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MARINE BIOLOGY

A Set of Optical Methods for Studying Marine Phytoplankton

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Abstract—The results of integrated optical measurements of Black Sea water samples using a spectrophotometer, laser spectrometer, and fluorometer with pulse-modulated excitation light are discussed. A linear correlation between the intensities of chlorophyll absorption at 673 nm and chlorophyll fluorescence (680–750 nm) is observed. Phycoerythrin-containing organisms are recorded in phytoplankton in layers below 20 m. The data of 1-week monitoring of phytoplankton abundance and functional activity in Golubaya Bay with a Mega-25 flow fluorometer are described.

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INTRODUCTION

Assessment of phytoplankton spatial and temporal dynamics is a key task for gaining insight into how marine ecosystems function. The main approach to studying phytoplankton is still bathymetric water sampling at different horizons and subsequent laboratory assay. Rapid tests providing primary information about the phytoplankton content and taxonomic composition directly on board a vessel play a special role in this kind of research. These data make it possible to adjust the sampling depth and perform additional sampling at specific sites discovered on the route of a research vessel.

The optical methods adapted to field conditions should have sufficient sensitivity reserve and require a minimum of preparatory manipulations with the sample. Among these methods is recording of the chlorophyll fluorescence [4, 5] and absorption spectra of suspended particles on fiber optic filters [8]. A comparatively new approach used in oceanological studies is to record the fluorescence spectra of phytoplankton and organic matter dissolved in water [1] using small-sized spectrophotometers and powerful semiconductor light sources.

In this work, we analyze data obtained by assaying the same seawater samples with three photometric devices: a spectrophotometer, pulse-modulated fluorometer, and laser spectrometer.

MATERIALS AND METHODS

Water specimens (over 30) were sampled in June 2014 in Golubaya Bay, the Black Sea (Fig. 1); approximately half of them were from the surface layer (0 and 5 m) and the remaining samples taken at some stations were from deeper horizons (17–45 m).

Absorption spectra were recorded in the range of 400–800 nm with a portable spectrophotometer designed at the Department of Biophysics, Biology Faculty, Moscow State University. A light beam (incandescent lamp) is directed into an integrating sphere (d = 38 mm) and projected to a round-shaped orifice (d = 10 mm) on its opposite side. Fiber optic filters with sedimentary phytoplankton cells are placed into this orifice. The filters are pressed to the sphere from the outside with a diffusely reflecting plate (fluorilon). The beam from the sphere is recorded with a USB2000+ spectrometer (Ocean Optics, United States). In this scheme, the integrating sphere collects the light reflected from the object. The zero line of the device was recorded with a moistened clean GF/F filter. The reflection spectra were recalculated to the absorption spectra assuming that all light losses relative to the zero line recorded by the sensor are determined by light absorption on its way from the front surface of the filter to the reflecting plate and back into the sphere.

The fluorescence spectra of chlorophyll *a* and dissolved organic matter were recorded with an LS-2 laser spectrometer. The device was designed at the Ocean Optics Laboratory with the Shirshov Institute of Oceanology, Russian Academy of Sciences, and has two sources of laser pumping radiation with wavelengths of λ_1 = 401 nm and λ_2 = 532 nm. The fluorescence and Raman scattering radiation induced by the laser beam on water molecules are collected by an

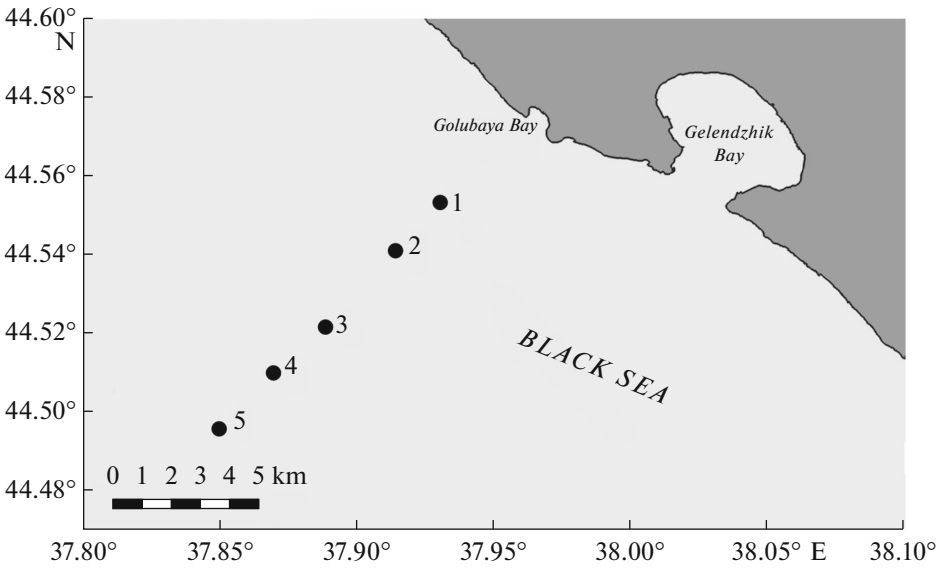

Fig. 1. Sampling region.

optic system with its axis orthogonal to the optic axes of laser beams. A ZhS-11 yellow glass filter with a thickness of 3 mm (for $\lambda_1 = 401$ nm) or a special dichroic filter (for $\lambda_2 = 532$ nm) is placed in front of the input lens of the optic system to eliminate the scattered radiation at the operational wavelength of the laser. The radiation via an FA2206 (Ocean Optics) flexible light guide is input to a USB4000 spectrometer (Ocean Optics; entrance slit, 0.2 mm). The recorded spectral range was 200–850 nm.

The chlorophyll fluorescence intensity in water samples was recorded with a Mega-25 fluorometer, designed at the Department of Biophysics, Moscow State University, described in detail earlier [2]. The source of excitation light ($\lambda_{max} = 455$ nm) had pulsed modulation to determine the fluorescence at the open (F_o) and closed (F_m) reaction centers of photosystem II. The F_o and F_m values were used to calculate $F_v/F_m = (F_m - F_o)/F_m$, the relative variable chlorophyll fluorescence, which is equal to the quantum yield of the highest possible primary photosynthesis processes under the given conditions. The Mega-25 fluorometer also operated in flow mode (for 7 days in September 2014) at the pier of the Southern Department of the Shirshov Institute of Oceanology (Golubaya Bay); water was sampled at a depth of 1 m.

RESULTS AND DISCUSSION

Phytoplankton in early June 2014 was represented by diatoms and dinoflagellates typical of this season, as is demonstrated by the characteristic positions of the bands in the absorption spectra and the ratio of their heights (Fig. 2a; depth, 17 m). On the other hand, phycoerythrin-containing forms were observed in phytoplankton at all stations where additional samples were taken from a depth below 20 m. The changes in the pigment composition of the samples are illustrated by the case study of station 2, where water was sampled from depths of 0, 5, 17, 22, 33, and 45 m. Omitting some intermediate variants, we show here only two typical absorption spectra of plankton particles, namely, the spectra for the "upper" (17 m) and "lower" (45 m) layers (Fig. 2a). The samples from the upper layers (0, 5, 17, and 22 m) contain a brown-colored group of algae, which includes diatoms and dinoflagellates, almost indistinguishable according to their absorption spectra (Fig. 2a; depth, 17 m). The short-wavelength absorption bands of chlorophyll *a* and *c* (Soret bands) are in the range of 420–460 nm. The absorption in the range of 480–540 nm is determined by the carotenoids fucoxanthin and/or peridinin. Long-wavelength absorption bands of chlorophyll *a* and *c* are in the range of 600–700 nm.

The chlorophyll concentration in the samples from depths of 33 and 45 m (Fig. 2a; depth, 45 m) decreases compared with the above layers. Note that an additional narrow absorption band appears at 545 nm, which is characteristic of phycoerythrin-containing cyanobacteria, red algae, and cryptophytes. The filter with sedimentary cells in this case was a shade of pink.

An analogous qualitative change in the pigment composition of samples was observed in the fluorescence spectra (recorded with an LS-2 device). When fluorescence is excited with a green laser, the emission spectrum contains a Raman scattering band at 650 nm, chlorophyll fluorescence band at 680 nm, and phycoerythrin fluorescence band at 560 nm, the intensity of which increased down to the 45 m layer (Fig. 2b). Independent quantitative estimates of the phycoerythrin content in samples according to the LS-2 and

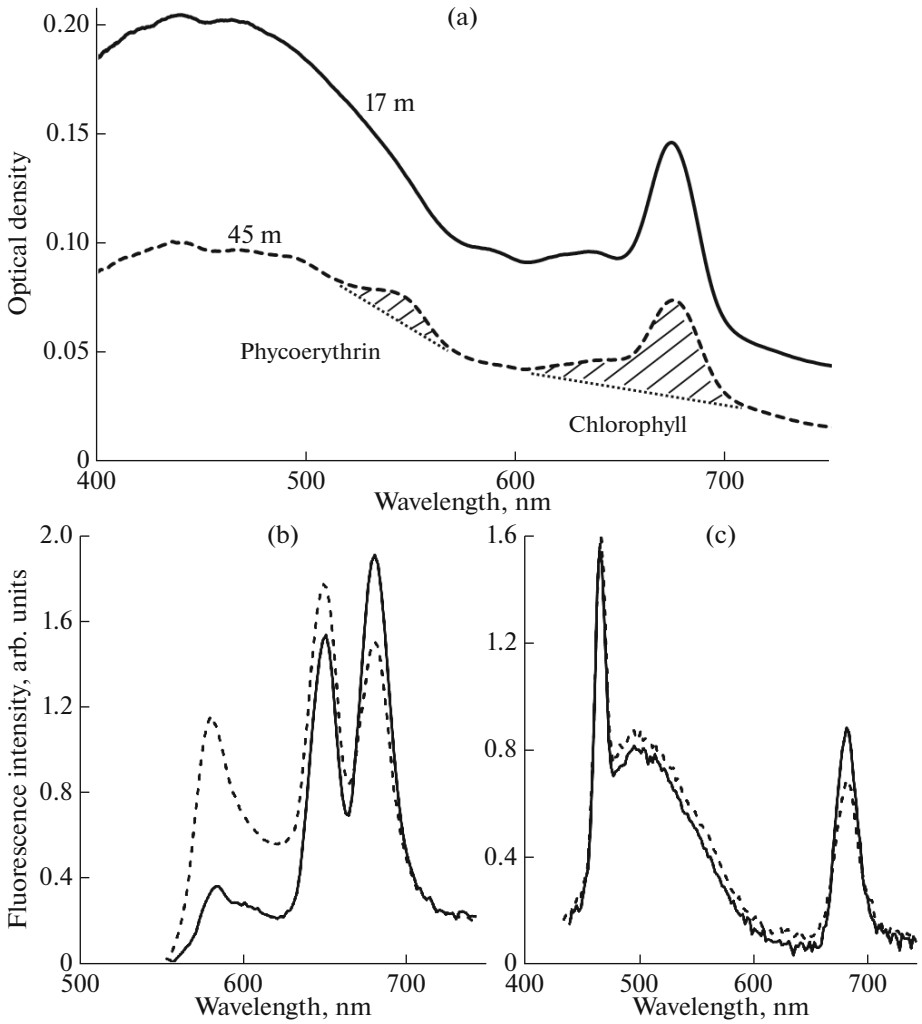

Fig. 2. Absorption and fluorescence spectra of samples from "upper" (solid line) and "lower" (dashed line) water layers: (a) absorption spectra; (b) fluorescence spectra with green laser (532 nm) excitation; and (c) fluorescence spectra with violet laser (401 nm) excitation.

absorption spectra (the hatched area in Fig. 2a) poorly correlated with one another (data not shown). However, laser spectrometry as a qualitative technique for phycoerythrin detection is undoubtedly more convenient, since it does not require any sample filtration before the assay. Owing to the second laser (401 nm), the LS-2 device is also suitable for a rapid assessment of the organic matter content (Fig. 2c).

Figure 3 shows the correlation between the chlorophyll fluorescence intensity determined with a Mega-25 fluorometer and the absorption of suspended matter at 673 nm determined spectrophotometrically on a GF/F. The chlorophyll fluorescence intensity in the assayed samples (over 30 samples) changed fivefold (Fig. 3). The chlorophyll fluorescence intensity and chlorophyll absorption at 673 nm display a linear correlation in this range (Fig. 3). A high correlation coefficient ($R = 0.95$) suggests close values of the chlorophyll fluorescence quantum yield in different samples, which suggests a homogeneous phytoplankton taxonomic composition in the examined water area.

The changes in chlorophyll fluorescence parameters recorded in a flow mode (Mega-25 fluorometer) made it possible to monitor the phytoplankton dynamics in the coastal area (200 m, Golubaya Bay). Figure 4a shows the change in the major parameters (F_o and F_v/F_m) over 1 week. The change in the phytoplankton content, be it an increase or a decrease, appears as a proportional change in both F_o and F_m. We also used the level of F_o as a characteristic of phytoplankton abundance. As is evident from Fig. 4a, the phytoplankton content at the same site of water area can vary twofold and more during 1 day (the interval of 0–1 days in the plot of F_o dynamics).

The phytoplankton content (F_o) insignificantly changed only during 1 day of observation, namely, the

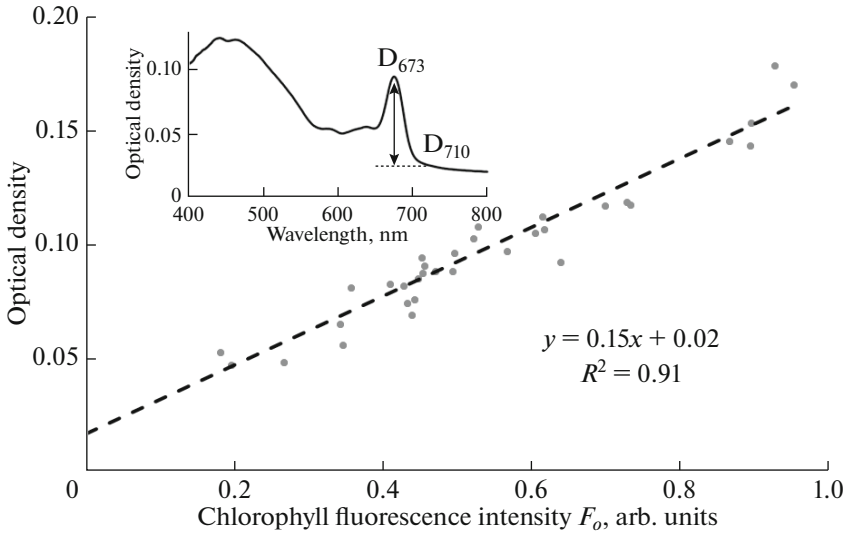

Fig. 3. Correlation between chlorophyll fluorescence intensity and absorption in water samples. Inset (left upper corner): estimate of chlorophyll intrinsic absorption in spectrum recorded in presence of terrigenous suspensions.

interval of days 6–7 (Fig. 4a). The dynamics of fluorescence parameters over this day is detailed in Fig. 4b. The efficiency of the primary photosynthesis was approximately 0.5 from midnight to 7 a.m. (Fig. 4b, curve F_v/F_m). This value cannot be regarded as high, since a value of over 0.65 is characteristic of most algae in the absence of mineral nutrient deficiency. It is also known that laboratory algal cultures cease increasing their population at $F_v/F_m < 0.3$ [7]. Thus, the obtained F_v/F_m values suggest a moderate deficiency of mineral nutrients during the observation period.

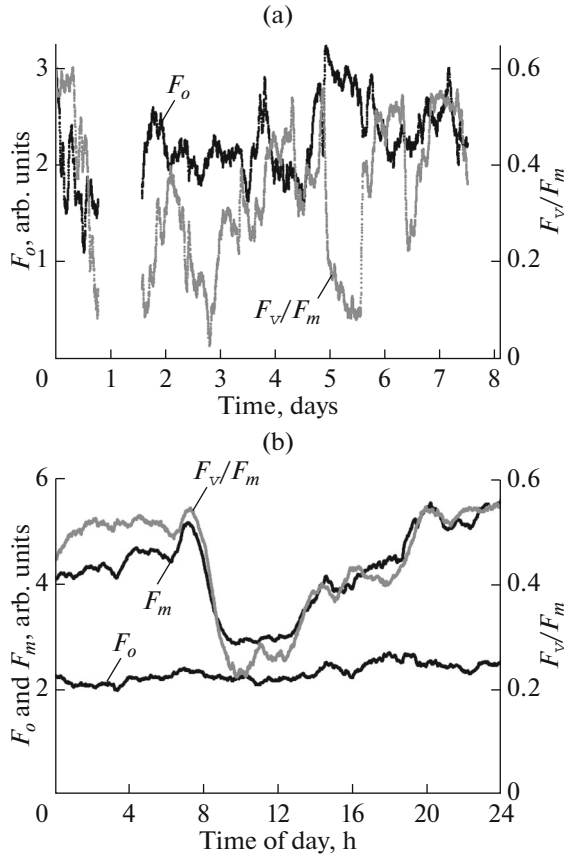

Fig. 4. Dynamics of chlorophyll fluorescence parameters in the Golubaya Bay: (a) during 1 week and (b) during 1 day (interval between days 6 and 7 in panel a).

After 8 a.m., the efficiency of photosynthesis rather rapidly decreased twofold (F_v/F_m decreased from 0.5 to 0.25; Fig. 4b). The cause of such a decrease is nonphotochemical quenching of chlorophyll excited states [3, 6] as suggested by a decrease in F_m. After sunset, the nonphotochemical quenching in algal cells relaxes. In this process, the intensity of maximal fluorescence increases (Fig. 4b, curve F_m) and the efficiency of photosynthesis restores it initial level of 0.5 (Fig. 4b, curve F_v/F_m).

CONCLUSIONS

The performed study shows an inhomogeneous spatial distribution of phytoplankton as well as a rapid change in the abundance of algal plankton even within a small water area. The abundance of phytoplankton under such conditions can repeatedly increase and decrease at the same site during 1 day (Fig. 4a); thus, only rapid tests utilizing fluorescence intensity of samples (chlorophyll and phycobilins) allow for an adequate assessment of the content of microalgae. A reliable determination of the chlorophyll content in absolute units requires that a fluorometer is calibrated in situ using seawater specimens (Fig. 3).

Qualitative matching of the data obtained using laser spectrometer (fluorescence intensity at 560 nm) and spectrophotometer (absorption intensity at 545 nm) is

evident in the water samples containing phycoerythrin. However, laser spectrometry under field conditions considerably exceeds the absorption measurements on filters in efficiency.

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