RESEARCH METHODS
AND FACILITIES

Assessment of Vertical Profiles of Phytoplankton Photosynthetic Activity by the Fluorescence Method

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Abstract—Vertical profiles of phytoplankton primary production (PP), chlorophyll *a*, and fluorescence measured near the surface in the central Baltic Sea in September 1995 featured similar variations related to the diurnal dynamics of solar radiation. Growth of irradiance from 50 W/m^2 in the morning hours to a maximum value of 300 W/m^2 about the noon was correlated with the increase in photoinhibition of the measured variables in the upper water layers. In the afternoon hours, when irradiance decreased, further development of photoinhibition was observed. When the surface irradiance was not over 150 W/m^2, as well as under a higher light intensity but only in the first hours of photoinhibition, the primary production profiles calculated on the basis of the data on fluorescence and illumination were similar to those of in situ measured PP. A comparison of the calculated PP values with those measured in the water column shows a good correlation between them, with the correlation coefficient being equal to 0.85.

INTRODUCTION

Assessment of the productivity of aquatic biological systems is necessary to understand the factors forming the basis of environmental changes both in a local region and on a global scale. In this connection, the elaboration of methods for express and precise measurements of the productivity of aquatic systems is of great importance because it is determined first by phytoplankton productivity at the basis of trophic chains.

As a rule, microalgal photosynthesis is estimated by the rate of photosynthetic utilization of radioactive inorganic carbon [26] or, less often, by the change in oxygen concentration in a sample [7, 18, 29]. These methods are labor-consuming and call for incubation of samples in a closed volume that results in the production of artifacts (the so-called *flask effect*). Therefore, in addition to the direct methods for primary production measurements, more simple indirect procedures were widely employed. They are based on empirical and theoretical models of photosynthesis and allow us to estimate PP by keeping a record of photosynthetic parameters, such as biomass, solar energy, inorganic nitrogen, temperature, etc. [5, 10, 24, 27].

The estimation of chlorophyll *a* fluorescence is a popular hydrobiological method [13, 14, 25] for measuring phytoplankton photosynthesis without any action on its physiological condition [10–12, 17]. To assess the vertical distribution of microalgal primary production in real time, a submersible fluorometer was designed [8], which permits assessment of such photosynthetic parameters as the light absorption efficiency by algal cells and the quantum efficiency of photosynthesis. The results obtained with the use of this instrument agreed closely with the primary production values measured by the direct method. However, using fluorescence for assessment of the quantum efficiency of photosynthesis under conditions of natural light requires application of rather complicated instruments.

This study deals with the results of primary production calculations. The algorithm is based on the model for primary photosynthetic processes suggested by Kiefer and Mitchell [15]. In this model, photosynthetic parameters, such as the light-absorptive capacity of phytoplankton and the efficiency of primary photosynthetic reactions, were estimated by fluorescence in phytoplankton by means of a PrimProd submersible fluorometer that we constructed. This instrument allows us to record the constant fluorescence Fo and the relative variable one Fv/Fm in a dark chamber with the pump-and-probe method [20]. The model implies a parameter that is impossible to estimate from microalgal fluorescence. This is the value of the light intensity, which semisaturates photosynthesis $I_{1/2}$. It can be found by means of calibration of fluorescence and irradiance against the primary production values measured by the direct method. The challenges facing us were the following: (1) to measure $I_{1/2}$, (2) to study its diurnal and horizontal fluctuations, and (3) to estimate the action of them upon the accuracy of primary production calculation by the method suggested.

METHODS

Theoretical Background of the Procedure

The photosynthetic rate in Kiefer's model is directly proportional to the absorbed light (in parentheses) and

the quantum efficiency of photosystem 2 hyperbolically related to the irradiance:

$$V_{\mathrm{ph}} = (ISn_{\mathrm{RC}})\frac{q_z I_{1/2}}{I + I_{1/2}}, \qquad (1)$$

where I is the integrated light spectrum, n_{RC} is the number of functional reactive centers in a sample volume unit, S is the light-absorptive capacity of antennas of an individual photosynthetic unit, q_z is the maximum quantum efficiency of photosystem 2, and $I_{1/2}$ is the light intensity under which the quantum efficiency of photosynthesis reaches half of its maximum value.

The variables $n_{\mathrm{RC}}S$ and q_z are related to the functioning of photosystem 2 and can be estimated by the fluorescent method. The constant fluorescence Fo is generally measured in order to estimate phytoplankton biomass and pigment concentrations (chlorophyll, carotinoids) in a sample [4, 9, 31]. This variable is linearly correlated to the absorptive capacity of phytoplankton (Sn_{RC}), if the quantum efficiency of fluorescence is constant and the spectral distribution of the fluorescence-induced pulse lamp is close to the solar spectrum:

$$n_{\mathrm{RC}}S = k\mathrm{Fo},$$

where k is a constant.

The variable Fv/Fm characterizes the maximum quantum efficiency of photosystem 2 or the quantum efficiency of photosynthesis under a low light [2, 3, 16]:

$$q_z = (\mathrm{Fm} - \mathrm{Fo})/\mathrm{Fm} = \mathrm{Fv/Fm} \ [2, 3, 16].$$

Moreover, the ratio Fv/Fm correlates to photoinhibition of photosynthesis [1, 6, 19], which causes a decrease in the number of functional reactive centers in algal cells n_{RC} [21, 28]. Thus, Fv/Fm is associated with photosynthesis under both low and high light and characterizes its intensity.

The value of $I_{1/2}$ is determined by the rate of the dark reactions limiting photosynthesis and cannot be measured fluorometrically. This value was found statistically as a constant for natural phytoplankton [30], but under laboratory conditions it was shown that it depended on the adaptation of microalgae to the action of various environmental factors [1]. We suppose that within a region with a certain water type this value is close to a constant and can be estimated by calibration of the fluorescence and irradiance data against those on primary production measured by the direct method, according to the following formula:

$$\mathrm{PP}(d) = tI(d)k\mathrm{Fo}(d)\frac{\mathrm{Fv/Fm}(d)I_{1/2}}{I(d) + I_{1/2}} \ (\mathrm{mgC/m}^3 \ \mathrm{h}), \ (2)$$

where $\mathrm{PP}(d)$, $I(d)$, $\mathrm{Fo}(d)$, and Fv/Fm (d) are the values of the measured primary production profiles, light intensity, constant and relative variables of fluorescence, respectively; k and $I_{1/2}$ are unknown values; d is the depth, and t is the time.

Recording of Data

The following variables were measured at two stations located in the Baltic Sea (56°03′ N, 19°13′ E and 55°20′ N, 18°07′ E) during a cruise of R/V *Oceania*, Institute of Oceanology, Polish Academy of Sciences, on September 9 and 12, 1995; (1) phytoplankton primary production, mgC/m^3 per hour; (2) chlorophyll *a* concentration, mg/m^3; (a) constant fluorescence Fo, relative units; (4) underwater irradiance and solar radiation above the surface, W/m^2.

Primary production was measured at eight levels down to a depth of 20 m using a standard procedure [26]. The measurements were started at 8:00 a.m. local time and were performed subsequently by means of two-hour in situ exposures of flasks five times during the light day at station 1 and seven times at station 2.

Chlorophyll *a* content was measured according to the standard spectrophotometrical method [7]. Water samples were collected from nine levels in the euphotic zone. Starting at 8:00 a.m., four measurements were carried out at each of two stations two hours apart.

Vertical profiles of Fo, Fv/Fm, and *I* were measured during the exposure of flasks by means of a PrimProd submersible fluorometer designed at the Department of Biophysics, Biological Faculty, Moscow State University. Fluorescence records are carried out by the pump-and-probe procedure [20]. When submersing a probe (generally, the rate of submersion was 30–50 cm/s), the water with microalgal cells enters into the dark meter chamber. Inside the chamber, a sample is exposed to a weak sounding flash of light, and then Fo is recorded in phytoplankton with the open reactive centers of photosystem 2. The next flash, one second later, was a saturating flash of light that changed reactive centers from the open state to the closed one. In 50 μs (the time comparable with that needed for the turnover of a reactive center) a sounding flash is fed into the measuring chamber and then the maximum fluorescence Fm is recorded. The spectrum of fluorescence activation lies within the wavelength range from 400 to 550 nm. The spectrum of fluorescence registration is from 680 nm.

The instrument is equipped by sensors of pressure (depth) and temperature, as well as by a quantum-meter gage of underwater irradiance. An IBM-compatible computer manages the operation of the instrument.

RESULTS

The shape of primary production and phytoplankton vertical profiles measured on a cloudy day at station 1 was unchanged practically throughout the day, when the mean light intensity was not more than 100 W/m^2. A typical result of measurements is shown in Fig. 1. The peak of microalgal productivity was near the surface and under irradiance of 100 W/m^2 phytoplankton productivity was as much as 40 mgC/m^3 per hour. Fluorescence profiles had no marked maxima in the water column, and fluorescence distribution was homoge-
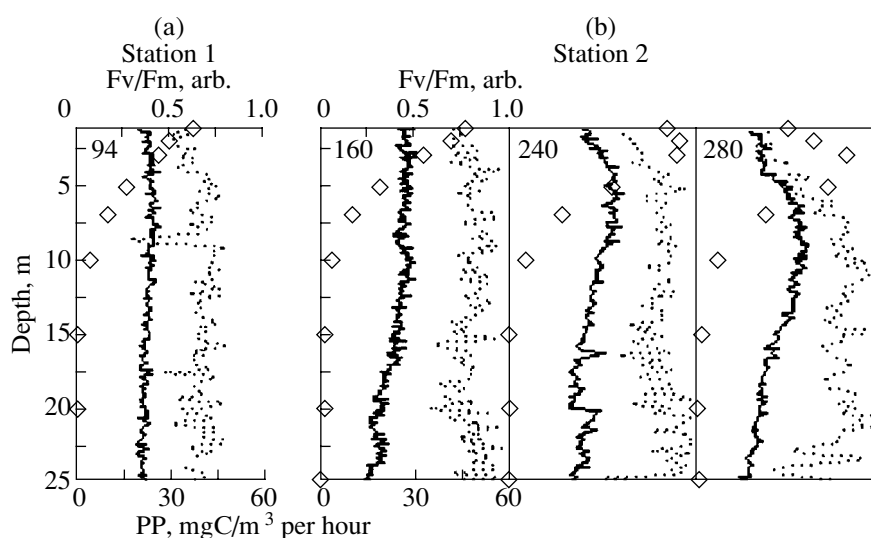
Fig. 1. Vertical distribution of primary production ($\diamond$), Fo (solid line), and Fv/Fm (dashed line) in the Baltic Sea : (a) at station 1 on a gloomy day (a typical result is presented) and (b) at station 2 on a clear day (three typical results for different daytime are presented). The values of light intensity above the surface (W/m²) averaged during the exposure of samples are presented next to the curves. The fluorescence and underwater irradiance were measured in the middle of exposure.

neous at all the levels of the euphotic zone down to 30 m. A slight decrease in fluorescence at the upper layers was observed only near noon (Fig. 1a).

Primary production and fluorescence values measured at station 2 during clear weather (I more than 200 W/m²) exceeded, on the average, the results obtained at station 1. Near the surface, their diurnal fluctuations were also observed. Typical results obtained in the morning, noon, and afternoon are shown in Fig. 1b. The maximum phytoplankton productivity was as great as 60 mgC/m³ per hour, but the level of peak location in the water column depended on the light above the water surface. Early in the day when the light intensity was less than 150 W/m², the maximum of productivity was positioned near the surface, as it was at station 1. About noon, when radiation reaches maximum values, namely, 300 W/m², primary production in the upper layers was decreased, which resulted in the descent of its maximum down to a depth of about 3 m. In the afternoon, the maximum productivity was observed at a level of 6 m when the surface irradiance was less than 250 W/m².

Microalgae fluorescence measured at station 2 in the morning hours in the euphotic zone was distributed evenly, although a slight reduction of Fo was registered with depth, as well as deviation from the average values of Fv/Fm in the water column at levels deeper than 20 m. At noon, a reduction in values of Fo and Fv/Fm near the surface and an increase of Fo in the range of depths from 5 to 10 m was observed. This was the reason for the lowering of the fluorescence peak to 5 m. In the latter half of the day, the depth of the fluorescence peak position increased to 10–13 m, whereas the light on the surface decreased as compared with its midday values.

The reduction of Fo in the upper layers and its increase at depths of 5–10 m at noon were respectively related to the decrease in phytoplankton biomass in the realm of high light intensities and to the growth of phytoplankon biomass in the range of optimal irradiance from about 50 to 100 W/m². This is confirmed by the results of comparison of Fo and chlorophyll a profiles, which have similar diurnal dynamics at station 2 (Fig. 2). The midday depression of Fv/Fm under high light, as it was noted above, indicates a photoinhibition of microalgal photosynthesis.

It is necessary to mark an almost total identity of the Fo and Fv/Fm diurnal dynamics in the upper layers and their relation to the changes in primary production. We also observed a similar depression of both parameters at the high light in the central deep areas of the Norwegian Sea and under laboratory conditions [1]. It was shown that there is a relation of the light-dependent changes of Fo to the inhibition of cellular development and an appropriate decrease in the microalgal concentration, as well as a correlation with photosynthetic photoinhibition.

Calculation of Phytoplankton Primary Production

To calculate the primary production using the procedure suggested in this study, it is necessary to determine the unknown photosynthetic parameter $I_{1/2}$ and the calibrating factor k (2) with the help of the data on the direct measurements of primary production. Thus, this method may be useful if in the test area these unknown variables vary slightly during a certain time span (day, month). In this situation it is enough to determine k and $I_{1/2}$ once, in order to carry out the estimation of the primary production only by fluorescence with a rather
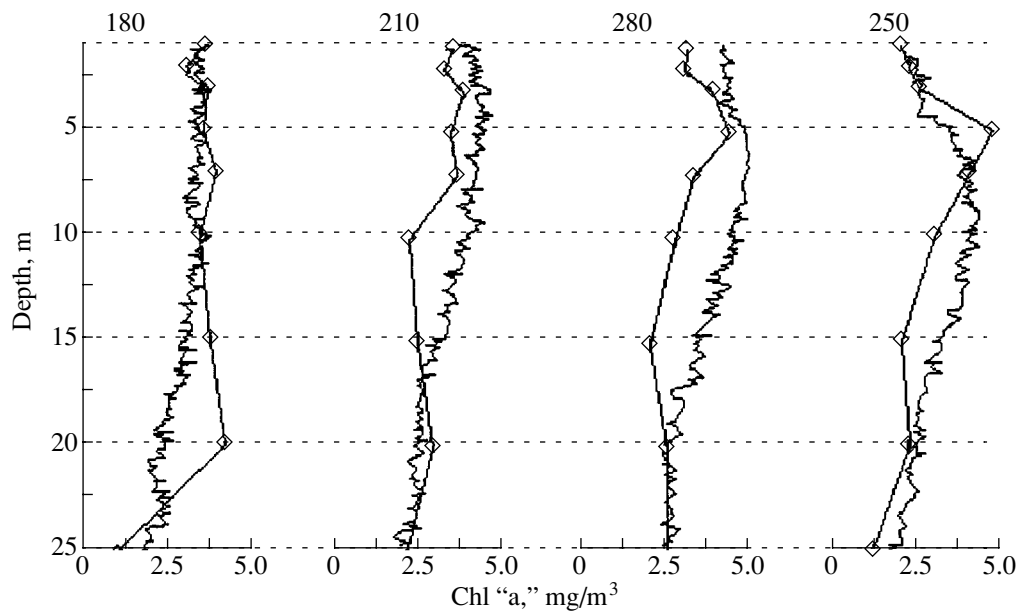
Fig. 2. Vertical distribution of chlorophyll *a* ($\diamond$) and Fo (solid line) obtained on a clear day in the central Baltic Sea. The values of light intensities above the surface (W/m^2) at the moment of measurements are shown next to the curves.

high accuracy during this period. We examined fluctuations of k and $I_{1/2}$ at two stations during the day.

Such unknowns as k and $I_{1/2}$ were found using formula (2) by substituting the data on primary production, light, and microalgae fluorescence. The results obtained are given in the table. In the columns, the ordinal numbers of measures, the starting time of sample exposure when determining primary production, light intensity over the surface, and the values of k and $I_{1/2}$ are shown. Standard deviations for k at stations 1 and 2 made up 9.7 and 11% and those for $I_{1/2}$ were 27 and 19%, respectively. The average value for k on station 1 was almost equal to that for station 2:

$$k_1 = 2.05 \times 10^{-3} \text{ (station 1)}$$

and

$$k_2 = 1.98 \times 10^{-3} \text{ (station 2)}.$$

Differences between the averages for $I_{1/2}$ were more substantial:

$$I_{1/2} = 28 \text{ (station 1)}$$

and

$$I_{1/2} = 41 \text{ (station 2)}.$$

These results point to more considerable fluctuations of $I_{1/2}$ as compared to k. They may be caused by differences in the species composition and physiological state of microalgae at both stations, whereas the scattering of k is related mainly to the measurement error.

The calculated primary production (CPP) profiles were obtained according to formula (2), by substituting the data on fluorescence and irradiance in the right-hand side of the equation, as well as the mean values of

k and $I_{1/2}$ values calculated from the results of separate measurements at two stations of the Baltic Sea, as well as the ordinal numbers of measurements, the starting time of sample exposure by the ^{14}C method, and irradiance above the water surface $I(0)$ at the moment of estimation of phytoplankton fluorescence

| Station 1					Station 2				
no.	time	$I(0)$	$k \times 10^3$	$I_{1/2}$	no.	time	$I(0)$	$k \times 10^3$	$I_{1/2}$
1	8	54	2.13	40	1	8	160	1.72	40
2	9	86	1.96	27	2	9	180	2.03	45
3	11	125	2.10	18	3	10	210	1.80	40
4	13	110	2.15	23	4	11	240	1.95	45
5	14	90	1.93	34	5	12	280	2.15	50
					6	13	250	1.79	45
					7	14	220	2.41	23

(a)
Station 1

(б)
Station 2
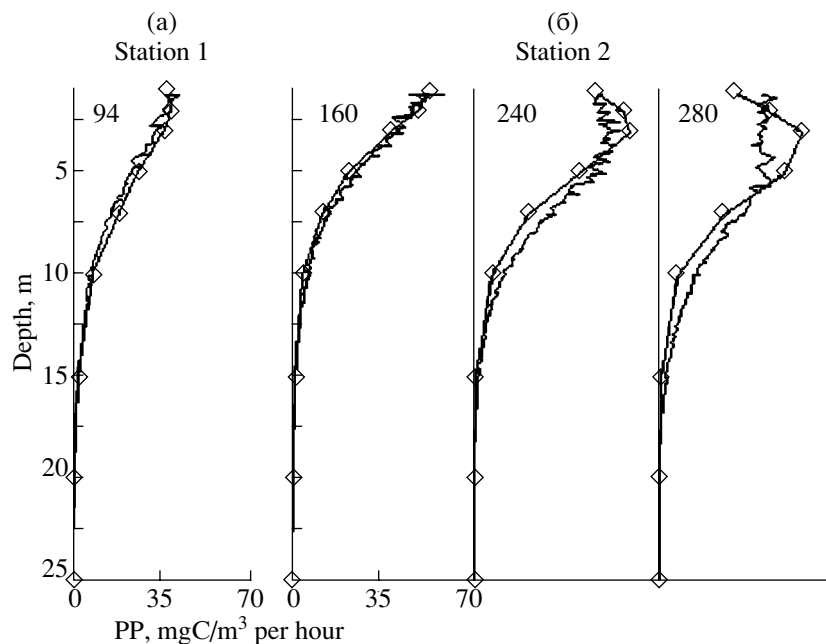
Fig. 3. Vertical profiles of the measured and calculated values of primary production. Results characteristic of two stations are given. The values of light intensity above the surface (W/m²) averaged for the time of two-hour exposure of samples by the ¹⁴C method are presented next to the curves.

k and $I_{1/2}$ at both stations. The measured and calculated relationships between the primary production and depth are given in Fig. 3. The results show a high correlation between the above variables for station 1 (Fig. 3a) and a slightly lower one for station 2 (Fig. 3b). CPP profiles derived from the results of the first four fluorescence measurements at station 2 almost coincided with those from the measured PP (Fig. 3b). The fourth and fifth

CPP profiles (second profile in Fig. 3) coincided with that of the measured PP near the surface, where the latter decreased. This regularity was absent for the latter two measurements carried out at station 2 after noon; namely, the near-surface CPP did not depend on depth. This fact is likely to be related to a decrease in the quantum fluorescence yield [22] and, as a result, with an underestimation of its values with reference to the actual chlorophyll concentration and efficiency of primary reactions of photosynthesis, which results in breaking of its correlation with microalgae productivity.

Despite the errors in PP calculations in the upper layers in p.m. hours, the coefficient of correlation between the calculated primary production within the water column under a unit area and that measured by the ¹⁴C method was rather high and equaled 0.88 (Fig. 4). This result is explained by complementary compensation for errors in calculations of PP near the surface where the CPP values were overestimated as compared to the measured production, as well as in the deep layers with the maximum productivity observed, where the underestimates of the CPP values were obtained.
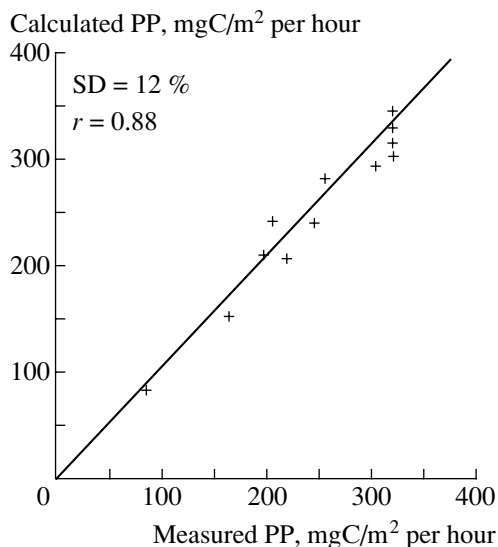
Fig. 4. Relationship between the calculated and the measured values of phytoplankton primary production under 1 m². Linear regression of this dependence and the standard deviation are given.

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