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Variability in the Condition of the Photosynthetic System of the Black Sea Phytoplankton

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Abstract—An analysis of the results of long-term multidisciplinary studies of the photosynthetic system of the Black Sea phytoplankton obtained using the fluorometric and spectrophotometric methods developed at the Chair of Biophysics of the Department of Biology, Moscow State University, is performed. The distributions of phytoplankton in the surface waters and over depth in the northeastern sector of the sea are presented. The abundance and the functional condition of the photosynthetic system of the populations of mass alga species and phytoplankton community in different ecological environments are compared. The regularities in the influence of insolation and oxidation stress on the parameters of the chlorophyll fluorescence are shown. The features of the use of the parameters of the chlorophyll fluorescence for the estimation of the production characteristics of natural phytoplankton are discussed together with the potential of luminescent and spectrophotometric methods, which can characterize the abundance, taxonomic composition, and functional condition of the photosynthetic system of individual cells, species, taxa, and phytoplankton community as a whole. The methodology of the use of a set of spectral methods of express analysis for phytoplankton monitoring of marine aquatic areas is proposed.

INTRODUCTION

The significant changes in the climatic conditions and intensification of the anthropogenic impact that have occurred over recent decades have led to changes in the structure of the Black Sea biocoenosis [18, 19]. Earlier, long-term and seasonal variations in the abundance, distribution, and structure of the phytoplankton community were noted [24]. Therefore, the performance of further studies aimed at the examination of the phytoplankton condition in the Black Sea represents an urgent task. In so doing, the methodological enhancement of these studies and acquisition of reliable results in the course of onboard operations are important.

One of the main parameters of the condition of planktonic algae that determines the primary production of the phytocoenosis is the efficiency of the functioning of the photosynthetic system. At present, great success is achieved in the use of luminescence methods for estimates of the phytoplankton abundance and functional condition of its photosynthetic system [2–4, 7–11, 17]. These methods provide high sensitivity and output, and allow one to perform measurements in situ in an on-line mode. The efficiency of the measurements of the fluorescence from vessels is especially important when studying mesoscale processes in marine ecosystems, which are distinguished by their high temporal variability.

The fluorescent methods are based on the fact that the chlorophyll existing in photosynthetic membranes serves as a kind of natural sensor of the alga cell condition. The successes in the studies of the mechanisms of primary photosynthetic processes and the wide use of fluorometric methods in the studies of alga physiology and ecology allowed scientists to recognize the relations of the chlorophyll fluorescence parameters to the characteristics of the photosynthetic system of the photosynthesizing organisms [3, 4, 8]. Under regular physiological conditions, 2–3% of the light absorbed by photosynthetic pigments is radiated in the form of fluorescence. About 90% of this fluorescence is radiated by chlorophyll a of photosystem 2. The energy of the light quanta absorbed by the light-collecting unit may be converted into the energy of separated charges, which is later utilized in the subsequent photosynthetic reactions or lost either through the emission of quanta of fluorescence or through diffuse heat radiation. The measurements of the ratio between the chlorophyll fluorescence intensity in light saturating the photosynthesis (F_m) and that under conditions that cause no changes in the condition of the photosynthetic system (F_0) allow one to determine the intensity of the primary photosynthetic processes from the relation $(F_m - F_0)/F_m = F_v/F_m$. The values of the fluorescence intensity F_0 are well correlated with the total contents of pigments of the photosynthetic system of phytoplankton within a wide range of conditions [13]. The efficiency of the primary photosynthetic processes (F_v/F_m) represents a dimensionless characteristic of the energy of photosynthesis similar to the coefficient of efficiency and independent of the species particularities [3, 4]. The maximum theoretically feasible value of the efficiency of light utilization in photosynthesis equals 0.84 at a zero minimum value. A utilization efficiency close to zero points to the separation of the light absorption processes from those utilizing its energy for the purposes of photosynthesis. This situation may be observed if the organism is dying or is severely damaged. The measurements of the values of F_v/F_m and of the intensity of the light absorbed by the photosynthetic pigments of algae help to determine the rate of their photosynthesis and its changes under the influence of external factors in the natural environment [1].

The action of excessive light intensities, which saturate photosynthesis, on the photosynthetic system causes a reconstruction of the light-collecting proteinpigment complex. This reconstruction results in an increase in the probability of heat dissipation of the energy of the light absorbed by pigments. In this way, the photosynthetic system protects itself from being damaged by oxidation caused by an excess of excited states of chlorophyll [25]. This phenomenon is commonly referred to as nonphotochemical extinguishing. It represents an adaptive response of the photosynthetic system to excessive insolation.

In order to implement the research on natural phytoplankton, at the Chair of Biophysics of the Department of Biology, Moscow State University, we designed a set of fluorescence instruments that included an onboard fluorometer, submersible and flow-through fluorometers, a microfluorometer, and a device for the measurement of thermoluminescence. In this paper, we present the results of the studies of the conditions of the photosynthetic system of the phytoplankton community in the near-shore and open-sea regions of the northeastern part of the Black Sea with the use of this set of instruments.

MATERIALS AND METHODS

The expeditionary studies were performed using the facilities of the Southern Branch of the Shirshov Institute of Oceanology, Russian Academy of Sciences, in the near-shore waters of the Black Sea in May–September 1998–2004 and in November 2000.

The measurements of the fluorescence parameters F_0 and F_v/F_m of natural phytoplankton under natural conditions with simultaneous recording of temperature and underwater irradiance were performed with the help of the submersible pulse fluorometric probe in the 0- to 100-m layer [10]. The same chlorophyll fluorescence parameters, as well as the coefficient of nonphotochemical fluorescence extinguishing of chlorophyll, were measured in natural water samples without preprocessing with the onboard portable fluorometer [17]. The measurements of the chlorophyll fluorescence parameters of individual cells of microalga were conducted with the pulse microfluorometer [16, 17].

In 2004, we used a flow-through fluorometer for the measurement of the fluorescence of phytoplankton chlorophyll from a moving vessel. This device, as well as the fluorometric probe and the onboard fluorometer,

automatically measures the fluorescence intensity F_0 , which is related to the phytoplankton abundance, and the value of F_v/F_m , which shows the efficiency of light utilization in the photosynthetic system of algae. The water collection and its delivery to the measuring device were implemented via a silicon hose. The hose with a filter at its end (with a mesh size of 0.5 mm) was installed in a metallic tube, which was placed overboard near midship. The receiving filter was submerged into the water 40–50 cm below the surface. The water flow was produced by the vacuum created by a waterjet pump installed behind the measuring chamber of the device, which provided nativity of the alga cells. The measuring chamber 200 ml in volume provided for bubble evacuation from the measuring volume. The velocity of the water flow was 0.5 l/min and the time of water passage over the hose up to the measuring chamber was less than 2 min. The measurements of the fluorescence parameters were performed every minute, while the time of recording of F_0 and F_v/F_m was 10 s. The flow-through fluorometer was supplied with a GPS sensor in order to determine and record the coordinates and time. The recording of all the values measured and the device control were implemented following a program specially elaborated by us; it allows one to visualize and save all the information obtained. With respect to the characteristics and parameters of the fluorometric unit, this device is similar to the onboard fluorometer [17].

At present, measurement of the pigment composition on filamentous glass filters using the spectrophotometric technique is widely used. This kind of analysis should be performed immediately after the phytoplankton sedimentation over the filter in order to avoid destructive changes in the pigment composition. Meanwhile, under expeditionary conditions, the use of large stationary laboratory devices supplied with integrated spheres for operations with dispersing samples is too inconvenient. In order to operatively determine the contents of the algal pigments in water samples and on filters as well as in pigment extracts, we elaborated and designed an onboard spectrophotometer based on a USB 2000 portable spectrophotometer (Ocean Optics, Inc.). This instrument has the following characteristics: the spectral range 370–1100 nm, the mass of the instrument <3 kg, and the measurement accuracy 0.005 optical density units at an optical density of the sample of 1 unit. The instrument is supplied with an integrating sphere. The typical absorption spectra of algae immobilized on GF/F filters are presented in Fig. 1.

RESULTS AND DISCUSSION

In the summertime, the greatest values of phytoplankton concentration are mostly confined to the shelf zone and to the waters of the Rim (Main Black Sea) Current. With respect to the vertical, the domain of enhanced values of phytoplankton concentration in these regions is located in the warm upper mixed layer,



Fig. 1. Absorption spectra of GF/F filters with alga from different depths immobilized on them. Station 1649 (44°30.85' N, 37°55.91' E), June 23, 2004, cruise 62 of R/V Akvanavt.



Fig. 2. Depth distribution of the parameters of phytoplankton fluorescence (F_0 -chl and F_v/F_m). The data were obtained in situ on May 17, 2002, in a transect near Golubaya Bay during a cruise of R/V Akvanavt with the help of a pulse fluorometric probe.

whose thickness may reach 40 m (Fig. 2). In the colder waters at the periphery of the divergence zone, one observes a regular decrease in the fluorescence intensity of phytoplankton pigments in the surface layer and a significant rearrangement of the vertical distribution of phytoplankton. The thickness of the upper mixed layer in these regions is reduced down to 18–20 m, while the depth of the maximum phytoplankton content reaches 35–40 m. In this case, the curve of the vertical distribution of the intensity of chlorophyll fluorescence (F_0) in the cold waters of the divergence zone features a maximum confined to the lower part of the seasonal thermocline (30–40 m). The maximum of the photo-

OCEANOLOGY Vol. 45 Suppl. 1 2005

synthetic pigment contents at these depths seems to be caused by an accumulation of diatomaceous phytoplankton. This kind of distribution is described in [18]. The vertical profiles of chlorophyll fluorescence reflect the most characteristic features of the distributions of pigments and phytoplankton biomass that have previously been noted during the hydrobiological studies in the northeastern part of the sea [19, 20, 24].

The absorption spectra of the phytoplankton immobilized on filters (Fig. 1) feature a significant absorption in the region of 540 nm characteristic of the algae collected in June 2004 from depths of 30–50 m. The absorption in this spectral band is typical of one of the



Fig. 3. Record of the changes in the fluorescence parameters F_0 and F_v/F_m obtained on June 24, 2004, in the southern transect from the near-shore station 1651 (43°25.77' N, 39°51.72' E) to station 1660 (43°08.41 N, 38°58.94' E) located at a distance of 55 miles from station 1651. Cruise 62 of R/V Akvanavt. The measurements were performed continuously with 1-min intervals from 9 a.m. to 9 p.m.

pigments of cyanobacteria—phycoeritrine [2, 3]. These data show that, in the composition of the phytoplankton community at depths of 30–50 m, a significant proportion of cyanobacteria is observed. Similar data on the presence of blue-green algae at a depth of approximately 40 m were also obtained in August 2003. As compared to other taxonomic groups of planktonic algae, the chlorophyll fluorescence of cyanobacteria features the lowest quantum yield [3]. This may explain the small contribution of blue-green algae to the fluorescence characteristics of phytoplankton. The changes in the absorption spectra of the filters may help to account for this phenomenon.

Knowledge of the condition of the phytoplankton community of large aquatic areas is impossible without information on the distribution of the waters with respect to the alga abundance and their photosynthetic activity. It is especially important to acquire these kinds of data in the course of ship movement in order to recognize gradient zones and to choose the location of the stations aimed at detailed phytoplankton examination. For the purpose of measuring the distribution and photosynthetic activity of natural phytoplankton populations over the water area, we applied the flow-through fluorometer, which allows one to measure the parameter of the chlorophyll fluorescence F_0 and the efficiency of photosynthesis F_v/F_m from a moving vessel in an online mode. Figure 3 presents a record of the fluorescence parameters measured that were obtained on June 24, 2004, over the southern transect on cruise 62 of the R/V Akvanavt. On this day, the cloudiness was variable and sometimes it rained. Therefore, the photosynthesis efficiency in this transect was significantly higher than on sunny days, because the photosynthesis efficiency falls under a high insolation. These conditions caused virtually no changes in the phytoplankton abundance parameter F_0 under the action of light. The transect discussed is represented by 840 individual measurements, each of which was obtained through averaging more than 100 fluorometer readings. These kinds of measurements allow one to reliably determine the spatial inhomogeneity of the distribution and photosynthetic activity of the phytoplankton of the surface waters.

During our summertime observations in 1998–2004, the phytoplankton distribution mostly retained the above-described structure. In selected cases, two peaks were recognized, one of which, as a rule, was located in the area of the thermocline and the other was



Fig. 4. Depth distributions of (a) temperature and chlorophyll fluorescence parameters (b) F_0 and (c) F_v/F_m in the zone of the shelf edge (semidiurnal 500 m) off Golubaya Bay in August–September 2000 and 2002.

observed below the thermocline. The fundamental uniformity in the distributions of the phytoplankton abundances may feature variations in different years. A comparison of the probing results obtained in 2000 and 2002 showed (Fig. 4) that, at the end of the summer of 2002, the ecological condition of phytoplankton in the northeastern part of the Black Sea underwent a significant change. In the absence of noticeable mesoscale variations, the waters of the upper water layer (0-25 m)over the entire northeastern sector of the sea were heated up to a temperature of 25° or higher. Under these conditions, the distribution of the pigment contents and the efficiency of the photosynthetic processes over depth also changed. The maximum values of both parameters of the phytoplankton condition turned out to be located 10–15 m deeper than in the preceding years. The algae collected in the upper layers (0-10 m)could not restore their photosynthetic functions even after a long adaptation in the dark. This allows one to suggest that they suffered irreversible photodamage. This suggestion is also supported by the weakening of the protective system of extinguishing excessive excited states of chlorophyll observed in these algae. Indeed, in August 2002, in the deep-water part of the shelf, the values of the coefficient of nonphotochemical fluorescence extinguishing for the phytoplankton community of the upper mixed layer were significantly lower than those observed in 2000. This reduction of the potentialities of adaptation of the photosynthetic system to the action of highly intensive light seems to have been caused by the changes in the temperature regime in the surface water layers in August 2002.

In the near-shore waters, the high summertime photosynthetic activity defined from the fluorescence parameter (F_v/F_m) , as a rule, corresponds to the maximum of the phytoplankton abundance. This is characteristic of the quasi-stationary conditions of the dwelling of the phytoplankton population (mineral feeding, light regime, etc.). The photosynthetic activity determines the rate of the alga growth and the production parameters of phytoplankton. Earlier, the decrease in the cell growth rate under the inhibition of the activity of the primary photosynthetic reactions registered by the changes in the fluorescence parameter, which was induced by unfavorable conditions or toxicants, was shown using microalga cultures [8, 16]. The presence of a dependence of the photosynthetic activity on the phytoplankton abundance was also often observed when measuring the Black Sea phytoplankton under stationary hydrological conditions (Fig. 5). Along with this, when the environmental conditions change rapidly, a disagreement between the efficiency of photosynthesis and the alga biomass value is possible. This situation is typical of the initial succession stage of the phytocoenosis and was observed by us in the cold waters at the periphery of the divergence zone [17]. The terminal succession stage is characterized by a low efficiency of photosynthesis at large values of the phytoplankton biomass, which corresponds to the conditions of a suppressed alga growth.

The phytoplankton community is nonuniform with respect both to the species composition and to the functional activity of individual cells. A part of the algae can actively develop and feature a high efficiency of photo-



Fig. 5. Relation between the efficiency of photosynthesis F_v/F_m and the chlorophyll content calculated from F_0 . The data were obtained in situ with a pulse fluorometric probe at 14 stations in the northeastern part of the Black Sea during a cruise of R/V Akvanavt on May 17–21, 2002.

synthesis, while the other part of the phytocoenosis may be suppressed. The condition of the photosynthetic system of individual cells and of the heterogeneity of their populations may be determined using the methods of pulse microfluorometry [15–17]. In the near-shore part of the Black Sea in the region of Gelendzhik and Golubaya bays in August-September 2000, mass species were represented by Thalassionema nitzsnhioides, Dactyliosolen fragilissimus, Pseudonitzschia seriata, and Gymnodinium wulffii. These species were chosen for an analysis of the cell distribution with respect to the parameter of efficiency of photosynthesis F_v/F_m (Fig. 6). The representatives of diatoms *Th. nitzsehio*ides and D. fragilissimus were found in a suppressed condition. About half of these cells were characterized by an extremely low level of efficiency of photosynthesis. Judging from the data obtained from alga cultures and natural populations [3, 8, 16], the growth in cell abundance of algae stops when the mean value of F_v/F_m decreases down to 0.3. Therefore, the distribution of the cells of Th. nitzsehioides and D. fragilissimus with respect to the relative fluorescence parameter allows one to forecast decreases in the abundance of these species. The majority of the cells of *P. seriata* and the dinoflagellate G. wulffii featured a high intensity of photosynthesis, pointing to conditions favorable for these species and allowing one to expect changes in the abundance of these species. Indeed, two weeks after the beginning of the survey, a significant growth in the relative content of G. wulffii cells was observed, while cells of Th. nitzsehioides and D. fragilissimus were encountered significantly more rarely. In this way, the use of the microfluorometric technique allowed us to acquire data for estimating the functional condition of



Fig. 6. Distribution of the cells of mass alga species over the values of the chlorophyll fluorescence parameter F_v/F_m in the nearshore zone of the northeastern part of the Black Sea in August 2000.



Fig. 7. Values of the fluorescence parameters of phytoplankton F_0 and F_{v}/F_m obtained with a flow-through fluorometer at a stop of the vessel at station 1606 (43°08.08' N, 38°09.52' E, June 13, 2004) from 4:30 a.m. to 2:00 p.m.

the photosynthetic system of the cells of the microalgal population and to formulate a short-term forecast of the dynamics of their abundance in the phytocoenosis under consideration.

In the surface waters, significant changes in the process of photosynthesis of alga and, correspondingly, in the fluorescence parameters may be induced by intense insolation. These processes, referred to as the processes of the midday photosynthesis depression or photoinhibition, are caused by the nonphotochemical extinguishing of the excited states of chlorophyll [25]. Owing to this reason, when determining the phytoplankton abundance by the readings of fluorescence sensors, one should take account of the influence of insolation on the fluorescence of the phytoplankton of the region considered through introducing correcting coefficients. The degrees of photoinhibition and decrease in the F_0 value for different group of alga and under different environmental conditions may differ significantly. In Fig. 7, we present typical results of the measurements of the phytoplankton fluorescence performed when the ship stopped at station 1606 from 4:30 a.m. to 2:00 p.m.

OCEANOLOGY Vol. 45 Suppl. 1 2005

using the flow-through fluorometric technique. With the insolation growth in the daytime, a decrease in the F_0 value is observed. The decrease in F_0 is somewhat correlated with the fall in F_v/F_m , which reflects the photosynthetic activity.

With the decrease in the illumination in the evening, the values of F_0 and F_v/F_m rebounded. In the surface layers, the degree of F_0 inhibition was the greatest; it fell sharply with depth together with the decrease in the underwater irradiance. The degree of F_0 fall in the daytime depended on the illumination, the rate of vertical water mixing, and the physiological condition of phytoplankton. In particular, significant differences are observed in the diurnal changes of F_0 in waters differing in their trophic properties. Under a good mineral food provision (in the near-shore regions), the F_0 values in the surface layer vary only slightly throughout the day depending on the insolation level. In the open parts of the Black Sea, under a moderate nutrient provision and in the absence of mixing, a significant decrease in the F_0 value (down to 40%) caused by photoinhibition is observed in the daytime. A significant drop in the flu-



Fig. 8. Changes in the (a) fluorescence intensity of the phytoplankton chlorophyll F_0 and (b) fluorescence parameter F_v/F_m depending on the adaptation time in the dark for water samples collected from the sea surface at stations 1604–1606 on cruise 61 of R/V *Akvanavt* on June 13 at 10:45 a.m., 12:35 p.m., and 3:15 p.m., respectively.

orescence F_0 was described for intense irradiation of microalga cultures at a deficiency of nitrogen [22]. In addition, the diurnal variations in the fluorescence F_0 and the efficiency of photosynthesis may also be affected by the taxonomic composition of algae. For example, the high content of coccolithophorids, which are resistant to photoinhibition, results in smaller changes in the chlorophyll fluorescence parameters with respect to the irradiance intensity [12, 14].

In order to estimate the degree of influence of insolation on the chlorophyll fluorescence intensity F_0 , it is necessary to perform adaptation of phytoplankton in the dark and to examine the processes of reparation of the photodamage. The curves of the F_0 recovery after sample placement in the dark for selected stations are presented in Fig. 8. It is seen that, in the dark, the values



Fig. 9. Changes in the values of F_{0} , F_m , and F_v/F_m and in the intensity of high-temperature thermochemiluminescence (TL) at 120° in the phytoplankton collected at 7 a.m. from the surface layer off Golubaya Bay of the Black Sea under the action of intense light (2000 μ M quanta m⁻² s⁻¹).

of F_0 increase and the activity F_v/F_m is restored. The time of recovery comprises a few hours. The experiments on inhibition of the protein synthesis in phytoplankton cells showed that the reparation process is strongly controlled by the synthesis of chloroplast proteins. The excessive irradiation leads to damage of some of the proteins of the reaction centers of the alga photosynthetic system [3, 8]. The partial destruction of the reaction centers and the energy expenditure on the protein reparation decrease the growth rate and productivity of algae.

The high insolation leads to a decrease in the chlorophyll fluorescence parameter F_v/F_m . The fall in the intensity of the photosynthetic processes under the action of excessive light may be both reversible and irreversible. The rapid (within 15–20 min) reversible recovery after stopping the excessive light is related to the regulation mechanisms preventing the reaction centers from receiving an excitation owing to the increase in the heat dissipation of the chlorophyll excited states. The slow (over 2–4 h) recovery of the performance of the photosynthetic system after photoinhibition is related to the processes of resynthesis of the proteins of the reaction centers. The degree of photodamage of the reaction centers may also be determined from the recovery of the fluorescence parameters in the course of dark adaptation (Fig. 8).

The processes of the regulation of the photosynthetic system are extremely important for the protection against the appearance of large amounts of reduced products of the light stage of photosynthesis that are not used in the biosynthetic reactions. Interacting with oxygen, these products generate active forms of oxygen and induce the processes of peroxide lipid oxidation, which lies in the basis of the universal mechanism of cell damage [6, 8].

The content of the peroxide lipid oxidation products in cells of photosynthesizing organisms may be determined by the intensity of high-temperature thermolu-

minescence at 120° [21, 23]. During the high daytime insolation, the surface waters may accumulate significant amounts of lipid peroxides. The changes in the fluorescence parameters of chlorophyll and in the intensity of thermoluminescence of phytoplankton samples under the action of enhanced light intensities are shown in Fig. 9. During the initial period (1–20 min) of intense irradiation, the photosynthetic efficiency $F_{\rm v}/F_{\rm m}$ decreases in response to the light action. At this stage, the changes in the photosynthetic system condition are reversible. Meanwhile, with further irradiation (after 20 min), the products of peroxide oxidation of lipids begin to accumulate in the membrane structures of the alga chloroplasts, which leads to a growth in thermochemiluminescence. According to the data obtained on cultures, cells are destroyed precisely during this period [21].

CONCLUSIONS

The use of the set of fluorometric methods allows one to perform a detailed examination of the condition of the photosynthetic system of phytoplankton and to reveal the mechanisms of the spatiotemporal variability of the phytocoenosis. The in situ measurements of the fluorescence parameters, in contrast to traditional hydrobiological methods, allow one to obtain information on the phytoplankton content and efficiency of its functioning in an on-line mode, which is important from the point of view of comparison between the satellite and sea-truth observations. Meanwhile, in contrast to the satellite observations, which are restricted to the phytoplankton content in the surface layer, the use of the submersible pulse fluorometric probe provides the possibility to relate the changes in the physiological condition of the phytocoenosis in the water column to the changes in the hydrodynamical structures in the surface waters. The flow-through fluorometry yields information on the variability in the abundance and photosynthetic activity of phytoplankton from a moving vessel in an on-line mode, which allows one to distinguish gradient zones and to outline mesoscale structures in the sea. Detailed studies of the heterogeneity of the populations and of the dynamics of the changes in selected groups of algae with the use of the microfluorescence analysis of the photosynthetic activity of individual alga cells helps one to estimate the phytocoenosis condition and to predict its further development.

An analysis of the response of the primary processes of photosynthesis of phytoplankton to the illumination regime allows one to reveal the light conditions to which phytoplankton cells are adapted and to estimate adaptive potentialities of these organisms. The recording of the high-temperature thermochemiluminescence makes it possible to follow the process of peroxide lipid oxidation in the membranes of phytoplankton cells and to determine the resistance of the algae to damage of their photosynthetic system and the efficiency of performance of its antioxidant system. A comprehensive analysis of the changes in the Black Sea ecosystem under the action of climatic and anthropogenic factors is impossible without long-term systematic studies of the condition of its phytoplankton community. The modern express methods proposed in this paper seem to be most promising for acquisition of data on the variability of the phytocoenosis of the Black Sea.

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