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A Study of the Variability of the Parameters for the Model of the Calculation of the Rate of Phytoplankton Photosynthesis by the Fluorescent Method from the Example of the Baltic Sea

T. K. Antal[1], P. S. Venediktov[1], D. N. Matorin[1], B. Wozniak[2], and A. B. Rubin[1]

[1] *Moscow State University, Moscow, Russia*

[2]*Institute of Oceanology, Polish Academy of Sciences, Sopot, Poland*

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Abstract—In this study, we examined the possibility to determine the rate of phytoplankton photosynthesis in situ with a submersible pump-and-probe fluorometer. A biophysical model was suggested to describe the relationships between the photosynthesis, the underwater irradiation, and the intensity of phytoplankton fluorescence induced by an artificial light source. The fluorescence parameters Fo and Fv/Fm were used to determine the coefficient of light absorption and the efficiency of the primary reactions of phytoplankton photosynthesis. Two parameters of the model which cannot be measured by this method, namely, the coefficient of proportionality (k) between the fluorescence yield Fo and the light absorption phytoplankton capacity, as well as the light intensity semisaturating photosynthesis ($I_{1/2}$), were found by means of calibrating the fluorometer in terms of the primary production values. The latter were measured by the radiocarbon modification of the flask method at 23 stations in different areas of the Baltic Sea in the period from May to September inclusively. It was shown that the standard deviations of these parameters in situ did not exceed 20%, and the values of the phytoplankton photosynthesis rate determined by the fluorescent method with the use of the averages of these parameters correlated well with the measured values ($r = 0.89$). The accuracy of determination of the primary production values decreased, for the most part, because of the decrease in the fluorescence quantum yield under the conditions of a hyperoptimal irradiance and variations of the $I_{1/2}$ parameter related mainly to the phytoplankton concentration.

INTRODUCTION

The contribution of the gross primary production of phytoplankton is about 95% of the total production in the oceans and 40% of the global carbon assimilation. It should be noted that the microalgae biomass is about 2% of the total mass of plants. This fact points to the high efficiency of light energy conversion by microalgae cells [14]. The measurements of the rate of phytoplankton photosynthesis give us the possibility to estimate the aquatic biosystem productivity on a local and global scale, as well as to study the mechanisms of the influence of environmental factors, including those of anthropogenic origin, on it.

Photosynthesis of microalgae is estimated by the rate of radiocarbon assimilation by cells [37] or by the changes in the water-soluble oxygen concentration [26]. These methods are reasonably labor consuming, and, when used, artifacts associated with the long-term isolation of phytoplankton cells in flasks [9], with the difference between net and gross photosynthesis [6], as well as with metal toxicity [15], arise. Therefore, at present, the development of methods that enable one to avoid these problems and to rapidly and continuously estimate the rate of phytoplankton photosynthesis, without affecting their physiological condition, is an urgent task. To some extent, the methods for chlorophyll *a* fluorescence registration are helpful in the solution of these problems [16, 18]. The relationship between the chlorophyll *a* fluorescence and photosynthesis values is described by a number of biophysical models of the primary processes of photosynthesis [22, 39].

In this study, we consider the possibility for determination of the rate of carbon assimilation by phytoplankton Vc (μmole C m^{-3} s^{-1}) in terms of fluorescence measured by the pump-and-probe method [25, 29] and excited by an artificial source of light. The basis for this model was the light–photosynthesis dependence [19], which was described through the coefficient of sunlight absorption by microalgae suspension and the efficiency of the light energy conversion absorbed during photosynthesis. In terms of the fluorescence parameters Fo and Fv/Fm, the coefficient of light absorption by microalgae (the light absorption capacity) and the efficiency of the primary photosynthetic reactions were determined. In the model, there are two parameters that cannot be measured by the method suggested. The first is the coefficient of proportionality k between the fluorescence yield Fo and the phytoplankton light absorption capacity, as well as the light intensity semisaturating photosynthesis $I_{1/2}$. The latter depends on the dark reactions limiting the rate of pho-

tosynthesis. These parameters were determined by means of calibrating the fluorometer with respect to the rate of carbon assimilation by alga cells measured by the radiocarbon method. In this study, the variations of the k and $I_{1/2}$ parameters were examined in the different areas of the Baltic Sea in the warm period of the year (May to September inclusively), depending on the accuracy of the measurements, abiotic factors, and phytoplankton characteristics. In addition, the possibility of using these parameters as constant values while estimating the primary production of phytoplankton in the test area during the season was examined.

METHODS

Abbreviations:

RC—reaction center;

PhS—photosystem;

Fo—constant of chlorophyll fluorescence, relative units;

Fv/Fm—relative fluorescence variable, relative units;

Chl—chlorophyll *a* concentration, mg Chl m^{-3};

CPP and PP—primary production values of phytoplankton calculated in terms of fluorescence and measured by the direct method, respectively, mgC m^{-3} per unit time;

a_{PSP}—coefficient of light absorption by the photosynthetic pigments of photosystem II of microalgae suspension (the light absorption capacity), m^{-1};

a_{fl}—coefficient of light absorption by algal suspension cells exciting Fo, m^{-1};

a_s—coefficient of light absorption by algal suspension cells, m^{-1};

k—coefficient of proportionality between Fo and the coefficient of light absorption by phytoplankton, namely, $k = a_{PSP}/\text{Fo}$, relative units, k_m—average value over the water column;

E—factor determined by the difference between the coefficients of absorption of the underwater irradiance by phytoplankton and the light exciting Fo, namely, $E = a_{PSP}/(a_{PSP})_{fl}$;

ϕ_{Fo}—quantum yield of Fo;

$I_{1/2}$—intensity of the light semisaturating the rate of photosynthesis, μE m^{-2} s^{-1}, $I_{1/2}m$—average value over the water column;

z—depth, m.

Model structure for the PP calculation in terms of the phytoplankton fluorescence parameters. The basis for this model is the photosynthesis–light relationship [19]

$$V_c(I) = a_{PSP}\phi(I)I, \tag{1}$$

where a_{PSP} (m^{-1}) is the coefficient of absorption of the underwater irradiance by photosynthetic pigments of photosystem II in a microalgae suspension (PSP—photosynthetic pigments [8]); ϕ (μmol C μE^{-1}) is the efficiency of the light energy absorbed by these pigments; and I is the underwater irradiance in the range of photosynthetically active radiation (μE m^{-2} s^{-1}).

The ϕ value is proportional to the relative amount of the functional (f) and open (q_P) reaction centers of photosystem II in algal cells, to the efficiency of the photochemical light energy conversion in an open reaction center (ϕ_{RC}, μmol electrons μE^{-1}), and to the efficiency of the electron transfer from H_2O to CO_2 (ϕ_e, μmol C (μmol electrons)$^{-1}$):

$$V_c(I) = a_{PSP} f q_P(I)\phi_{RC}\phi_e I. \tag{2}$$

Determination of the a_{PSP} Values and the Product ($f\phi_{RC}$) in Terms of Phytoplankton Fluorescence

The fluorescence signal Fo excited by an artificial light source in open microalgae reaction centers can be found from the following equation:

$$F_0 = GI_{fl}(a_{PSP})_{fl}\phi_{\text{Fo}}, \tag{3}$$

where I_{fl} is the intensity of the exciting light (in our fluorometer, $I_{fl}(\lambda)$ is fairly evenly distributed over the spectral band 400–550 nm), a constant; $(a_{PSP})_{fl}$ is the coefficient of absorption of the light exciting fluorescence by photosynthetic pigments of photosystem II in the algal suspension, averaged over the range of 400–550 nm; ϕ_{Fo} is the fluorescence quantum yield in the cells with open reaction centers of photosystem II; and G is the coefficient determined by the geometric characteristics and sensitivity of the signal sensor, a constant.

Taking into account that $(GI_{fl})^{-1}$ = const, the coefficient of absorption of the underwater irradiance by the pigments of photosystem II in the microalgal suspension is related to the fluorescence in the following manner:

$$a_{PSP} = \text{const}\phi_{\text{Fo}}^{-1}EF_0 = k(\phi_{\text{Fo}}, E)F_0, \tag{4}$$

where $E = a_{PSP}/(a_{PSP})_{fl}$ and k is the coefficient of proportionality that depends on ϕ_{Fo} and E.

The efficiency of the photochemical light energy conversion in an open reaction center of photosystem II is expressed through the ratio of fluorescence parameters, namely, $\phi_{RC} = (F_m - F_0)/F_m = F_v/Fm$ [23]. It was also shown that the decrease in the Fv/Fm value corresponds to a reduction of the fraction of the functional reaction centers of photosystem II (f) in phytoplankton cells [24, 25]. Such a reduction can be observed due to the destruction processes in photosystem II with a deficiency of nutrients [12, 17] and/or hyperoptimal irradiance (photoinhibition) [28, 38]. Thus, the product of the parameters ϕ_{RC} and f is proportional to the relative yield

of the variable fluorescence of microalgae adapted to the natural irradiance:

$$f\phi_{RC} = F_v/F_m. \quad (5)$$

Parameters q_p and ϕ_e

It is well known that the photochemical energy conversion in photosystem II occurs only in open reaction centers. The relative concentration of the open centers q_p was derived from the model for the light-dependent transition of the photosystem II reaction centers from the open to the closed condition [20]:

$$q_p(I) = I_{1/2}/(I + I_{1/2}), \quad (6)$$

where $I_{1/2}$ is the light intensity, at which a half of the reaction centers are in the closed condition.

The ϕ_e value was estimated in the following manner. Reduction of the CO_2 molecules requires direct transportation of four electrons from photosystem. Therefore, theoretically, the ϕ_e value may be as great as 0.25, but the electrons are partially consumed for the reduction of nitrates and sulfates [8, 27], for the cyclic electron flow around photosystem I [31, 36] and photosystem II [11], as well as for reduction of O_2 [3]. The correlation of this parameter with the maximum quantum yield of carbon fixation allows us to accept that ϕ_e is roughly constant [21, 30] and does not exceed 0.16 for phytoplankton under natural conditions [5]. Therefore, we admit that ϕ_e is equal to 0.16.

Substituting (4)–(6) into equation (2) and entering the coefficient $6.9 = 12 \times 10^{-3}$ (μC μmolC^{-1}) × 3600 (s h^{-1}), we write the vertical distribution of the rate of phytoplankton photosynthesis (mgC m^{-3} h^{-1}) through the fluorescence parameters:

$$V_c(z) = 6.9k(z)F_0(z)F_v/F_m(z)\frac{I_{1/2}}{I(z) + I_{1/2}}I(z), \quad (7)$$

where z is the depth.

Determination of k and $I_{1/2}$. The unknown k and $I_{1/2}$ parameters were determined by correlating the phytoplankton primary production values (mgC m^{-3} h^{-1}) measured by the radiocarbon method with the data on fluorescence and the underwater irradiance according to the formula

$$\mathrm{PP}(z) = 6.9\sum_{i=1,}^{n}\left(k_m F_0(z)F_v/F_m(z)\frac{I_{1/2m}}{I(z) + I_{1/2m}}I(z)\Delta t\right)_i, \quad (8)$$

where n is the number of fluorescence and irradiance profiles observed over the time of the flask exposure at a station; Δt is the time between these measurements (h); and k_m and $I_{1/2m}$ are the averages within the water column in the euphotic zone for the k and $I_{1/2}$ parameters, respectively. They were calculated by approximation of the relationship between PP and the sea depth using the function with two unknown parameters (k_m and $I_{1/2m}$) written in the right-hand part of formula (8). At the first approximation carried out by the least square method with the use of the built-in procedures of the GIM program, rough values of the k_m and $I_{1/2m}$ parameters were found. After that, through varying these parameters, values were selected at which the PP–depth relationship was most exactly described by formula (8) (with the coefficient of correlation not less than 0.95).

Data registration. The data on the vertical distribution of fluorescence, irradiance, phytoplankton primary production, and chlorophyll *a* concentration were obtained during the following cruises to the Baltic Sea (13°10′–25°15′ N, 53°25′–58°10′ E):

(1) June–July 1993—cruise of R/V *Humboldt* by the "Plankton" Program; the data measured at seven stations in the southern and eastern coastal waters of the Baltic Sea are presented;

(2), (3), (4), and (5)—May 1993, September 1993, May 1994, and September 1995, respectively. These are cruises of R/V *Oceania* of the Institute of Oceanology of the Polish Academy of Sciences. The data measured at 16 stations in the central and coastal parts of the Baltic Sea are presented.

Fluorescence measurement. The vertical distribution of fluorescence was measured in situ with a submersible pump-and-probe fluorometer developed at Moscow State University, Biological Faculty, Department of Biophysics. The instrument also provides determination of the density of the quanta flux in the range of photosynthetically active radiation (μE m^{-2} s^{-1}), temperature(°C), and sea depth (m). The fluorometer produces series of the subsequent pump-and-probe light impulses with a frequency of 2 Hz. The saturating (pump) flash emitting 1 J of light energy over 0.01 ms follows 1 s after the first probing flash (0.01 J 0.01 ms^{-1}), and then, after 50 μs, the second probing flash follows. The pulses are produced by a SSh-20 xenon lamp. Flashes are isolated from the sample by a SZS-22 blue–green light filter. The excitation spectrum of fluorescence is evenly distributed over the wavelength range from 400 to 550 nm.

When submerging the probe, water passively enters into it through a dark chamber, where, over 0.5 s, the fluorescence of the phytoplankton cells adapted to the underwater irradiance is measured. The velocity of submerging is 0.3–0.5 m s^{-1} allowing us to obtain vertical profiles of the values measured with a high resolution.

At illumination by the first probing flash, Fo is registered, i.e., the fluorescence yield at open reaction centers of photosystem II. The saturating light flash takes most of the reaction centers to the closed state, and in

the time comparable to the turnover time of a reaction center (0.1 ms), the second probing flash is delivered. The fluorescence (I1) corresponding to the maximum level of the fluorescence saturation with a short powerful flash is registered [35]. Fm is calculated according to the formula Fm = 1.4I1, where $1.4 = Fm_{DCMU}/I1$ is the ratio of the maximum fluorescence yield in the presence of diuron, an inhibitor of the electron transport to photosystem II, to the fluorescence yield measured instrumentally.

The fluorescence signal is registered with a FEU-68 detector after passing through a KS-17 boundary light filter with a transmission wavelength of $\lambda > 680$ nm.

The fluorescence signals, the underwater irradiance, temperature, and pressure (depth) registered are transmitted through a cable to the onboard controlling computer in the online mode.

Phytoplankton primary production. Phytoplankton PP was measured by radiocarbon modification of the flask method at 5–10 sea depths down to 30 m by the standard procedure [37]. The flasks were exposed *in situ* within the water column in cruise 1 for six hours; in cruises 2, 3, and 4 for four hours; and in cruise 5 for 2 h.

Chlorophyll a concentration. This was measured in the microalgae samples by the spectrophotometric method after filtration of a water sample through a membrane filter with a mesh size of 0.45 μm and extraction of the sediment obtained with acetone [34].

Marine algae such as diatoms *Phaeodactylum tricornutum* [Bohlin] and *Thalassiosira weissflogii* [Grunow], chrysophyta *Nephlochloris salina* [Cart], and green alga *Platymonas viridis* [Rouch.] were cultivated in bottles at a constant temperature of 20°C and illumination of 10 W m^{-2} in a Goldberg medium, which was prepared with artificial sea water [2].

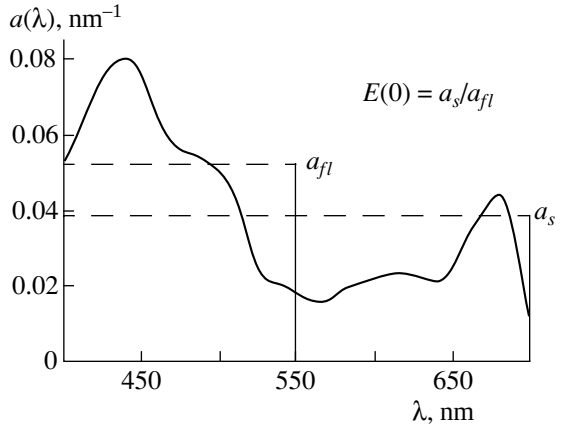

Fig. 1. Spectrum of the light absorption by phytoplankton suspension taken from the central part of the Baltic Sea. The absorption values are averaged over the spectral bands 400–550 nm (a_{fl}) and 400–700 nm (a_s).

RESULTS AND DISCUSSION

It was experimentally shown [10, 40] that the photosynthetic parameters ϕ_{Fo} and $I_{1/2}$ could vary at a stress impact of abiotic factors. Under natural conditions, the value of the ϕ_{Fo} parameter does not depend significantly on the effect of the environmental factors [32, 33], whereas the $I_{1/2}$ parameter depends mainly on the water temperature [7, 40]. We admit that these parameters are close to constant within the region with similar characteristics of the water temperature during a season. The k and $I_{1/2}$ values in the euphotic zone averaged over the water column, i.e., k_m and $I_{1/2m}$, respectively, were calculated (equation (8)) according to the data from 51 measurements of the vertical fluorescence profiles and the underwater irradiance at 23 stations in the Baltic Sea. The stations were carried out in the central and coastal (from the Gulf of Riga to Pomorskaya Bay) parts of the Baltic Sea and were characterized by an average chlorophyll *a* concentration within the water column from 0.7 to 10 mg m^{-3}. The results obtained are given in the table.

Variations in the k_m values. The average value of this parameter at all the stations was 5.6×10^{-5} (standard deviation $SD = \pm 17\%$).

The dispersion of the k values can be related to the factor $E = a_{PSP}/(a_{PSP})_{fl} \sim a/a_{fl}$ (see formula (4)), which depends on the attenuation of the underwater light (at a certain depth) and that of the excited phytoplankton fluorescence. The value E depends, for the most part, on the taxonomic composition of microalgae and their physiological condition. Figure 1 shows an example of the calculation of the E value for the phytoplankton exposed to solar light with respect to the light spectrum absorbed by the suspension of microalgal cells. Owing to the averaging of the absorption values over the spectrum in the spectral band 400–700 nm, we found the coefficient of solar light absorption (a_s) and, in the spectral band 400–550 nm, the coefficient of absorption of light exciting fluorescence (a_{fl}). For example, the E values calculated in such a way in four species of the sea microalgae varied from 0.6 to 0.75 (the data are not presented). These microalgae refer to three widespread taxonomic groups such as diatoms *Ph. tricornutum* and *Th. weissflogii*, chrysophyta *N. salina*, and green algae *P. viridis* grown under optimal conditions at low irradiance (<1 W m^{-2}), as well as after a 20-h cultivation in a KNO_3-free medium. For the samples of natural phytoplankton from the Baltic Sea, the E value is equal to 0.74. The experimental values did not exceed unity due to the fact that the coefficient of absorption of the color blue by the sea algae was usually higher than that of solar light because of the great content of carotinoids absorbing the light in the blue region. Therefore,

Calculation of the k_m and $I_{1/2m}$ values at the stations in the Baltic Sea. Numbers of cruises and stations, dates, areas, and time ranges (hours of local time) are presented

Cruise	Stations	Date	Area	$k_m \times 10^5$, relative units				$I_{1/2m}$, μE m^{-2} s^{-1}			
				Time range, h				Time range, h			
				3–6	6–9	9–12	12–15	3–6	6–9	9–12	12–15
1	1	June 23, 1993	6	–	4.64	5.48	–	–	155	167	–
	2	June 26, 1993	6	5.60	4.92	4.8	5.60	134	164	135	161
	3	June 28, 1993	4	5.28	5.48	6.20	5.20	170	134	144	128
	4	June 30, 1993	3	5.60	5.32	4.32	4.44	176	116	107	124
	5	July 1, 1993	3	5.00	5.52	–	–	113	100	–	–
	6	July 9, 1993	2	4.72	4.60	5.88	5.60	188	95	134	139
	7	July 10, 1993	2	5.00	4.80	4.44	5.00	170	134	140	155
2	8	May 13, 1993	1	–	–	4.92	–	–	–	124	–
	9	May 14, 1993	1	–	–	5.68	–	–	–	118	–
	10	May 15, 1993	4	–	–	5.80	–	–	–	178	–
	11	May 10, 1993	6	–	–	8.44	–	–	–	145	–
3	12	September 28, 1993	6	–	–	5.48	–	–	–	164	–
	13	September 29, 1993	6	–	–	7.24	–	–	–	104	–
	14	September 30, 1993	6	–	–	7.96	–		–	110	–
4	15	May 9, 1994	1	–	–	5.60	–	–	–	125	–
	16	May 11, 1994	6	–	–	8.60	–	–	–	115	–
	17	May 13, 1994	5	–	–	5.28	–	–	–	140	–
5	18	September 8, 1995	3	–	–	6.08	5.48	–	–	98	127
	19	September 9, 1995	3	–	–	5.32	–	–	–	145	–
	20	September 10, 1995	1	–	5.40	5.48*	6.20*	–	148	190*	121*
	21	September 12, 1995	1	–	–	5.88	–	–	–	133	–
	22	September 13, 1995	1	–	5.60	5.76*	7.44*	–	180	165*	138
	23	September 14, 1995	1	–	–	5.12	–	–	–	131	–

Note: Areas: 1—central waters; 2—Gulf of Riga; 3—Coast of Lithuania; 4—Gulf of Gdansk; 5—Coast between Gulf of Gdansk and Pomorskaya Inlet; 6—Pomorskaya Inlet.

* Results averaged over several measurements.

we can admit that, within the upper water layers (not deeper than 1 m), where the spectrum of the underwater irradiance is close to that of solar light, the E value for natural phytoplankton varies from 0.6 to 0.75.

The changes in the spectrum of the underwater irradiance with depth are accompanied by changes in the E value; namely, it occurs according to the equation $E(z) = E(0)\varphi(z)$, where $E(0)$ corresponds to the E value at the water surface and $\varphi(z)$ describes the E value variations with depth. In clear waters, the contribution of red light to the spectrum of the underwater irradiance decreases with depth and has to cause a rise in the E value from 0.60–0.75 at the surface to about 1.00 at a depth of 20 m and deeper, where the spectrum of the fluorescence excitation and that of the underwater irradiance almost coincide (the data are not presented). Therefore, we can expect that the E values averaged over the water column, on which the k_m values depend, vary in the range from 0.7 to 1.0.

Within the upper intensively mixed layer of the Baltic Sea, the taxonomic composition of phytoplankton within the water column is homogeneous, and the pattern of the vertical distribution of the chlorophyll *a* concentration represents variations in the phytoplankton concentration and light absorption over the profiles. At low irradiance at the surface ($I(0) < I_{1/2}$), one can admit that $\phi_{Fo}(z)$ is constant, therefore, $E(z) = \text{const}\,\text{Chl}(z)/F_0(z)$. We estimated the E value at a depth of 1 m using the results of 11 measurements of Fo and

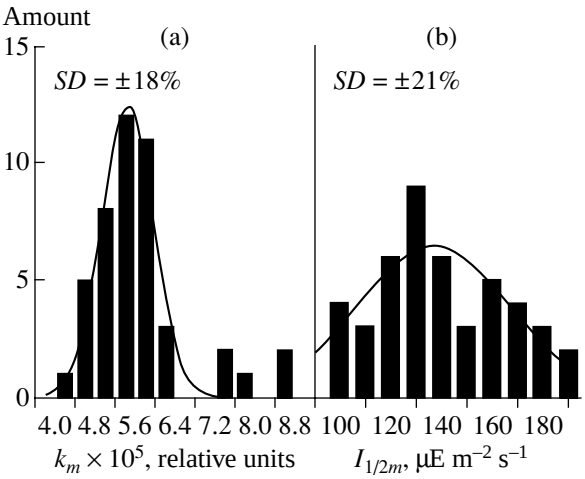

Fig. 2. Histograms of the distributions of (a) k_m and (b) $I_{1/2m}$ values calculated by formula (8) from experimental data on fluorescence, underwater irradiance, and phytoplankton productivity (by the radiocarbon method) at 23 stations in the Baltic Sea.

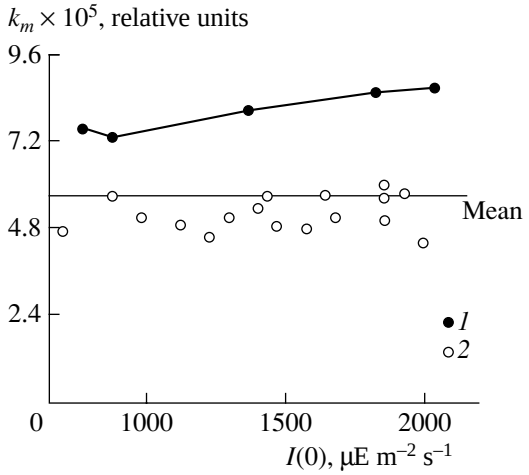

Fig. 3. Dependence of the k_m parameter on the irradiance at the sea surface $I(0)$ and its value averaged over 23 stations in the Baltic Sea. *1*—$k_m > 7 \times 10^{-5}$; *2*— $k_m < 7 \times 10^{-5}$.

the chlorophyll *a* concentration under low irradiance according the formula $E(1) = \frac{\mathrm{Chl}(1)\mathrm{Fo}(20)}{\mathrm{Chl}(20)\mathrm{Fo}(1)} E(20)$, where $E(20) = 1$. The values obtained varied from 0.83 to 1.1, which exceeded the $E(0)$ values calculated from the spectra of light absorption by algal cultures. This result can be caused by the marked decrease in the contribution of the red light to the spectrum of the underwater irradiance already at a depth of 1 m. Therefore, the average E value over the water column is close to unity, shows weak variations at different stations, and has no pronounced effect on the dispersion of the k_m values.

As we can see from the histogram of the distribution of k_m values given in Fig. 2a, the rather great standard deviation of this parameter was, to a great extent, determined by the $k_m > 7 \times 10^{-5}$ values, which significantly exceeded the average for the entire station (5.6×10^{-5}). Figure 3 illustrates the relationship between the k_m values and those of the irradiance at the surface $I(0)$ constructed by the results of the measurements that were conducted at the stations where a light-dependent depression of the primary production and phytoplankton fluorescence was registered near the sea surface. As is shown in the figure, a weak positive correlation between the k_m values and those of the surface irradiance was observed only in the case when the k_m values exceeded 7×10^{-5}. These values were obtained at stations 11, 13, 14, 16, and 22 (below, they will be denoted as stations*). Figure 4a shows the relationship between the ratio PP/(FoFv/Fm) and the underwater irradiance typical of stations* and the other 17 measurements performed at the stations with PP and Fo depressions within the upper layers. At high values of the underwater irradiance ($I(z) > I_{1/2}$), this ratio is proportional to the product ($kI_{1/2}$), because the equation PP/(FoFv/Fm) ~ const($kI_{1/2}$) (see formula (7)) is admitted. As we can see from the figure, at the values $I > 400$ μE m^{-2} s^{-1}, the product ($kI_{1/2}$) increased at stations*, which caused an overestimation of the calculated k_m values at these stations. At other stations, the changes in the ($kI_{1/2}$) product were independent of $I(0)$. The reason for the light-dependent changes in the ($kI_{1/2}$) product could be the decrease in the ϕ_{Fo} values and/or the increase in the $I_{1/2}$ values under the adaptation of phytoplankton to the hyperoptimal irradiation.

In [4] it was shown that the $I_{1/2}$ parameter depended on the optical depth and had a rise near the water surface. However, this relationship is characteristic of oligotrophic areas and, to a lesser extent, of mesotrophic areas; in eutrophic areas such as the test area in the Baltic Sea it is absent. Therefore, the increase in the product ($kI_{1/2}$) in the subsurface waters at stations* was most likely related to the vertical variations in the quantum fluorescence yield. This was confirmed when comparing the profiles of Fo and the chlorophyll *a* concentration. As mentioned above, in the intensively mixed waters, the vertical distribution of the chlorophyll *a* concentration is proportional to that of the light absorption and, correspondingly, to the phytoplankton fluorescence, namely, $\mathrm{Chl}(z) = \mathrm{const}k(z)\mathrm{Fo}(z)$. The character of the vertical distribution of the chlorophyll *a* concentration was homogeneous at stations*, where $k_m > 7 \times 10^{-5}$, while the subsurface Fo values were two–four times smaller than at a depth of 15 m and deeper (Fig. 4b). This pointed to an increase in the k values in the subsurface waters. The rise in the values of the k parameter was related to the decrease in the fluorescence quantum yield at a high intensity of the underwater irradiation. It is interesting that four of five stations*, at which the

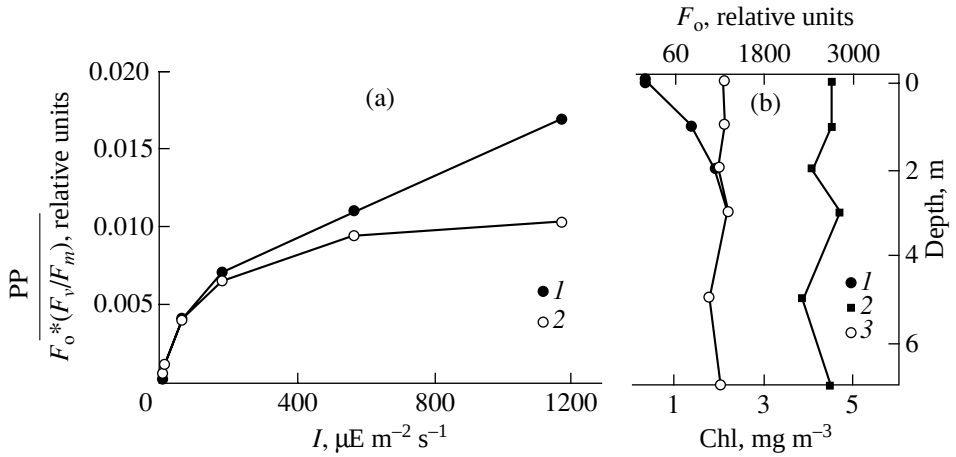

Fig. 4. (a) Relationships between the ratio PP/(FoFv/Fm) and the underwater irradiance *I* observed at the stations in the Baltic Sea during the midday depression of Fo and PP within the upper water layers typical of the stations with *1*— $k_m > 7 \times 10^{-5}$ and *2*—$k_m < 7 \times 10^{-5}$. (b) Example of the vertical distribution of the Fo values and chlorophyll *a* concentrations at the stations with $k_m > 7 \times 10^{-5}$: *1*— Fo; *2*—Chl; *3*—result obtained after correcting Fo with regard to Chl.

growth of the *k* values was observed near the surface, were located in Pomorskaya Inlet (the Odra River estuary). Therefore, the characteristic light-dependent decrease in the ϕ_{Fo} values in the subsurface waters was likely to be related to the features of the phytoplankton physiological condition in this area, which are explained by its exposure to anthropogenic factors. After conversion of the k_m values with regard to the vertical Fo profiles corrected by the chlorophyll *a* concentration at five stations*, as is shown in Fig. 4b, the standard deviation of this parameter for all the measurements decreased from 17 to 9% as compared to the value calculated before. This points to a reasonably great contribution of the light-dependent variations of the ϕ_{Fo} to the dispersion of the k_m parameter. Therefore, when calculating this parameter, it is desirable to take into account the necessity of a correction of the Fo and chlorophyll *a* concentration profiles and to carry it out. At other stations at which a Fo depression was registered in the subsurface waters, the chlorophyll *a* concentration decreased proportionally to the fluorescence value within the upper layers. In this case, the ratio Chl(*z*)/Fo(*z*) did not depend on the depth or show insignificant variations. Thus, the vertical variations of the Fo values at most of the stations reflected the changes in the concentration of microalgae and their light absorption capacity rather than the quantum yield of fluorescence, which, in general, agrees with the data published in [32, 33]. They show that the ϕ_{Fo} value for the natural phytoplankton is close to constant, in particular, in the Baltic Sea.

Thus, only at 5 stations of 23 studied in the Baltic Sea was the dispersion of the k_m parameter mostly related to the light-dependent variations of ϕ_{Fo}. After correction of the fluorescence profiles with respect to those of the chlorophyll *a* concentration, the dispersion of the k_m values decreased almost twofold. Similarly, the average of the k_m values for all the measurements, which comprised 5.4×10^{-5}, insignificantly decreased. Later, the above value was used as a constant when calculating the phytoplankton primary production values in the area studied.

Variations of the $I_{1/2}$ value. In column 6 of the table, the calculated values (according to formula (8)) for the $I_{1/2}$ ($I_{1/2m}$) averaged over the water column are given. The maximum and minimum $I_{1/2m}$ values were equal to 98 and 190, respectively; the average calculated with regard to all the measurements was 137 μE m^{-2} s^{-1}, and the standard deviation was 22%, which points to a greater extent of variation of this parameter as compared to k_m (see Fig. 2b). The $I_{1/2m}$ value did not show any correlation with the daily dynamics of the irradiance (see also [1]), but it had a tendency to decrease with the growth of the chlorophyll concentration (Fig. 5a).

The result of a polynomial fit of the relationship between the $I_{1/2m}$ values and the average chlorophyll *a* concentration over the water column (Chl_m) at the stations of the Baltic Sea is:

$$I_{1/2m} = 171 - 14.7Chl_m + 0.8(Chl_m)^2. \quad (9)$$

When comparing Figs. 2b and 5b, we can see that the standard deviation of the $I_{1/2m}$ value decreased from 22 to 16%, if we estimate the dispersion of this value with respect to the values determined by formula (9) rather than with respect to the average value derived from the data of all the measurements, namely, 137 μE m^{-2} s^{-1}. Thus, the $I_{1/2m}$ variations were related to the different chlorophyll *a* concentration at the test stations (i.e., this parameter depends on the trophic status of the area), as well as to the error in determination of the $I_{1/2m}$ values.

Estimation of the primary production values with the use of the averages for the k_m and $I_{1/2m}$

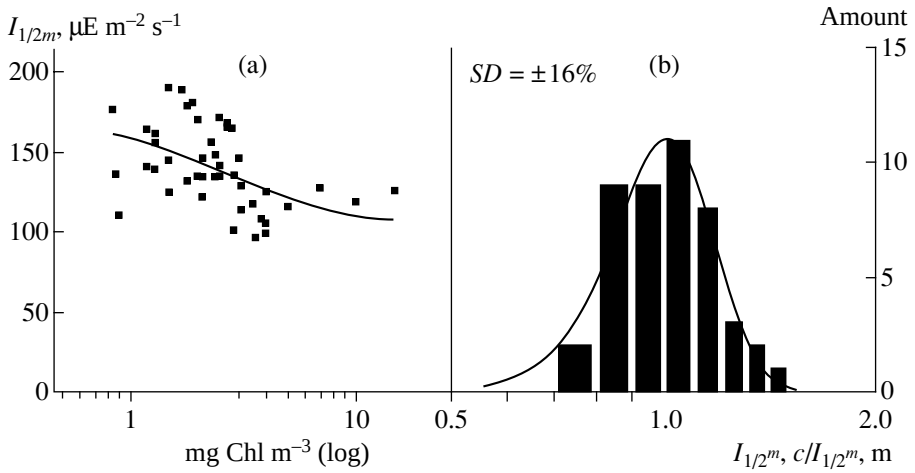

Fig. 5. (a) Relationship between $I_{1/2m}$ and the chlorophyll *a* concentration averaged over the water column and (b) histogram of distribution of the ratio of the $I_{1/2m}$ values calculated by formula (8) to those determined by formula (9).

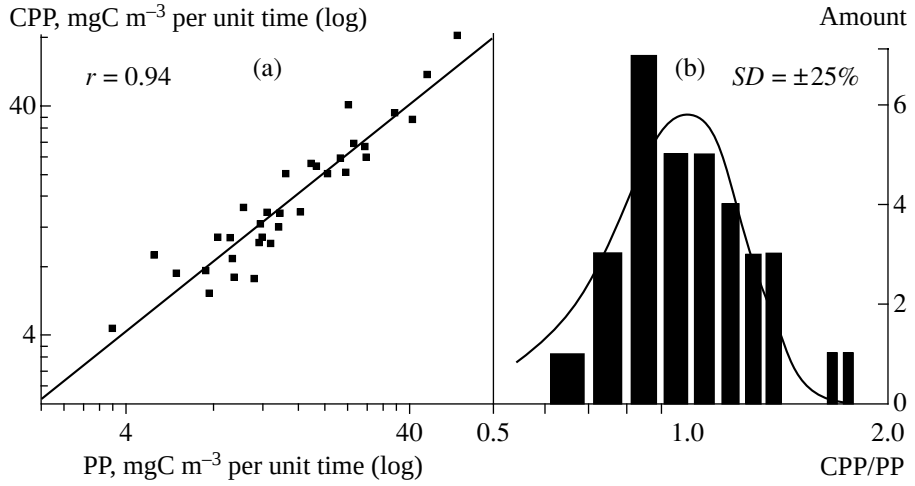

Fig. 6. (a) Relationship between the phytoplankton primary production value calculated by formula (8) averaged over the water column and that measured by the radiocarbon method at the stations in the Baltic Sea and (b) histogram of distribution of the CPP/PP ratios.

parameters measured throughout the Baltic Sea. At the stations in the Baltic Sea, the estimation of the phytoplankton primary production value within the water column per unit area (CPP, mgC m^{-2} h^{-1}) was carried out by substituting the data on the fluorescence and the underwater irradiance, as well as the k_m value equal to 5.4×10^{-5} and the $I_{1/2m}$ values derived from formula (9), into the right-hand part of formula (8), and then by integrating the CPP(*z*) values over depth. In this case, the effect of the light-dependent reduction of the ϕ_{Fo} values within the subsurface layers was taken into account, and, in addition, a corresponding correction of the Fo profiles was performed at five stations*, as described above. The CPP values calculated in such a way correlated well with the production values measured by radiocarbon modification of the flask method. The coefficient of correlation was 0.94, and the root-mean-square deviation was ±25% (Fig. 6). The CPP values calculated without correcting Fo with respect to the chlorophyll *a* concentration, but by substituting the $I_{1/2m}$ value equal to 137 into formula (8), were correlated more poorly with the measured production, namely, $r = 0.89$ (the results are not presented). Both results point to the rather high accuracy of the estimation of the rate of phytoplankton photosynthesis with the help of the fluorescent method suggested, but the best result was observed when the data on the vertical chlorophyll distribution were used.

Since the method suggested for determination of phytoplankton production gave good results at 23 stations in the coastal and central parts of the Baltic Sea in the late spring, in the summer, and in the early fall in different years, we can believe that the k_m and $I_{1/2m}$ parameters are applicable to the estimation of the pro-

ductivity in the Baltic Sea during the warm periods of the year by the fluorescent method.

In conclusion, it should be noted that this method allows us to estimate rapidly the rate of microalgae photosynthesis in a continuous mode. However, there is the necessity to calibrate the method by the results obtained during the measurements of the production with the use of labor-consuming standard procedures. Some fluorescent methods for PP determination do not demand such a calibration [13], but the estimation, for example, of the q_p parameter from the fluorescence parameters (formula (2)) with an adequate accuracy requires technically complicated instruments. The method suggested for the estimation of the photosynthetic phytoplankton production is appropriate for use in two cases: (a) in a specific area of the sea or the ocean, where monitoring has been conducted over many years; (b) in a detailed survey (for example, on revealing the mesoscale structures) with a great number of test stations, when the use of standard methods is possible only at reference stations for calibration of the fluorescent method.

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