

**Measurement of
phytoplankton
photosynthesis rate
using a *pump-and-probe*
fluorometer***

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Abstract

In this work we have studied the possibility of determining the rate of phytoplankton photosynthesis *in situ* using a submersible *pump-and-probe* fluorometer in water areas differing in their trophic level, as well as in climatic and hydrophysical characteristics. A biophysical model was used to describe the relationship between

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photosynthesis, underwater irradiance, and the intensity of phytoplankton fluorescence excited by an artificial light source. Fluorescence intensity was used as a measure of light absorption by phytoplankton and for assessing the efficiency of photochemical energy conversion at photosynthetic reaction centers. Parameters of the model that could not be measured experimentally were determined by calibrating fluorescence and irradiance data against the primary production measured in the Baltic Sea with the radioactive carbon method. It was shown that the standard deviation of these parameters *in situ* did not exceed 20%, and the use of their mean values to estimate the phytoplankton photosynthetic rate showed a good correlation between the calculated and measured data on primary production in the Baltic ($r = 0.89$), Norwegian ($r = 0.77$) and South China ($r = 0.76$) Seas.

1. Introduction

Photosynthesis of phytoplankton can be measured as the rate of radioactive carbon assimilation (Steemann-Nielsen 1952) or as the increase in the concentration of soluble oxygen in a sample (Williams & Jenkinson 1982, Langdon 1984). These methods are rather labor-consuming, and their application involves numerous artifacts owing to the prolonged isolation of phytoplankton in bottles (Eppley 1980), the difference between net and gross photosynthesis (Bender et al. 1987), and metal toxicity (Fitzwater et al. 1982). The application of chlorophyll fluorescence methods avoids these problems and allows gross photosynthesis of phytoplankton to be measured continuously in real time without their physiological state being affected (Kolber et al. 1990, Green et al. 1992). The relationship between chlorophyll *a* (C_a) fluorescence and photosynthesis is described in a number of biophysical models of the primary processes of photosynthesis (Weis & Berry 1987, Genty et al. 1989, Kiefer & Reynolds 1992). The aim of our work was to elaborate the methodology of determining the rate of photosynthesis *in situ* using theoretically justified biophysical models. The model of carbon assimilation P_c [$\mu\text{M C m}^{-3} \text{ s}^{-1}$] by phytoplankton used in our work is based on the light dependence of photosynthesis (Jassby & Platt 1976) and can be described by the following product:

$$P_c = \bar{a}_{pl, PSP} \Phi(E) E, \quad (1)$$

where:¹

$\bar{a}_{pl, PSP}$ [m^{-1}] – mean coefficient of solar irradiance absorption by phytoplankton photosynthetic pigments (PSP) in the 400–700 nm spectral range (PAR) (after Dubinsky et al. 1986),

¹For the reader's convenience, we append a list of symbols denoting the physical quantities used in the text. The nomenclature and denotations are in line with the conventions employed in the subject literature.

$\Phi(E)$ [$\mu\text{M C } \mu\text{E}^{-1}$] – efficiency (quantum yield) of the conversion of absorbed energy in photosynthetic reactions,

E [$\mu\text{E m}^{-2} \text{ s}^{-1}$] – total irradiance in the PAR range.

The value of Φ is proportional to the relative number of functionally active (f) and open (q_p) PS II reaction centers in algal cells, to the efficiency of photochemical conversion of light energy in open reaction centers (Φ_{RC}), [$\mu\text{M electron } \mu\text{E}^{-1}$], and to the efficiency of electron transfer from H_2O to CO_2 (Φ_e), [$\mu\text{M C } (\mu\text{M electron})^{-1}$]:

$$P_c = \bar{a}_{pl, PSP} f q_p(E) \Phi_{RC} \Phi_e E. \quad (2)$$

Some parameters of equation (2), like $\bar{a}_{pl, PSP}$ or f , can be determined by measuring fluorescence parameters F_0 and F_v/F_m by the *pump-and-probe* method (Mauzerall 1972, Kolber et al. 1990) in phytoplankton adapted to ambient light; alternatively, by substituting the photosynthetic rate measured by the radiocarbon method for P_c in formula (2), or by measuring light absorption by algae.

In this work, we investigated the variation of these indirectly measured parameters in the Baltic Sea. The possibility of applying the mean values of these parameters to determine the primary production of phytoplankton in the Baltic, Norwegian, and South China Seas was also studied.

2. Methods

2.1. Determination of $\bar{a}_{pl, PSP}$, f , and Φ_{RC} from phytoplankton fluorescence characteristics

The intensity of fluorescence excited by an artificial light source, with open reaction centers (RC) in algae, can be found from the equation

$$F_0 = G I_{fl} \bar{a}_{pl, PSP, fl} \Phi_{F_0}, \quad (3)$$

where:

I_{fl} – total intensity of the exciting flash (in our fluorometer, $I_{fl}(\lambda)$ was nearly uniformly distributed over the 400–550 nm spectral range), (constant),

$\bar{a}_{pl, PSP, fl}$ [m^{-1}] – coefficient of exciting flash absorption by PSP, averaged over the 400–550 nm spectral range,

Φ_{F_0} – quantum yield of fluorescence in cells with open RC,

G – coefficient defined by geometric characteristics and the sensitivity of the fluorescence light sensor, (constant).

Taking into account the fact that $(G I_{fl})^{-1} = \text{const}$, the coefficient of solar irradiance absorption by algae can be related to the fluorescence intensity as follows:

$$\bar{a}_{pl, PSP} = \text{const } \Phi_{F_0}^{-1} A F_0 = k(\Phi_{F_0}, A) F_0, \quad (4)$$

where:

$$A = \bar{a}_{pl, PSP} / \bar{a}_{pl, PSP, fl},$$

$k(\Phi_{F_0}, A)$ – a proportionality coefficient, which can be determined by intercalibration – see below.

The photochemical efficiency of an open PS II reaction center can be determined from the ratio of fluorescence parameters

$$\Phi_{RC} \approx (F_m - F_0) / F_m = F_\nu / F_m$$

(Klughhammer 1992). It was shown that the decrease in the F_ν / F_m ratio corresponds to part of the decrease in the fraction of functioning PS II reaction centers (f) (Kolber et al. 1988, 1990), a process induced by excessive irradiation (Long et al. 1994, Vassiliev et al. 1994) (photoinhibition) and/or limitation of phytoplankton growth by mineral nutrients (Falkowski et al. 1989, Green et al. 1992). Thus, parameters Φ_{RC} and f are proportional to the relative yield of variable fluorescence of chlorophyll in phytoplankton adapted to natural radiation. We therefore assume that

$$f \Phi_{RC} = F_\nu / F_m. \quad (5)$$

2.2. Determination of q_p and Φ_e

It is known that photochemical conversion of light energy in PS II takes place only in open reaction centers. The relative concentration of open centers q_p can be found from the model of the light-dependent transition of reaction centers between the open and closed states. We used the model expressed by the Michaelis-Menten equation, which was proposed among others by Kiefer & Mitchell (1983):

$$q_p(E) = E_{1/2} / (E + E_{1/2}), \quad (6)$$

where $E_{1/2}$ is the light irradiation, at which half of the RC are in the closed state – see below.

The value of Φ_e was estimated from the following considerations. To reduce one molecule of CO_2 , 4 electrons should be transferred from H_2O . Theoretically, therefore, Φ_e may be as high as 0.25; however, a certain fraction of the electron flow is consumed for nitrate and sulfate, for cyclic electron transport around PS I and PS II, as well as for O_2 reduction (Slovacek et al. 1980, Dubinsky et al. 1986, Falkowski et al. 1986, Myers 1987, Laws 1991). Comparison of Φ_e with the maximum quantum yield of carbon fixation leads to the assumption that Φ_e is approximately

constant (Kiefer et al. 1989, Morel 1991) and is not over 0.16 for natural phytoplankton (Bannister & Weidemann 1984). Hence, we assume that $\Phi_e = 0.16$.

By substituting 4, 5, 6, in eq. (2) and introducing the coefficient $6.9 = 12 \times 10^{-3} [\text{mg C } (\mu\text{M C})^{-1}] 3600 [\text{s h}^{-1}] \Phi_e$, the equation for the vertical profile of the algae photosynthesis rate $[\text{mg C m}^{-3} \text{ h}^{-1}]$ can be written as

$$P_c(z) = 6.9 k(z) F_0(z) F_\nu/F_m(z) \frac{E_{1/2}}{E(z) + E_{1/2}} E(z), \quad (7)$$

where z is depth [m].

2.3. Estimation of k and $E_{1/2}$

The unknown parameters k and $E_{1/2}$ were found by comparing the primary production of phytoplankton P^c $[\text{mg C m}^{-3} \text{ time}^{-1}]$ measured by the radiocarbon method, and fluorescence and irradiance measurements, according to the formula

$$P^c(z) = 6.9 \sum_{i=1}^n (k_m F_0(z) F_\nu/F_m(z) \frac{E_{1/2m}}{E(z) + E_{1/2m}} E(z) \Delta t)_i, \quad (8)$$

where n is the number of fluorescence and irradiation profiles measured for the period of bottle exposure at a station;

Δt [h] is the time period between these measurements;

k_m and $E_{1/2m}$ are the respective values of the parameters k and $E_{1/2}$ averaged in the water column. They were calculated by approximating the P^c versus z dependence with eq. (8) by the least squares method.

Parameter k was also estimated under laboratory conditions by calibrating F_0 against the coefficient of exciting flash absorption by phytoplankton taken at a natural concentration ($C_a = 0.1\text{--}10 \text{ mg m}^{-3}$). Parameter k was determined from formula (4) for $A = 1$. The value of $\bar{a}_{pl, PSP, fl}$ was measured with a laboratory instrument. Light from the KGM 150/24 halogen lamp of a slide projector passed through an SZS22 glass filter and a 0.2-m-long dark chamber containing the sample, and the output quantum flux density was measured with a laboratory-made quantum sensor. Calculations were done using the formula $\bar{a}_{pl, PSP, fl} [\text{m}^{-1}] = 1/0.2 (I_{n,c} - I_n)/I_{n,c} = 5 (I_{n,c} - I_n)/I_{n,c}$, where I_n is the intensity of light passed through a suspension of algae of concentration n ; $I_{n,c}$ is the same for a suspension of algal cells bleached by illumination in the presence of 1 mM hydroxylamine.

For laboratory experiments, marine algae were grown on Goldberg medium prepared with artificial sea water in flasks at constant temperature in light (Lanskaya 1971).

2.4. Data recording

2.4.1. Material

The vertical distribution of irradiance, fluorescence, primary production of phytoplankton, and chlorophyll concentration were measured in the Bay of Nhatrang in the South China Sea ($12^{\circ}09'–12^{\circ}18' N$, $109^{\circ}12'–109^{\circ}20' E$) and during cruises in the Baltic ($13^{\circ}10'–25^{\circ}15' N$, $53^{\circ}25'–58^{\circ}10' E$) and Norwegian Seas ($64^{\circ}15'–70^{\circ}20' N$, $4^{\circ}40' W–4^{\circ}30' E$):

- (1) June–July 1993 – the cruise of the r/v 'Humboldt', according to the 'Plankton' program; measurements at 7 stations near the southern and eastern coasts of the Baltic Sea.
- (2) May 1993 – the cruise of r/v 'Oceania', Institute of Oceanology PAS; measurements at 4 stations in central and coastal waters of the Baltic Sea.
- (3) September 1993 – the cruise of r/v 'Oceania', Institute of Oceanology PAS; measurements at 3 stations in central and coastal waters of the Baltic Sea.
- (4) May 1994 – the cruise of r/v 'Oceania', Institute of Oceanology PAS; measurements at 3 stations in central and coastal waters of the Baltic Sea.
- (5) September 1995 – the cruise of r/v 'Oceania', Institute of Oceanology PAS; measurements at 6 stations in central and coastal waters of the Baltic Sea.
- (6) June–July 1997 – cruise of r/v 'A. Petrov', VNIRO of the RAS; measurements at 13 stations in the deeps of the central Norwegian Sea.
- (7) March 1998 – measurements at 8 stations in the Bay of Nhatrang in the South China Sea.

2.4.2. Measurements

Vertical profiles of *in situ* fluorescence were recorded with a 'Prim Prod' submersible *pump-and-probe* fluorometer designed at the Biophysical Department of the Faculty of Biology of Lomonosov Moscow State University. The instrument also recorded irradiance in the PAR region [$\mu E m^{-2} s^{-1}$], temperature, and depth. The fluorometer generates sequential pump and probe flashes at a frequency of 2 Hz. The saturating (pump) flash of 1 J/0.01 ms power per duration was given 1 s after the first probe flash (0.01 J/0.01 ms), and the second probe flash follows the pump flash after 50 μs . The impulses were generated by an SSh-20 (MELZ, Russia) xenon

lamp. The flashes are isolated from the sample by a light blue-green filter SZS-22. The spectrum of the fluorescence excitation is distributed practically evenly within the range of wavelengths from 400 to 520 nm.

During probe submersion, external water passively enters an open dark chamber in which the fluorescence of phytoplankton cells adapted to underwater radiation is measured every 0.5 s. The probe submersion rate was 0.3–0.5 m s⁻¹, which allowed for resolution depth profiles.

The first probing flash measures F_0 , the fluorescence intensity with open PS II centers. The subsequent saturating flash converts most of the RC to the closed state, and the second probing flash, which follows within 50 μ s, a time comparable to the reaction center turnover time, measures the fluorescence, which corresponds to the $I1$ level of fluorescence saturation (Schreiber et al. 1995). F_m is calculated according to the formula $F_m = 1.4 \times I1$, where $F_{m,DCMU}/I1 = 1.4$ is the ratio of the maximum fluorescence obtained in the presence of DCMU, an inhibitor of electron transport in PS II, to the fluorescence yield measured by the PrimProd.

After passing through a KS-17 cut-off glass filter, the fluorescence signal is recorded by photomultiplier-68.

The recorded fluorescence signals as well as the underwater irradiance, temperature and pressure (depth) are transmitted in real time via a cable-rope connected to a personal computer.

The primary production of phytoplankton was measured in the Baltic Sea with the radiocarbon method at 5–10 horizons down to a depth of 30 m using a routine method (Steemann-Nielsen 1952), a modification of it in the Norwegian Sea (Sorokin 1960), and by the oxygen method in the Bay of Nhatrang and Norwegian Sea (Vinberg 1969). During measurement with the radiocarbon method, bottles were exposed for 6 hours during the 1st cruise, for 4 hours during the 2nd, 3rd and 4th cruises, for 2 hours during the 5th cruise, and for 6 hours during the 6th cruise.

The chlorophyll a content was determined with a standard spectrophotometric method (Bender et al. 1987).

3. Results

To determine the rate of phytoplankton photosynthesis according to formula 7, it is necessary to estimate the unknown quantities $k(\Phi_{F_0}, A)$ and $E_{1/2}$ and their variability in the regions studied. As experiments have shown (Ernst et al. 1986, Dera 1995, Woźniak et al. 1997), photosynthetic parameters Φ_{F_0} and $E_{1/2}$ vary under the stress action of abiotic factors. In natural phytoplankton, according to Ostrowska et al. (2000a and b), the parameter Φ_{F_0} does not depend significantly on environmental factors (with the exception of the fluorescence photoinhibition that is possible

Table 1. Values of k_m and $E_{1/2m}$ calculated at given time intervals from 51 profiles of phytoplankton production, fluorescence, and underwater irradiation at 23 stations in the Baltic Sea. The cruise and station numbers, dates and areas of measurements are also given

Cruise	Station	Date	Area	$k_m \times 10^5$ [relative units]				$E_{1/2m}$ [$\mu\text{E m}^{-2} \text{s}^{-1}$]			
				3-6	6-9	9-12	12-15	3-6	6-9	9-12	12-15
1	1	23.06.1993	5	–	4.64	5.48	–	–	155	167	–
	2	26.06.1993	5	5.60	4.92	4.8	5.60	134	164	135	161
	3	28.06.1993	4	5.28	5.48	6.20	5.20	170	134	144	128
	4	30.06.1993	3	5.60	5.32	4.32	4.44	176	116	107	124
	5	01.07.1993	3	5.00	5.52	–	–	113	100	–	–
	6	09.07.1993	2	4.72	4.60	5.88	5.60	188	95	134	139
	7	10.07.1993	2	5.00	4.80	4.44	5.00	170	134	140	155
2	8	13.05.1993	1	–	–	4.92	–	–	–	124	–
	9	14.05.1993	1	–	–	5.68	–	–	–	118	–
	10	15.05.1993	4	–	–	5.80	–	–	–	178	–
	11	10.05.1993	5	–	–	8.44	–	–	–	145	–
3	12	28.09.1993	5	–	–	5.48	–	–	–	164	–
	13	29.09.1993	5	–	–	7.24	–	–	–	104	–
	14	30.09.1993	5	–	–	7.96	–	–	–	110	–
4	15	09.05.1994	1	–	–	5.60	–	–	–	125	–
	16	11.05.1994	5	–	–	8.60	–	–	–	115	–
	17	13.05.1994	6	–	–	5.28	–	–	–	140	–
5	18	08.09.1995	3	–	–	6.08	5.48	–	–	98	127
	19	09.09.1995	3	–	–	5.32	–	–	–	145	–
	20*	10.09.1995	1	–	5.40	5.48*	6.20*	–	148	190*	121*
	21	12.09.1995	1	–	–	5.88	–	–	–	133	–
	22*	13.09.1995	1	–	5.60	5.76*	7.44*	–	180	165*	138*
	23	14.09.1995	1	–	–	5.12	–	–	–	131	–

Areas: 1 – central waters, 2 – the Gulf of Riga, 3 – the Lithuanian coast, 4 – the Gulf of Gdańsk, 5 – the Pomeranian Bay, 6 – the coastal waters between 4 and 5; * the results were averaged over several measurements.

in phytoplankton from overexposed shallow waters under intense natural irradiance), while parameter $E_{1/2}$ can change to some degree with the trophic type of water and does change mainly with the temperature of the water body (Morel 1991, Dera 1995, Antoine & Morel 1996, Woźniak et al. 1997). We presume that it is nearly constant in regions with similar temperature (Woźniak et al. 1992). The mean values of k and $E_{1/2}$ in the water column – k_m and $E_{1/2m}$ – were calculated (formula 8) at 23 stations

in the central and coastal areas of the Baltic Sea (from the Gulf of Riga to the Pomeranian Bay), where the average concentration of C_a in the water column varies from 0.7 to 10 mg m⁻³. The data are given in Table 1.

3.1. Variation of k_m at the Baltic Sea stations

The mean value of this parameter at the Baltic Sea stations was 5.6×10^{-5} (standard deviation SD = $\pm 17\%$).

The k_m variation can be related to the factor A (see formula 4), which is induced by the differences in blue light and solar radiation absorption by marine phytoplankton. The value of A depends mainly on the taxonomic composition of the algae and their physiological condition. For example, A calculated *in vivo* from absorption spectra (as shown in Fig. 1a) for three taxonomic groups – diatomea *Phaeodactylum tricornutum*, yellow-green algae *Nephrochloris salina*, and green algae *Platymonas viridis* – grown under optimum conditions and at low irradiation, elevated temperatures, or nitrogen deficiency, varied from 0.6 to 0.75. For samples of natural phytoplankton from the Baltic Sea, $A = 0.74$. The experimental value of A was < 1 due to the fact that the absorption coefficient of marine algae for blue light is usually much higher than their absorption coefficient averaged over the PAR region: $\bar{a}_{pl, PSP, fl} > \bar{a}_{pl, PSP}$. Thus, it can be expected that, to a first approximation, the values A for natural phytoplankton should vary from 0.6 to 0.75 in the upper water layers, where the irradiance spectrum is close to that of solar radiation.

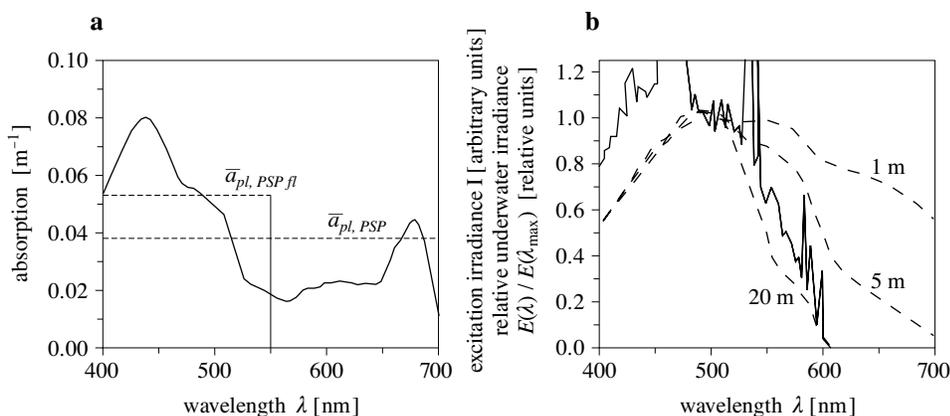


Fig. 1. Exemplary spectrum of phytoplankton light absorption coefficient from the central Baltic Sea. Values of absorption coefficients are averaged over the 400–550 nm ($\bar{a}_{pl, PSP, fl}$) and 400–700 nm ($\bar{a}_{pl, PSP}$) spectral regions (a). Spectrum of the light used to excite chlorophyll fluorescence in the PrimProd fluorometer (solid line), and spectral distributions of underwater irradiance in the sea at different depths (dashed lines – data by M. Ostrowska and R. Hapter) (b)

Changes in the spectrum of underwater irradiance with depth are accompanied by changes in A : $A(z) = A(0) \times T(z)$, where $A(0) = \bar{a}_{pl, PSP} / \bar{a}_{pl, PSP, fl}$ is the value of A at the water surface and $T(z)$ is the depth dependence of A . In clear water, the attenuation of red light with depth must lead to an increase in $A(z)$ from 0.6–0.7 at the surface to 1 at 20 m and greater depths, where the spectra of the probing flash and underwater irradiance are similar (Fig. 1b). Thus, it can be expected that mean values of A in the water column, which affect k_m , should be higher than 0.7 and vary to a lesser extent than at the surface. As a first approximation we can assume that $A = 1$ and does not influence the value of k_m (see section 4).

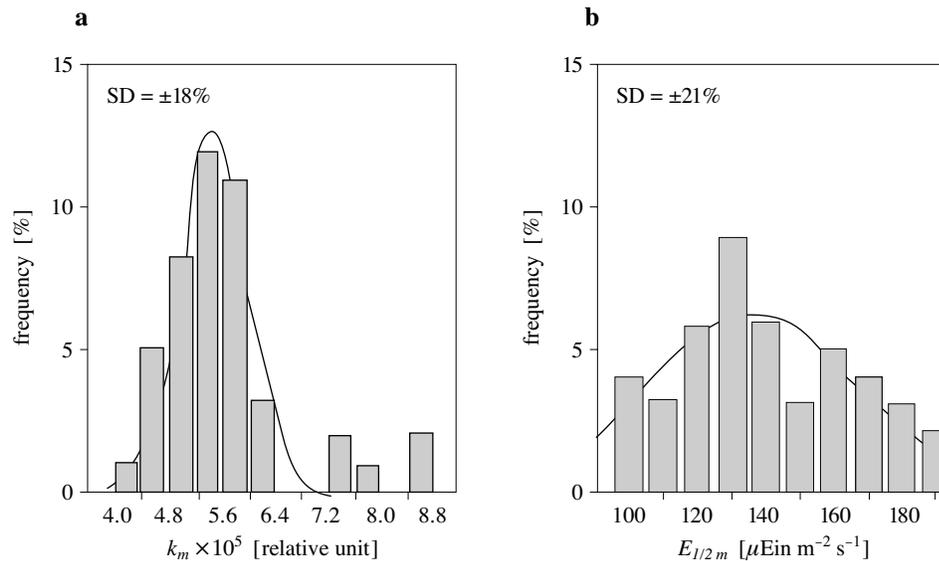


Fig. 2. Histograms of k_m (a) and $E_{1/2m}$ (b) distribution, determined for the Baltic Sea stations

As can be seen from the histogram of k_m distribution (Fig. 2a), the high standard deviations of this parameter were due mainly to the occurrence of k_m values in the range $k_m > 7 \times 10^{-5}$. Figure 3 shows the dependence of k_m on $E(0)$ for stations with a distinct surface inhibition of P^c and phytoplankton fluorescence. As can be seen from Fig. 3, there is a weak positive correlation between k_m and surface irradiance only for $k_m > 7 \times 10^{-5}$, which were measured at stations 11, 13, 14, 16 and 22.

Vertical profiles of C_a were uniform at stations where $k_m > 7 \times 10^{-5}$, and F_0 decreased 2–4 times in surface water. Taking into account the fact that A changes only slightly with depth, our data indicate a light-dependent decrease in Φ_{F_0} in the upper layers under intense irradiance, which is why

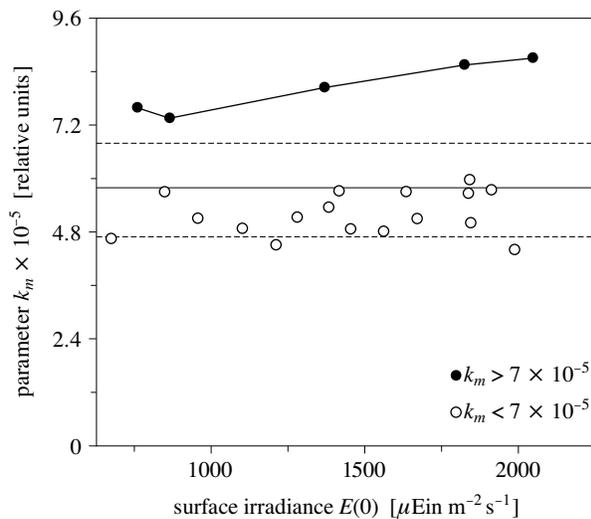


Fig. 3. Dependence of k_m on surface irradiance $E(0)$ for the Baltic Sea stations. Dashed lines show the standard deviation band

the calculated k_m values at these stations were overestimated. It should also be noted that 4 of the ‘5-area’ stations were investigated at different times in the same area of the Baltic Sea – the Pomeranian Bay (Oder mouth). The recalculation of k_m at these stations to take into account the vertical distribution of F_0 resulted in a reduction of the standard deviation of this parameter by 17 to 9%, as compared to that calculated previously. This indicates a rather considerable contribution of light-dependent changes in Φ_{F_0} to the dispersion of k_m . At the other 18 stations, where the noon depression of fluorescence was also recorded, both F_0 and C_a were reduced in surface water. The vertical profiles of F_0 at most stations thus demonstrated the distinct depth dependence of phytoplankton concentration and its absorption capacity, but not of Φ_{F_0} , which confirms the assumption that Φ_{F_0} is roughly constant in natural phytoplankton. The low level of Φ_{F_0} , which is not typical of the study area as a whole, could be related to the characteristic physiological state of the phytoplankton in the Pomeranian Bay.

Therefore, the variation of k_m at 23 stations of the Baltic Sea was due mainly to light-dependent inhibition Φ_{F_0} at 5 stations.

3.2. Variation of $E_{1/2}$ at Baltic Sea stations

Column 6 of Table 1 gives $E_{1/2}$ values ($E_{1/2m}$) averaged over the water column in the Baltic Sea calculated according to formula 8. The minimal and maximal values of this parameter are 98 and 190, respectively; the mean

value for all stations was $137 \mu\text{E m}^{-2} \text{s}^{-1}$, and the standard deviation was 22%, which indicates a greater variation in this parameter in comparison to k_m (Fig. 2b). $E_{1/2m}$ did not correlate with daily changes in solar irradiance (see also Antal et al. 1999); however, $E_{1/2m}$ did tend to decrease with chlorophyll concentration (Fig. 4a).

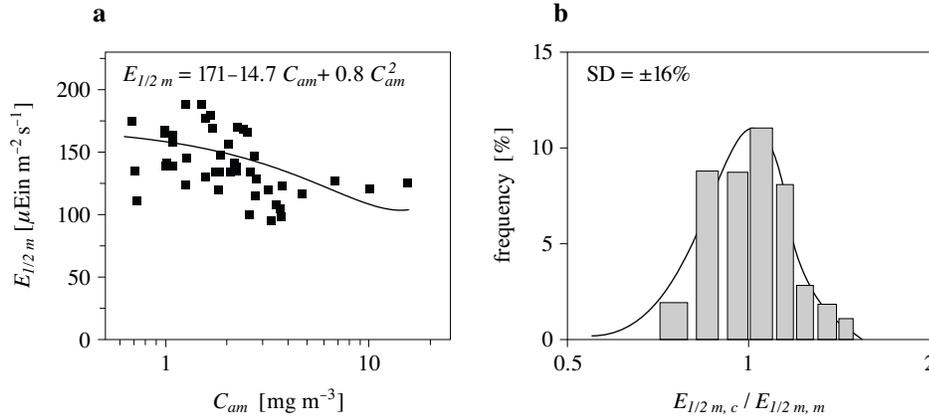


Fig. 4. The dependence of $E_{1/2m}$ on the chlorophyll a C_{am} content averaged over the water column and polynomial regression of this dependence (a); histogram of distribution of the ratio of $E_{1/2m,c}/E_{1/2m,m}$ (index c – calculated, using polynomial regression values of $E_{1/2m}$, index m – observed values of this parameter) (b)

The result of the polynomial regression of the dependence of $E_{1/2m}$ on the average content of chlorophyll a in the water column (C_{am}) is

$$E_{1/2m} = 171 - 14.7 C_{am} + 0.8 (C_{am})^2, \quad (9)$$

Comparison of Figs. 2b and 4b shows that the degree of $E_{1/2m}$ variation decreases from 22% to 16% when the standard deviation of this parameter is calculated with respect to values of $E_{1/2m}$ determined from formula 9, but not with respect to the mean value for all stations. Thus, the variation of $E_{1/2m}$ at stations in the Baltic Sea was partly due to an error in determining this parameter, as well as to the variation in chlorophyll a content at the stations. This indicates that there is some range of variation for this parameter, depending on the trophicity of the investigated waters.

3.3. Primary production of phytoplankton, PP_c

The primary production of phytoplankton, PP_c , was calculated by substituting fluorescence, underwater irradiance, and parameters $k_m = 5.4 \times 10^{-5}$, and $E_{1/2m}$, determined from formula 9, in the right-hand side of formula 8 and by integrating $P_c(z)$ over depth. The effect of the

light-dependent decrease in Φ_{F_0} was also taken into account. PP_c calculated in this way is well correlated with the production measured directly; the coefficient of correlation $r = 0.94$ and the standard deviation is $\pm 25\%$ (Fig. 5). When PP_c was calculated by substituting the value of $E_{1/2m} = 137$ in formula 8 without the light-dependent decrease in Φ_{F_0} being taken into account, it was slightly less well correlated with the measured production: $r = 0.89$. However, both results indicate that the suggested fluorescence method yields a fairly accurate estimate of the rate of phytoplankton photosynthesis.

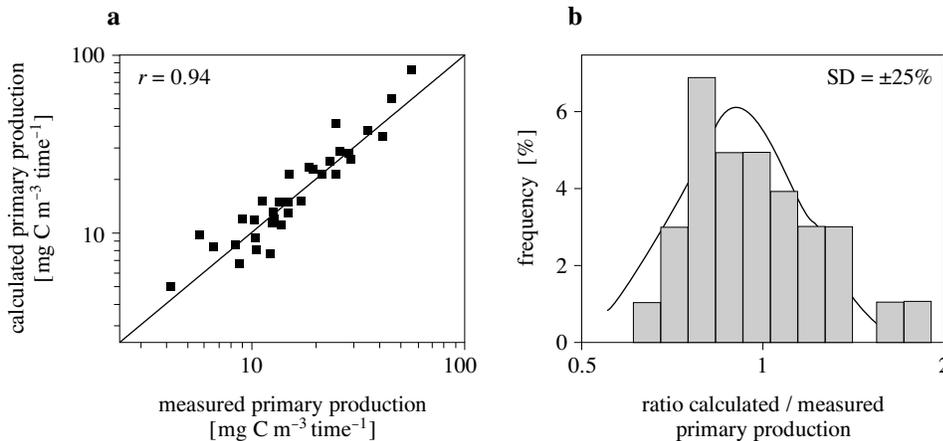


Fig. 5. Dependence of the calculated primary production of phytoplankton on the measured value averaged over the water column at Baltic Sea stations (a), histogram of the distribution ratio of the calculated productions to the measured values (b)

This method of determining the primary production of phytoplankton showed good results at 23 stations of the Baltic Sea in coastal and central waters in spring, summer, and autumn in different years. It seems likely that it can be applied successfully to the estimation of productivity in the Baltic Sea.

We also investigated the possibility of determining PP_c in other climatic zones, which differ from the Baltic Sea in trophicity and hydrophysical characteristics. PP_c was calculated in central mesotrophic stratified waters of the Norwegian Sea, where C_a , averaged over the water column, varied between stations from 0.20 to 0.49 mg m^{-3} , and in the oligo-mesotrophic, stratified, coastal waters of the South China Sea (the Bay of Nhatrang), where the chlorophyll content varied from 0.025 to 0.25 mg m^{-3} . In the calculations, we substituted the parameters k_m and $E_{1/2m}$ in formula 8 with the average values for the Baltic Sea: 5.4×10^{-5} and 137,

respectively. The calculated and measured primary production are slightly less well correlated with each other than in the Baltic Sea: $r = 0.77$ (radiocarbon method) and 0.70 (oxygen method) in the Norwegian Sea and 0.76 in the Bay of Nhatrang (oxygen method). Comparison of F_0 and C_a profiles showed that there were no abrupt changes in Φ_{F_0} . Thus, the lower correlation, as compared to the Baltic Sea, may be related to variations in $E_{1/2}$ and to the low accuracy of direct measurement of P^c in these regions: samples were collected from only two horizons and the samples were incubated on board ship. Furthermore, P_c measured by the oxygen method was only qualitatively correlated with PP_c , exceeding it threefold on average (see Sapozhnikov et al. 2000). As described above, the fluorometer probe was calibrated against radiocarbon methods, which gives lower values as compared to those obtained with the oxygen method, owing to differences in the methods of calculation (Naletova & Sapozhnikov 1995) and measurement (Koblentz-Mischke & Vedernikov 1977).

Measurement of parameter k by direct calibration of fluorescence data in terms of light absorption by phytoplankton allows for an independent estimation of PP_c in phytoplankton and its comparison with the data obtained by direct measurements. We measured F_0 as a function of absorption under laboratory conditions in green (*Chlorella vulgaris*), diatomic (*Thalassiosira west.*), and yellow-green (*N. salina*) algae (data not shown). The dependencies were linear at $C_a < 10 \text{ mg m}^{-3}$. Values of k , as determined at $A = 1$ by the non-linear regression of this dependency, varied only slightly within the range $8\text{--}9 \times 10^{-5}$. When the decrease of A under natural conditions in surface water (see above) was taken into account, the upper and lower limits of k were equal to 6.4×10^{-5} and 9×10^{-5} , respectively, which is slightly above the radiocarbon data values, which lie within the range $4.32\text{--}6.20 \times 10^{-5}$ (without taking into account the values $k > 7 \times 10^{-5}$, see Table 1).

The photosynthetic rate calculated with the use of the average value $k = 7.7 \times 10^{-5}$, which was determined by this method, is about 1.5 times higher than the radiocarbon data result, but only half as great as the result obtained using the oxygen method data.

4. Conclusion and final remark

Primary production determined by the pump-and-probe fluorometer correlated well with that measured by direct methods at stations in the Baltic, Norwegian, and South China Seas: application of this method takes into account changes in Φ_{F_0} in various marine areas. Hence, because of its efficiency and speed, the method presented in this paper could be used as an alternative to the traditional method.

Using the PrimProd fluorescence method for estimating primary production, one has to bear in mind inconveniences and simplifications that are the cause of certain inaccuracies, in particular, the following:

- Firstly, this method requires calibration by traditional measurements and the determination of the constant k_m (related to the specific absorption coefficient) and the constant $E_{1/2}$ (related to the shape of the light-photosynthesis curve) in equation (8). This is why this method is not universal.
- Secondly, when using this method we assume that the fluorescence F_0 is the measure of the mean coefficient of the total photosynthetic pigment light absorption. While this assumption is correct, it should be realized that this mean absorption coefficient, $\bar{a}_{pl, PSP, fl}$, is in reality the mean absorption coefficient with the weight of the spectrum of the exciting light:

$$\bar{a}_{pl, PSP, fl} = \text{const} \times F_0 \quad \text{where} \quad F_0 = \Phi_{fl} \frac{\int_{\Delta\lambda} a_{pl, PSP}(\lambda) I(\lambda) d\lambda}{\int_{\Delta\lambda} I(\lambda) d\lambda}, \quad (10)$$

where Φ_{fl} is the quantum yield of fluorescence, and $\Delta\lambda$ is the spectral range of the exciting light.

On the other hand, to determine the energy absorbed by pigments we have to use a similar absorption coefficient $\tilde{a}_{pl, PSP}$ averaged with the weight of the spectrum scalar irradiance in the sea $I_\lambda(\lambda)$:

$$\tilde{a}_{pl, PSP} = \frac{1}{I} \int_{400 \text{ nm}}^{700 \text{ nm}} a_{pl, PSP}(\lambda) I_\lambda(\lambda) d\lambda, \quad \left(I = \int_{400 \text{ nm}}^{700 \text{ nm}} I_\lambda(\lambda) d\lambda \right). \quad (11)$$

Unfortunately, these absorption coefficients ($\bar{a}_{pl, PSP, fl}$ and $\tilde{a}_{pl, PSP}$) are not proportional. In reality, as a result of changes in $I_\lambda(\lambda)$ spectra with depth, the ratio $\tilde{a}_{pl, PSP}/\bar{a}_{pl, PSP, fl}$ is also strongly depth-dependent. Moreover, this ratio depends on the trophicity. Let us denote this ratio as A_{mod} :

$$A_{\text{mod}} = \frac{\tilde{a}_{pl, PSP}}{\bar{a}_{pl, PSP, fl}}.$$

As we can see in Fig. 6, the parameter A_{mod} differs for different trophic types of sea (we assume the surface chlorophyll a concentration $C_a(0)$ to be the trophic index of the sea) and also varies with depth. These changes are significant, especially in regions of high and low trophicity. This illustrates the imprecision of the assumption in eq. (8) that parameter k_m is constant for all depths in the sea.

Therefore, the assumption of a constant value of k_m for all depths in eq. (8) can be the source of significant errors.

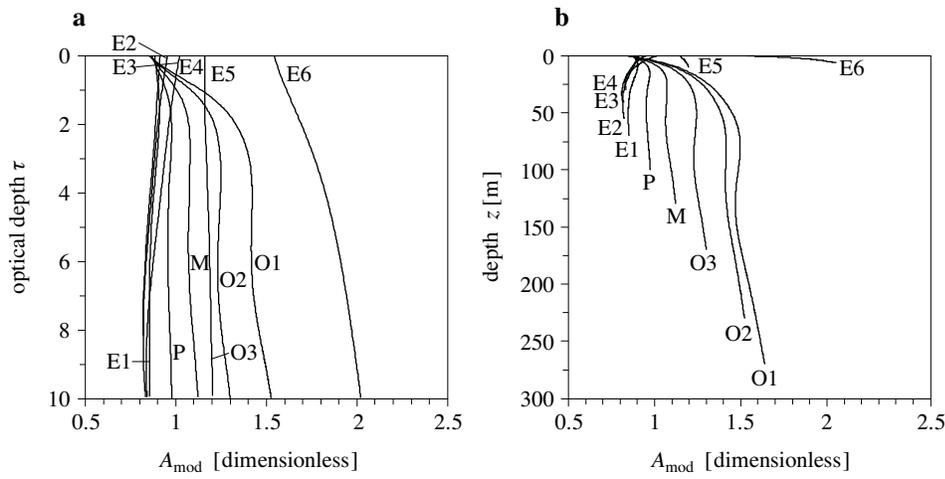


Fig. 6. Computed (using the model according to Ostrowska 2000) depth profiles of the ratio $A_{mod} = \tilde{a}_{pl, PSP} / \tilde{a}_{pl, PSP, fl}$ for optical (a) and real (b) depths for different trophic types of sea. Surface chlorophyll a concentrations $C_a(0)$ [mg tot.chl a m^{-3}] were assumed to represent the water trophic type index (according to Woźniak et al. 1992): O1 - $C_a(0) = 0.035$; O2 - $C_a(0) = 0.07$; O3 - $C_a(0) = 0.15$; M - $C_a(0) = 0.35$; P - $C_a(0) = 0.7$; E1 - $C_a(0) = 1.5$; E2 - $C_a(0) = 3.5$; E3 - $C_a(0) = 7$; E4 - $C_a(0) = 15$; E5 - $C_a(0) = 35$; E6 - $C_a(0) = 70$

- Thirdly, q_p is a function of temperature in the sea. The initially determined q_p as a function of the absorbed energy PUR_{PSP}^* (part of the Photosynthetically Usable Radiation due to photosynthetic pigments (per unit of chlorophyll a mass)) and temperature is given by the equation (Ostrowska 2000)

$$q_p = \frac{KPUR_{PSP}^*(temp)}{PUR_{PSP}^*} \left(1 - \exp \left[-\frac{PUR_{PSP}^*}{KPUR_{PSP}^*(temp)} \right] \right),$$

where $KPUR_{PSP}^*(temp)$ (the so-called ‘saturation irradiance’) depends on temperature according the Arrhenius law:

$$KPUR_{PSP}^*(temp) = KPUR_{PSP,0}^* Q_{10}^{temp/10^\circ C}.$$

The values of $KPUR_{PSP,0}^*$ (‘saturation irradiance’ at $0^\circ C$) and Q_{10} (a factor describing the increase in saturation irradiance caused by the temperature increase ($\Delta temp = 10^\circ C$)) should be determined empirically. To a first approximation (according to Ostrowska 2000): $KPUR_{PSP,0}^* = 8.39 \times 10^{-7}$ [Ein s^{-1} (mg tot. chl a) $^{-1}$], $Q_{10} = 1.9$ [dimensionless].

The authors intend to take into consideration all these remarks and modifications in their forthcoming papers.

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Appendix

List of symbols and abbreviations denoting the physical quantities

Symbol	Denotes	Units
$\bar{a}_{pl, PSP}$	mean coefficient of solar irradiance absorption by phytoplankton photosynthetic pigments (PSP) in the 400–700 nm spectral range (PAR)	m^{-1}
$\bar{a}_{pl, PSP, fl}$	mean coefficient of exciting flash absorption by PSP, averaged over the 400–550 nm spectral range	m^{-1}
$\tilde{a}_{pl, PSP}$	mean absorption coefficient averaged with the weight of the spectrum scalar irradiance in the sea	m^{-1}
A	ratio of the mean absorption coefficient: mean in PAR range and averaged over the 400–550 nm spectral range	dimensionless
A_{mod}	ratio of the mean absorption coefficient averaged with the weight of the spectrum scalar irradiance in the sea and averaged over the 400–550 nm spectral range	dimensionless
$A(0)$	value of A at the water surface	dimensionless
C_a	sum of chlorophylls a + pheo, or total chlorophyll (chl a + divinyl chl a) concentrations	$\text{mg tot. chl } a \text{ m}^{-3}$
C_{am}	averaged content of chlorophyll a in the water column	$\text{mg tot. chl } a \text{ m}^{-3}$
DCMU	an inhibitor of electron transport in PS II	
E	total irradiance in PAR range	$\mu\text{E m}^{-2} \text{ s}^{-1}$
$E_{1/2}$	light irradiation at which half of the RC are in the closed state	$\mu\text{E m}^{-2} \text{ s}^{-1}$
$E_{1/2m}$	value of parameter $E_{1/2}$ averaged in the water column	$\mu\text{E m}^{-2} \text{ s}^{-1}$

Appendix

List of symbols and abbreviations (continued)

Symbol	Denotes	Units
f	relative number of functionally active PSII reaction centers	dimensionless
F_0, F_m	<i>in vivo</i> phytoplankton fluorescence yield induced by a weak probe flash in the dark, under ambient light, and following a saturating flash, all measured in a light-adapted state	conv. units
F_v	variable fluorescence = $F_m - F_0$	conv. units
$F_{m,DCMU}$	fluorescence measured after adding inhibitor DCMU	conv. units
G	coefficient defined by geometric characteristics and sensitivity of the fluorescence light sensor, (constant)	
I_{fl}	total intensity of fluorescence excitation light	quanta $m^{-2} s^{-1}$
I_n	intensity of light passed through a suspension of algae of concentration n	quanta $m^{-2} s^{-1}$
$I_{n,c}$	intensity of light passed through a suspension of the algal cells of concentration n bleached by illumination in the presence of 1 mM hydroxylamine	quanta $m^{-2} s^{-1}$
$k(\Phi_{F_0, A})$	proportionality coefficient	dimensionless
k_m	value of parameter k averaged in the water column	dimensionless

Appendix

List of symbols and abbreviations (continued)

Symbol	Denotes	Units
$KPUR_{PSP}^*(temp)$	photosynthesis saturation PUR_{PSP} energy	$\text{Ein (mg tot. chl } a)^{-1} \text{ s}^{-1}$
$KPUR_{PSP,0}^*$	‘saturation irradiance’ (e.g. photosynthesis saturation PUR_{PSP} energy) at 0° C	$\text{Ein (mg tot. chl } a)^{-1} \text{ s}^{-1}$
n	number of fluorescence and radiation profiles measured for the period of bottle exposure at a station	
P_c	carbon assimilation by phytoplankton	$\mu\text{M C m}^{-3} \text{ s}^{-1}$
P^c	primary production of phytoplankton	$\text{mg C m}^{-3} \text{ time}^{-1}$
PP_c	primary production determined using the described method averaged in the water column	$\text{mg C m}^{-3} \text{ time}^{-1}$
PAR	photosynthetically available radiation	
PUR^*	photosynthetically usable radiation (per unit of chlorophyll a mass)	$\text{Ein (mg tot. chl } a)^{-1} \text{ s}^{-1}$
PUR_{PSP}^*	part of PUR^* due to photosynthetic pigments	$\text{Ein (mg tot. chl } a)^{-1} \text{ s}^{-1}$
PSP	photosynthetic pigments	
PS II	photosystem 2	

Appendix

List of symbols and abbreviations (continued)

Symbol	Denotes	Units
Trophic type symbols:		
O	oligotrophic	
M	mesotrophic	
P	intermediate	
E	eutrophic	
Q_{10}	factor describing the increase in saturation irradiance caused by a temperature increase $\Delta temp = 10^\circ\text{C}$	
q_p	relative number of functionally open reaction centers PS II in algal cells	dimensionless
r	correlation coefficient	dimensionless
RC	reaction center	
$T(z)$	depth dependence of A	dimensionless
z	depth in the sea	m
$\Phi(E)$	efficiency (quantum yield) of the conversion of absorbed energy in photosynthetic reactions	$\mu\text{M C } \mu\text{E}^{-1}$
Φ_e	efficiency of electron transfer from H_2O to CO_2	
Φ_{fl}	quantum yield of fluorescence	dimensionless
Φ_{F_0}	quantum yield of fluorescence in cells with open RC	dimensionless
Φ_{RC}	efficiency of photochemical conversion of light energy in open reaction centers	$\mu\text{M electron } \mu\text{E}^{-1}$
$\Delta\lambda$	spectral range of exciting light	nm