics of the Faculty of Biology of the Moscow State University (Moscow, Russia) and Institute of Marine Biology (Kotor, Yugoslavia) and according to the World Ocean Program (Russia).

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