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**OCEANOLOGY**

# Application of a Double-Flash, Impulse, Submersible Fluorimeter in the Determination of Photosynthetic Activity of Natural Phytoplankton

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In the past few years data testifying to the fact that abiotic factors substantially alter the efficiency of light-induced photosynthesis reactions of microalgae at the functioning level of photosystem 2 (PS2) reaction centers (RC), which put into effect water decomposition and oxygen release [1, 9, 11], has been collected. The change in the light stage under the action of environmental factors is necessary for correlating the formation processes of photosynthesis products and their utilization in biosynthetic processes in alga cells [1].

The methods based on measurement of chlorophyll luminescence [1–5, 10] are used to study photosynthesis in plants and algae. The intensity of fluorescence is used in oceanology as an indicator of chlorophyll concentration in reservoirs [8]. Photosynthetic activity may be evaluated by means of measuring the intensity of variable fluorescence dependent on PS2 [1, 4, 5, 7, 10]. On fluorescence excitement by a short and faint flash of light, during which each of the photosynthetic RC absorbs no more than one photon, energy excitated by any photosynthetic pigment of a light-collecting antenna is rapidly transferred into an RC pigment complex where primary conversion of the light energy takes place. The destiny of the excitation energy is determined by the relationship between rate constants of three concurring processes of chlorophyll molecule deactivation in an RC complex [5, 10]:

$$P^* \xrightarrow{K_f + K_d + K_{ph}} P, \qquad (1)$$

where $P$ and $P^*$ are quiescent and excited states of chlorophyll molecule a; $K_f$, $K_d$, and $K_{ph}$ are rate constants of radiating (fluorescence), nonradiating (heat dissipation), and photochemical (primary charge separation in RC) excitement deactivation. Quantum efficiencies of primary charge separation and fluorescence are calculated from the formulas

$$qZ = \frac{K_{ph}}{K_f + K_d + K_{ph}}, \quad qF_o = \frac{K_f}{K_f + K_d + K_{ph}}, \qquad (2)$$

respectively.

The values of these constants are determined by the molecular organization of PS2 reaction centers and are evidently independent of alga taxonomic affiliation. Under optimal conditions, constant $K_{ph}$ is maximum in these three coefficients at active PS2 reaction centers. As a result, excitation energy is used in photosynthetic reactions with quantum efficiency $qZ$ close to unity, and only a small part of the excitements (about 0.3%) is lost in the form of fluorescence. At the same time, a rate constant of photochemical deactivation of excitement

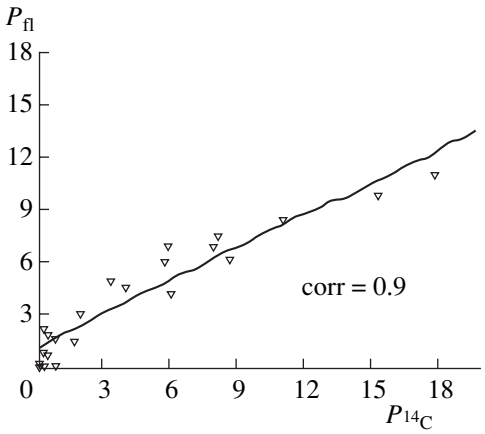


**Fig. 1.** Correlation between primary phytoplankton production ($P_{fl}$), calculated from fluorescence data on $F_o$, $F_v/F_m$ and illumination measured by submersible fluorimeter, and production determined *in situ* by a $^{14}C$ radiocarbon method ($P$, μg/ml). The data were obtained at the stations in the Baltic Sea at 9-hour exposures.

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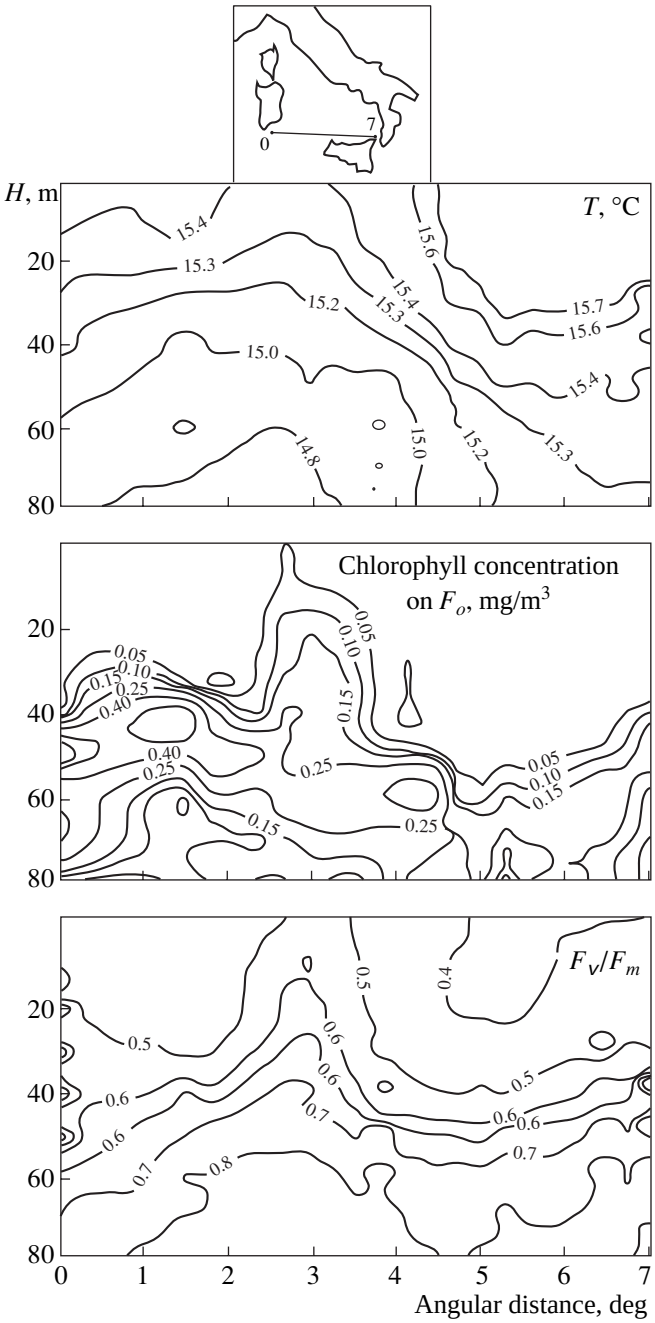


**Fig. 2.** In-depth distribution of fluorescence parameters ($F_o$, $F_v/F_m$) and temperature measured by a submersible fluorimeter along a section of the Tyrrhenian Sea between Corsica and the Strait of Messina during a cruise of the R/V *Moscow University.*

is equal to zero, and fluorescence efficiency rises to its maximum value ($F_m$):

$$qZ = 0, \quad qF_m = \frac{K_f}{K_f + K_d}. \tag{3}$$

If RC are closed (inactive), for example, in the case of ingression of the second excitement into RC with a time interval shorter than the RC cycle, while primary charges are not yet used in further reactions. The difference between fluorescence intensity at closed and open RC ($F_v = F_m - F_o$) is called the variable fluorescence [5, 7]; it corresponds to that part of the light energy involved in photosynthesis [10]. It is shown that the relationship between the variable and maximum fluorescence is equal to the quantum efficiency of a primary photochemical reaction of charge separation in photosynthetic centers [5, 10]:

$$(qF_m - qF_o)/qF_m = \frac{K_{ph}}{K_f + K_d + K_{ph}} = qZ. \tag{4}$$

Fluorescence intensity at open RC ($F_o$) may be determined by exciting a fluorescence by a short probing light flash of low intensity such that no more than one photon will enter each PS2 reaction center during this flash:

$$F_o = k \cdot qF_o. \tag{5}$$

Fluorescence intensity at closed RC ($F_m$) may be determined though an inhibition of electron flux by diuronide or with the help of double-impulse lighting, when a powerful flash of light transforming all RC in a closed state is first set off; then, fluorescence intensity is measured at an interval shorter than an RC cycle (<100 μs) [4–6]:

$$F_m = k \cdot qF_m. \tag{6}$$

Thus, measuring chlorophyll fluorescence intensities $F_o$ and $F_m$ in relative units, we can obtain a value of quantum efficiency of charge separation in photosynthetic RC:

$$qZ = F_v/F_m, \tag{7}$$

where $F_v = F_m - F_o$.

It is shown that the efficiency of charge decoupling in PS2 reaction centers is lower under unfavorable conditions [1, 5, 11]. A proportional dependence between $F_v/F_m$ and the photosynthetic production of phytoplankton cells is observed in numerous works [4, 9, 11].

To work with natural phytoplankton, we have designed a compact submersible impulse fluorimeter, which makes it possible to probe the fluorescence parameters while simultaneously registering water temperature and illumination. The device is composed of a submersible probe, board power pack, and an IBM-compatible computer controlling the measurement process. The registering part of a probe is composed of a photodetector (photomultiplier), amplifier, analog-digital converter, interface for communication with the computer, and two impulse sources of light with flash durations of 10 μs (the spectral region is between 400 and 480 nm). The first probing flash with an energy of 0.01 J ensures the measurement of background fluorescence ($F_o$). The last one allows us to evaluate chlorophyll content of natural phytoplankton after proper calibration. The second flash, with a photosynthesis saturating energy of 1 J, is put into operation before the probing flash. The powerful luminescence brings about an

increase in the fluorescence intensity to the level of $F_m$. The fluorimeter registers the degree of increase in the fluorescence intensity ($F_v = F_m - F_o$) induced by a powerful flash, which makes it possible to calculate the efficiency of light use by microalgae. The application of an underwater illumination monitor allows us to evaluate photosynthetic production, measuring variable fluorescence $F_v/F_m$ and illumination at the specific horizon.

The experiments carried out by us in the Baltic Sea (Fig. 1) have shown a high correlation between primary production, calculated from the fluorescence parameters and underwater illumination, and production determined *in situ* by the classical method with the application of $^{14}C$ isotope. The correlation gets better if the inhibition effect in surface horizons is taken into account.

All parameters are automatically measured by a probe, and data are displayed in real time in the form of plots reflecting either the vertical temperature profile, underwater illumination, alga concentration and activity at a fluorimeter submersion, or revealing the dynamics of these parameters in time or along the vessel's path. Using the fluorescence probe described above, we have constructed a deep-sea section extending from the southern tip of Corsica up to the Strait of Messina, passing the coast of Sicily (60 km). We have obtained three profiles of temperature, chlorophyll concentration ($F_o$), and phytoplankton photosynthetic activity $F_v/F_m$ (Fig. 2). The general phytoplankton distribution was characteristic of oligotrophic water with low alga concentrations and abundant algae at depths of 50 m and deeper. Phytoplankton activity was low in the surface water and increased with depth, reaching its maximum value at a depth of 60 m. We should note that the character of distribution of both phytoplankton concentration and its activity correlated with the distribution of water bodies of different temperatures. At the same time, we observed an increase in phytoplankton number and activity in the ascent zone of deeper cold water enriched in minerals. This example demonstrates the such application of such a probe for the study of dynamic characteristics of water systems as well as for a rapid evaluation of photosynthetic characteristics natural populations of phytoplankton.

## ACKNOWLEDGMENTS

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