Fluorescence "in situ" method for the determination of chlorophyll *a* concentration in sea

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Fluorescence
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MIROSŁAWA OSTROWSKA
Institute of Oceanology,
Polish Academy of Sciences,
Sopot

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Abstract

The paper presents a statistical analysis of experimental data concerning the effect of chlorophyll *a* concentration and optical depth in a sea on artificially induced phytoplankton fluorescence. Certain empirical correlations have been found between these quantities. They were applied for a development of fluorometric methods of determination of the chlorophyll *a* concentration on the basis of the "in situ" fluorescence. The results of preliminary experimental verification of these methods are also presented.

1. Introduction

This paper is already the 6th from the cycle devoted to the problem of applicability of fluorescence methods for marine photosynthesis investigation. In previous works from that cycle (Ostrowska and Woźniak, in press a, b; Woźniak and Ostrowska, in press, 1990a, 1990b), the review of the literature concerning the general body luminescence phenomena was presented and on that basis the sea water luminescence and related processes were characterized. The most important photosynthetic pigments together with their chemical and electron structures were discussed, and the individual, optical absorption and fluorescence properties of the particular pigments were characterized. Moreover, on the basis of long term investigations the pigment composition of phytocenoses in different areas of the World Ocean were characterized; the general optical absorption and fluorescence properties of the marine phytoplankton were also described.

After this initial stage of investigations, with this article we start the analysis and evaluation of the possibility of application of the fluorometric characteristics of pigments and phytoplankton photosynthetic apparatus for the investigations on various quantities describing the process of marine photosynthesis. We start from the concentration of the most important photosynthetic pigment in sea water, chlorophyll *a*, being the subject of this paper. This concentration is a measure of phytoplankton resources in sea water and also indicates sea production potential. Traditionally, in oceanographic investigations the chlorophyll *a* concentration is estimated with "in vitro" methods on water samples collected from various water depths in sea. This estimations are most often made by means of phytoplankton aceton extracts spectrophotometry (sea *e.g.* Strickland and Parsons, 1968; Jeffrey and Humphrey, 1975), or – in order to increase the accuracy – by means of fluorescence measurements of these extracts (Lorenzen, 1966, 1968; Yentsch and Menzel, 1963).

Both these methods disturb natural phytoplankton environment; moreover, they are time consuming because of complexity of laboratory works concerned with extraction. The obtained results concern points randomly scattered in time and space. Therefore more effective investigation methods enabling especially space-time concentration of the experimental data are searched for. Such possibilities are created among others by conctact physical methods based on observations of stimulated physical phenomena (mostly optical) occuring in phytoplankton. The measurements of physical characteristics of these phenomena allow indirectly to determine *e.g.* chlorophyll concentration.

One of the indirect remote optical measurement methods of determination of the chlorophyll concentration in sea is the measurement of the ordinary (short-life) fluorescence of sea water made by means of submerged fluorometers (Karabashev, 1987; Brown, 1980; Hundahl and Holck, 1980). These devices make a total measurement of the photoinduced luminescence of sea water in red spectral range. In this band the chlorophyll *a* fluorescence dominates over other components of the photoinduced emission of sea water (compare Fig. 12C in paper by Ostrowska and Woźniak, in press a). Investigations performed by many scientists (Karabashev, 1987; Fadeyev *et al.*, 1979) indicate, however, that there is no explicit correlation between fluorescence intensity and chlorophyll concentration when the results of measurements carried out on various natural phytocenoses are taken into account. This is due to the fact that natural chlorophyll fluorescence depends on many biotic and abiotic

marine parameters. One of the most important factors influencing the fluorescence intensity of sea water in the red area of the spectrum is the composition of phytoplankton pigments.

It is known that apart from "common" chlorophyll *a* fluorescence we deal with induced fluorescence caused by a transfer of excitation energy of other pigments supporting photosynthesis to the chlorophyll (Ostrowska and Woźniak, in press b). Therefore although if we measure the fluorescence in the red area of the spectrum, where practically only chlorophyll *a* both absorbs and emits light, the amount of the emitted energy depends also on energy from the short-wave spectral region absorbed by chlorophyll *a* and the other pigments. Concentration of pigments supporting photosynthesis is not constant; it varies with both depth and the biological type of waters (Woźniak and Ostrowska, 1990a). Due to this, the fluorescence abilities of phytoplankton – especially specific fluorescences – are different. That is why primary goal of this work was the determination of statistical relationships between fluorescence properties of the phytoplankton and the biological type of water masses (represented by chlorophyll *a* concentration), as well as the optical depth in sea. Knowledge of these relationships enables evaluation of chlorophyll *a* concentration on the basis of phytoplankton fluorescence measurements. The second important goal was therefore the evaluation of the accuracy of determination of chlorophyll *a* concentration by means of "in situ" fluorescence measurements. The assumed goals of the work were accomplished by means of statistical analysis of the experimental data.

2. Material and experimental methods

2.1. Experimental material

Experimental material collected by the team from the biophysics laboratory of the Institute of Oceanology Polish Academy of Sciences in the years 1987–1988 in various areas of the Norwegian Sea and the Baltic from the board of r/v "Oceania", including the First International Ecological Experiment Sopot 1987, was utilized in the research. The experimental data used in our work consisted, among others of:

ϕ – [arbitrary units characteristic for a given device] fluorescence intensity of sea water at different depths in the basin measured "in situ" in red band of radiation,

Ba $[mg/m^3]$ – chlorophyll a concentrations determined by means of spectrophotometric "in vitro" method in sea water samples from various depths of sea,

T – [units expressing fraction of luminescence just under the water surface at $z = 0$] luminescence transmission functions $PhAR$ in sea determined for the same depths as for chlorophyll a determination.

The total number of the measurement points (ϕ, Ba) was 186, including 100 for the Baltic and 86 for the Norwegian Sea. From this amount for 80 experimental points determined at the Baltic also the transmission function T was determined.

The analyzed experimental material, hence also the conclusions from the performed analysis, is characteristic for the following biological types of phytocenoses: mesotrophic, transient meso-eutrophic and eutrophic (see Tabl. 1 in paper by Woźniak and Ostrowska, 1990a). It is caused by the faet that for the analyzed cases the chlorophyll a concentration Ba varied in the range $0.2\ mg/m^3 < Ba < 40\ mg/m^3$.

Moreover, the experimental measurements were carried out in waters of temperatures from the range $2 \div 12\,^oC$. Because of the dependence of the fluorescence phenomena on temperature (even though this dependence in the range of temperatures found in seas is not significant), the obtained results should not be extrapolated for other temperature ranges.

2.2. The principle of fluorescence measurements by means of Q-fluorometer

Fluorescence intensity was determined by means of a submerged fluorometer of a Q-fluorometer type made in Denmark. Block diagram of this Q-fluorometer is shown in Figure 1 . It is a device used for measurements of common fluorescence in water directly from the vessel board. By means of this device, it is possible to measure both the horizontal, and owing to the installed depthmeter also vertical profiles of the fluorescence. The measurement idea consists in irradiating sea water with short flashes (10 Hz frequency) and immediate reception of the induced fluorescence at a 90^o angle. A xenon lamp is used as a light source. The UDT-500 photodiode is used as the fluoroescence detector. The device can be used for the determination of rhodamine B or chlorophyll concentration depending on the filters applied for both the incident and

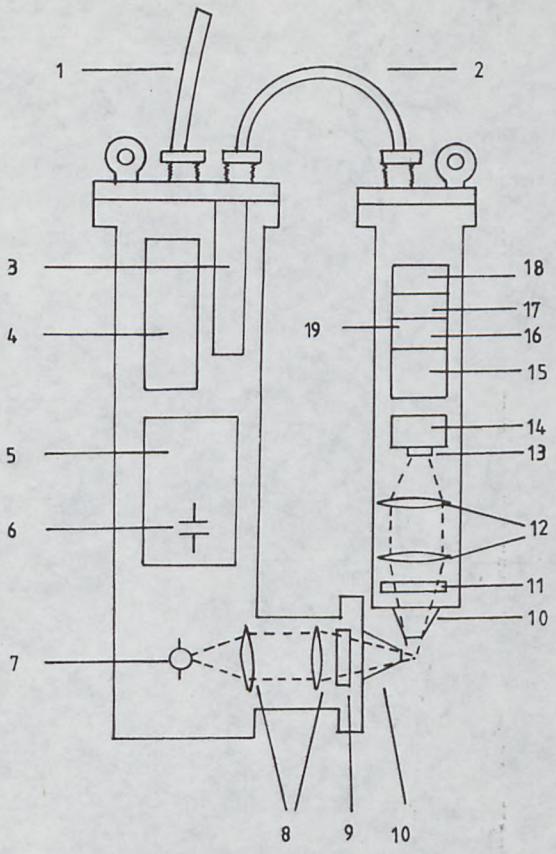


Figure 1: Block diagram of the Q-fluorometer (after Hundahl and Holck, 1980): 1 – main cable to deck, 2 – interconnection cable, 3 – depth transducer, 4 – power supply to electronic, 5 – power supply to lamp, 6 – discharge capacitor, 7 – xenon flash lamp, 8 – condenser filter, 9 – transmitter filter, 10 – plexiglass cones, 11 – receiver filter, 12 – condenser lenses, 13 – PIN photodiode, 14 – pre-amplifier, 15 – 10 Hz oscillator, 16 – peak detector, 17 – logarithmic amplifier, 18 – linear amplifier, 19 – internal switch

received light beams. Spectral transmission of these filters is illustrated in Figure 2. As one can see in the case of chlorophyll *a* (Fig. 2A), the fluorescence is induced by shortwave and middle part of the visible part of spectrum, *i.e.* in the absorption area of all photosynthetic pigments. On the other hand, fluorescence from the red area of spectrum is recorded – so it is characteristic for chlorophyll *a*. Hence, the described device serves for the determination of the induced fluorescence of phytoplankton, or more precisely, induced fluorescence of chlorophyll *a* under the conditions of a living plant.

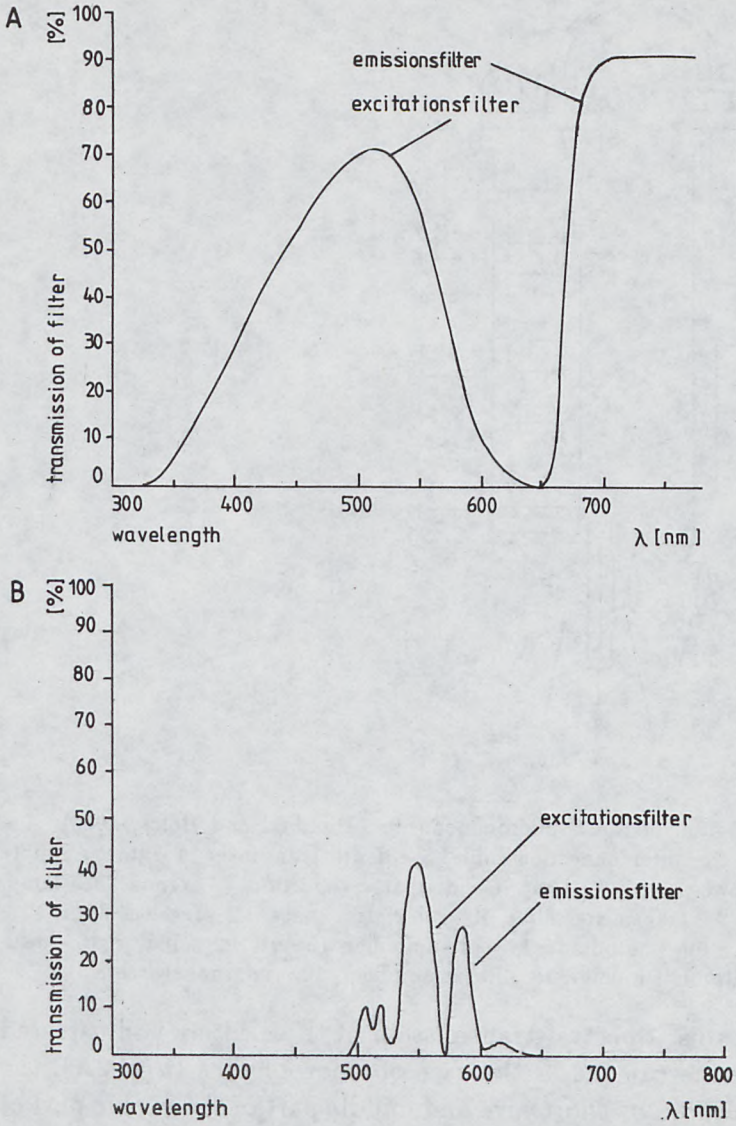


Figure 2: Transmission of the receiving and exciting beam filters of the Q-fluorometer: A – filters used for chlorophyll *a* concentration measurements, B – filters used for rhodamine *B* concentration measurements

2.3. Other experimental methods

Water samples for chlorophyll *a* concentration determinations were collected from various depths of sea using a 10 liter bathometer. The samples were filtered through SYNPOR No 5 filters of 0.6 μm pore diameter under the pressure of ca 400 mg Hg. The amount of the filtered water depended on the chlorophyll content. In the case of clear waters of the Norwegian Sea about 7–10 l of sea water was filtered, whereas in the case of rich in chlorophyll water from the Gulf of Gdańsk this volume was ca 1.5–2.0 l. After introductory drying, the filters were stored at a temperature of ca 0 ^{o}C.

Chlorophyll *a* concentration was determined by means of a standard spectrophotometric method (Strickland and Parsons, 1968) in 90% acetone extracts of the photoplankton. For Ba concentration evaluations we used:

- Jeffrey and Humphrey formula (1975) – in Norwegian Sea case:

$$Ba = (11.85\,D_{664} - 1.54\,D_{647} - 0.08\,D_{630})\,\frac{V_e}{V_0 l}; \tag{1}$$

- Formula recommended by SCOR-UNESCO (1966) in the Baltic case:

$$Ba = (11.6\,D_{665} - 1.3\,D_{645} - 0.14\,D_{630})\,\frac{V_e}{V_0 l}, \tag{2}$$

where:
D_λ – phytoplankton extracts' extinctions measured for various light wavelenghts λ;
V_e – extract volume [ml];
V_0 – sample volume [l];
l – lenght of the absorption cell disk [cm].
It should be emphasized that the results of Ba evaluations on the basis of these two formulas practically do not differ.

In order to determine the transmission function T, of $PhAR$ in sea, the results of direct spectral distributions of the irradiances $Ed(\lambda, z)$ measured of various depths were utilized. These measurements were carried out using underwater spectrophotometer constructed in the Institue of Oceanology Polish Academy of Sciences, with utilization of methods described in works: Woźniak and Montwiłł (1973), Woźniak *et al.* (1983). On the basis of $Ed(\lambda, z)$ distributions the deep sea irradiance profiles

of $PhAR$ were determined by integration over wavelength in the range 400–700 nm:

$$\eta_{PhAR}(z) = \int_{400nm}^{700nm} Ed(\lambda, z)d\lambda. \tag{3}$$

Then, the transmission functions $T(z)$ were determined for particular depths z in the sea, as ratios of irradiances of $PhAR$ at these depths to the surface irradiance:

$$T(z) = \frac{\eta_{PhAR}(z)}{\eta_{PhAR}(z=0)}. \tag{4}$$

In the following part of this work the determined volues of transmission $T(z)$ are treated as indexes of the optical depth of the basin.

3. Results and discussion

3.1. Dependence of fluorescence and specific fluorescence of phytoplankton on chlorophyll *a* concentration

Figure 3 shows the observed dependence of fluorescence ϕ and chlorophyll *a* concentration Ba, for all the experimental points (*i.e.* from various depths and various sea areas). The experimental point locations are approximated by an empiric curve obtained using the least squares method:

$$\phi = 23Ba + 182, \tag{5}$$

where:

Ba – chlorophyll *a* concentration in $[mg/m^3]$,

ϕ – fluorescence intensity expressed in relative units, characteristic for a given device.

Linear correlation coefficient for this relationship $r = 0.75$.

As it follows from Figure 3, the experimental dependence between the phytoplankton fluorescence intensity and the chlorophyll *a* concentration is characterized by a large scatter. Due to this, the fluorescence intensity measurements are not suitable for direct and precise evaluation of the chlorophyll *a* concentration. Irrespective of the scatter of the experimental results, however, one can find certain regularities in Figure 3. Apart from a general increase of the fluorescence with an increase of chlorophyll *a* concentration, a decrease of the ϕ/Ba ratio with an increase of the Ba can also be noticed. It is also confirmed by the form of the dependence (5). This fact should be interpreted – as it was already mentioned

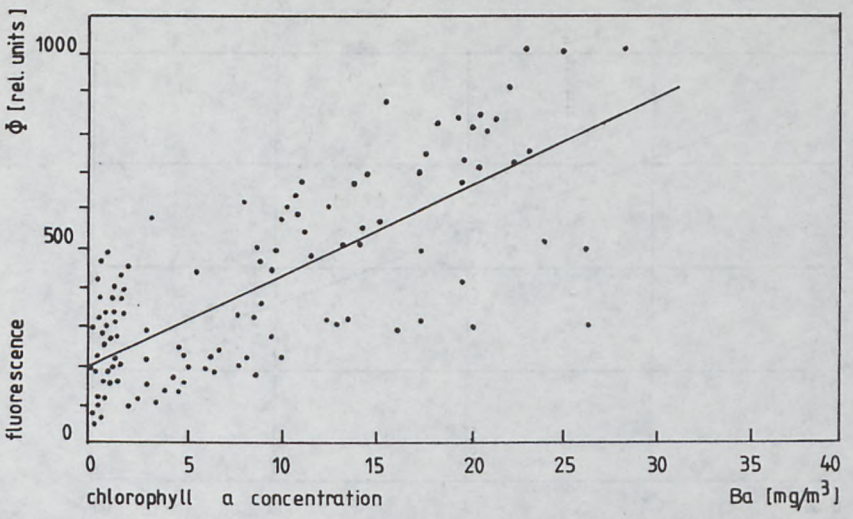


Figure 3: Experimental relationship between the fluorescence ϕ and chlorophyll *a* concentration Ba. Dots denote experimental points location for various depths and different sea areas; the straight line approximates the experimental points location by means of the least square method from formula (5)

in the introdution – as being due to various sets of photosynthetic pigments in the same seas, in case of different areas and seasons. As it is known, in biologically rich waters, characterized by large amounts of chlorophyll *a*, the ratio of the assisting pigments concentration to the amount of chlorophyll *a* is smaller than in the case of biologically lean waters (Woźniak and Ostrowska, in press – Figs. 5–7). It leads among others to an increase of specific absorption coefficients of phytoplankton in oligotrophic phytocenoses, relative to eutrophic basins (compare Woźniak and Ostrowska, 1990b – Figs. 6, 8–10). Hence, since the energy absorbed by pigments assisting photosynthesis is transferred to chlorophyll *a*, it can be expected that in the case of small concentrations of chlorophyll *a* – *i.e.* a relatively large amount of the assisting pigments, a higher intensity of fluorescence will occur relative to the chlorophyll *a* concentration than in waters containing phytoplankton more rich in chlorophyll *a*.

These regularities are shown in Figure 4. It presents in a logarithmic scale the experimental correlation of phytoplankton specific fluorescence ϕ_M and chlorophyll *a* concentration Ba, for different depths and for different sea areas. Specific fluorescence ϕ_M in this paper is understood as the ratio of the intensity of phytoplankton fluorescence ϕ to chlorophyll *a* concentration Ba:

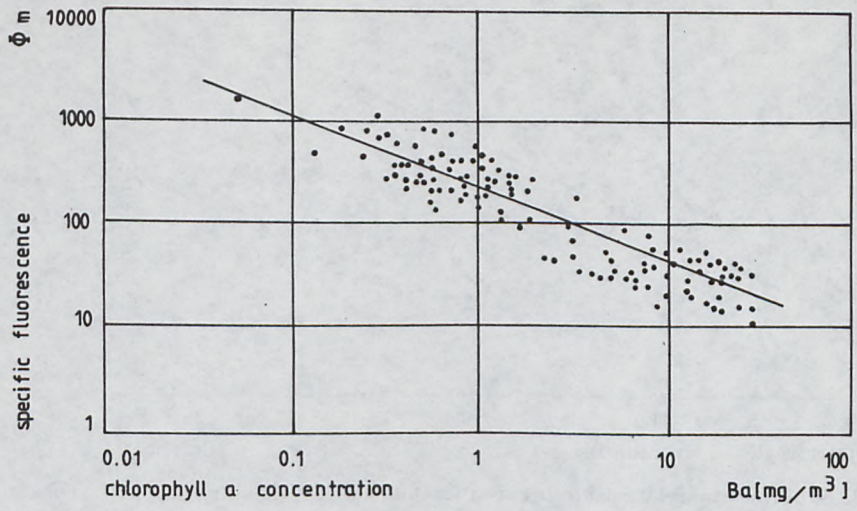


Figure 4: Experimental relationship between the specific fluorescence ϕ_M and chlorophyll a concentration Ba. Dots denote experimental points location; the straight line approximates the experimental points location by means of the least square method according to formula (8)

$$\phi_M = \phi / Ba. \tag{6}$$

The empirical dependence of $\log \phi_M$ versus $\log Ba$ was approximated by the following line:

$$\log \phi_M = a \log Ba + b. \tag{7}$$

The a, b and r values obtained from the least square method are:

$$a = -0.68, \qquad b = 2.32, \qquad r = 0.92.$$

Hence, the dependence (7) has the following form:

$$\log \phi_M = -0.68 \, log Ba + 2.32. \tag{8}$$

It follows from Figure 4, and the dependence (8) that the specific fluorescence decreases with chlorophyll a concentration increase, *i.e.* with a decrease of the assisting pigments amount. It confirms the previously suggested correlations. As a consequence, the dependence of the averaged fluorescence intensity ϕ on the chlorophyll a concentration Ba can be described with the following dimensionless function (after transformation of equation (8) and regarding dependence (6)):

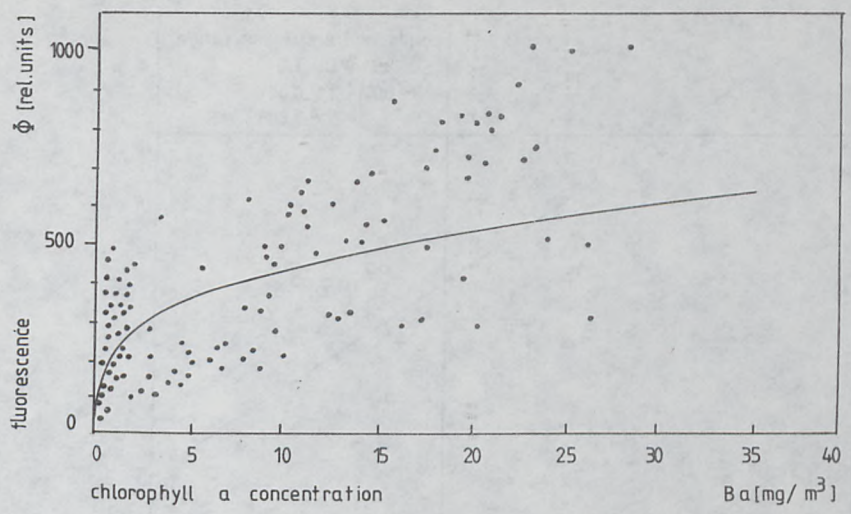


Figure 5: Fluorescence ϕ and chlorophyll *a* concentration dependence, described by formula (9). Dots denote experimental points location

$$\phi = 209 Ba^{0.32} \tag{9}$$

Figure 5 illustrates the diagram of the above dependence. It can be noticed that the statistically averaged phytoplankton fluorescence intensity increases with an increase of chlorophyll *a* concentration, the rate of this increase decreasing with a change from lean to rich basins.

3.2. Optical depth influence on concentration dependences of phytoplankton fluorescence.

Empirical correlations of the fluorescence properties of the phytoplankton and chlorophyll *a* concentration analysed above are characterized by a large scatter of experimental points. It is caused by a complex influence of numerous environmental agents determining the optical properties of the phytoplankton. Right now, a precise quantitative description of the influence of these agents on phytoplankton fluorescence is not possible. However, as the analysis of the collected experimental material showed, it is possible to determine certain quantitative relationships between the concentration dependences of the phytoplankton fluorescence and the optical depth in sea. The existence of these relations has been also demonstrated by Karabashev (1987) and Bekasova *et al.* (1987). Let

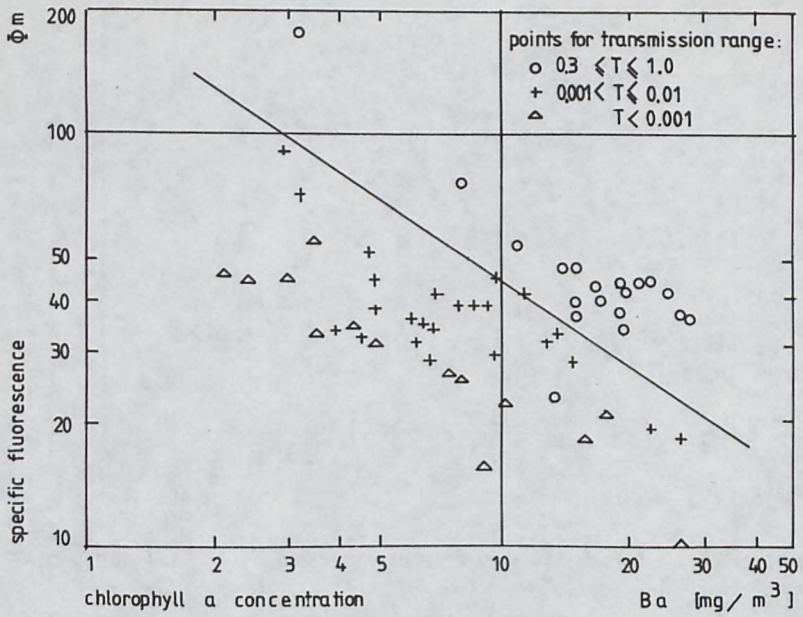


Figure 6: Experimental dependence of the specific fluorescence ϕ_M and chlorophyll concentration Ba for three exemplary optical depth ranges

us consider therefore the phytoplankton fluorescence as a function of two variables, *i.e.* chlorophyll *a* concentration and optical depth in sea. The experimental data for the Baltic obtained during the SOPOT '87 experiment was utilized (the total of about 100 measuring points).

An example of the optical depth influence on phytoplankton fluorescence in sea is shown in Figure 6. It illustrates the experimental correlations of the specific fluorescences ϕ_M on chlorophyll *a* concentration, for phytoplankton from three, exemplary optical depth ranges in sea. Transmission function T of the $PhAR$ has been taken as a measure of the optical depth. As one can see from Figure 6, the scatter of the experimental points of the $\log \phi_M$ versus $\log Ba$ dependence for separate phytoplankton groups with determined values of T is much smaller than in the case of all the points. The above relations for particular groups of T are characterized by a similar slope, close to an average slope for all the data. On the other hand, differences occur in absolute values of ϕ_M and they consist in parallel translations of these dependences with respect to each other. The maximum values of the specific fluorescence ϕ_M are observed for surface phytoplankton (group of points with T in

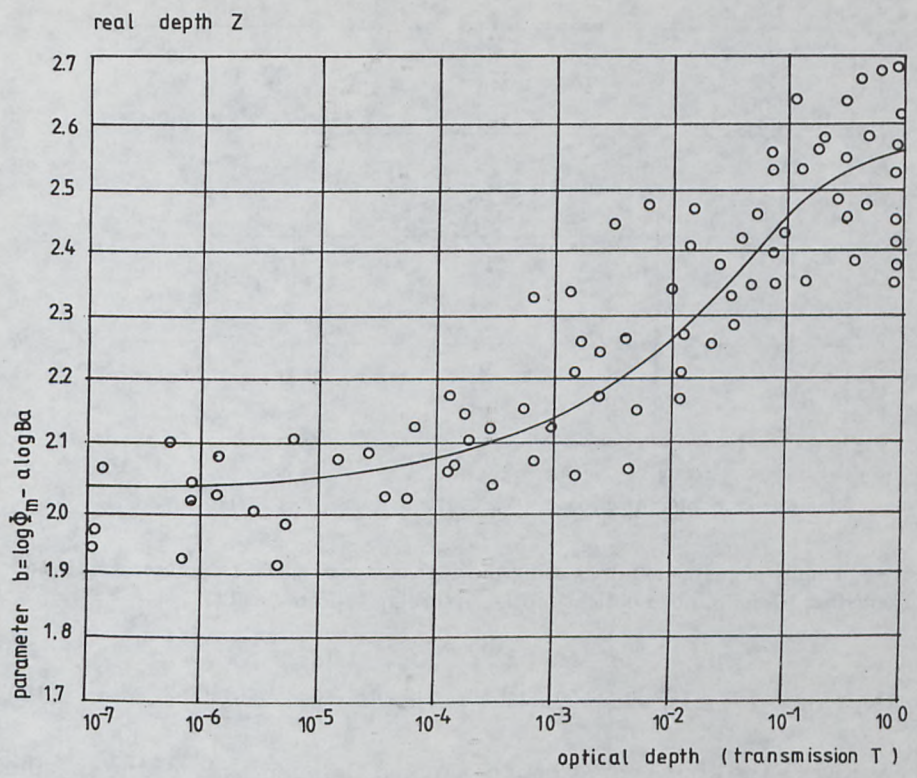


Figure 7: Experimental dependence of the parameter b (eq. 7) on transmission T. Dots denote experimental points location; straight line – the empirical approximation, according to eq. (10)

a range $0.3 \div 1.0$ in Figure 6). With an increase of depth (group of points with T in a range $0.0001 \div 0.01$ and $T = 0.001$ in Figure 6) the values of the specific fluorescence decrease.

Due to the above tendencies, in the following stage of the analysis of the optical depth influence on phytoplankton fluorescence, (eq. 7) was adopted for functions approximating the $\log \phi_M = \phi(\log Ba)$ dependence for various T. For slopes a of these functions the derived before, average for all the data value of the parametr $a = -0.68$ was taken. The parameter $b = \log \phi_M - a \log Ba$ was varied depending on the transmission T.

The experimental dependence of the parameter b on transmission T is illustrated in Figure 7. The location of the experimental data points is well approximated by an expression derived from nonlinear regression method:

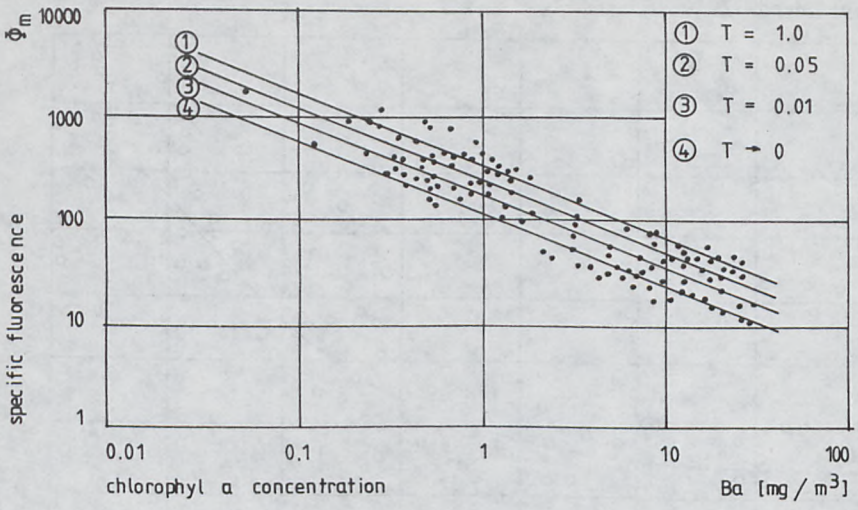


Figure 8: Family of curves approximating the dependence of specific fluorescence ϕ_M and chlorophyll a Ba for various optical depths, according to formula (11)

$$b = \log \phi_M - a \log Ba = 2.041 + 1.4 \log \left(\frac{10}{T}\right) e^{-\log\left(\frac{10}{T}\right)}. \tag{10}$$

According to the above equation, the empirical dependence of the specific fluorescence versus chlorophyll a concentration Ba, and optical depth expressed through transmission T, is of the following form:

$$\log \phi_M = -0.68 \log Ba + 2.041 + 1.4 \log \left(\frac{10}{T}\right) e^{-\log\left(\frac{10}{T}\right)}. \tag{11}$$

Therefore, the observed dependence $\log \phi_M$ versus $\log Ba$ can be approximated by a family of functions (11) for given transmissions. It is illustrated in Figure 8. Further transformation of eq. (11) (taking into account the relation (6) enables obtaining an empirical form of the dependence of fluorescence Ba on chlorophyll concentration Ba and the optical depth:

$$\phi = 110 \exp \left[0.515 ln \left(\frac{10}{T}\right) \exp(0.4343 ln T)\right] Ba^{0.32}. \tag{12}$$

The family of curves determined by means of the above equation, illustrated in Figure 9, showes the course of the dependences of phytoplankton fluorescence ϕ on chlorophyll a concentration of a sea depth Ba, for various optical depths represented by transmission T. As one can see from

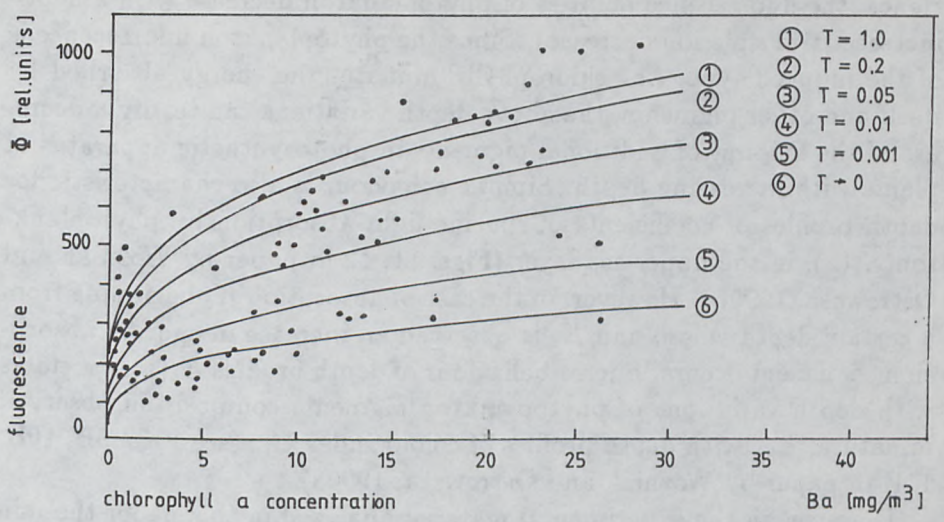


Figure 9: Family of curves describing the dependence of fluorescence ϕ and chlorophyll a concentration Ba, for various optical depths, according to formula (12)

Figures 8 and 9, the families of curves cover basicaly the entire area of the dependence ϕ_M versous Ba occupied with experimental points.

Let us discuss now the obtained results of statistical approximations. It follows from the form of relations (11) and (12) that at constant chlorophyll concentration, the phytoplankton from great depths is characterized by the smallest values of the fluorescence ϕ and specific fluorescence ϕ_M. The transmission at these depths approaches zero ($T \to 0$). After reaching this limits, the expressions for minimum fluorescence and specific fluorescence are the following (curves 1 in Figs. 8 and 9):

$$\phi_{M\,min} = 110 Ba^{-0.68}, \tag{13}$$

$$\phi_{min} = 110 Ba^{0.32}. \tag{14}$$

On the other hand, with an increase in depth these fluorescences increase and their maximum values characterize surface phytoplankton for $T = 1.0$ (see curves 6 in Figs. 8, 9). The expressions for ϕ and ϕ_M are:

$$\phi_{M\,max} = 360 Ba^{-0.68}, \tag{15}$$

$$\phi_{max} = 360 Ba^{0.32}. \tag{16}$$

Hence, the fluorescence abilities of phytoplankton decrease with a dept' increase (transmission decrease). Since the phytoplankton fluorescence is of the induced type, *i.e.* chlorophyll luminates the energy absorbed by itself and other pigments, the above depth variations can testify a decrease of the amount of additional pigments in photosynthetic apparatus of plants with increasing depth. Similar behaviour is also characteristic for depth profiles of coefficients of specific light absorption by phytoplankton $\widetilde{K}_c(z)$, in the upper sea layer (Figs. 11, 12 in paper by Woźniak and Ostrowska, 1990b). However, in the case of absorption $\widetilde{K}_c$ beginning from a certain depth where min $\widetilde{K}_c$ is observed an increase in specific absorption coefficient occurs. Such a behaviour of depth profiles $\widetilde{K}_c(z)$ correlates with depth variations of phytoplankton pigments composition observed in nature, *e.g.* with depth profiles of colour index C_{In} (see Figs. 9B, 10B, 11B in paper by Woźniak and Ostrowska, 1990a).

However, in the experimental material analyzed in this paper the min $\phi_M(z)$ did not occur at finite depths and the region of an increase in the specific fluorescence at great depths was not observed. How can we explain therefore the difference between the tendencies in the character of depth profiles of specific absorption and the specific phytoplankton fluorescence analyzed here in? The explanation probably concerns the experimental methods used. In the experimental part of this work, for the determination of the chlorophyll *a* concentration the standard spectrophotometric methods were used (see section 2.1.). By means of these methods the total chlorophyll *a* concentration is estimated, without non-active chlorophyll and pheophytin reduction. Hence (due to an increasing amount of these compounds with depth, see *e.g.* Vedernikov *et al.*, 1973), the total contents of chlorophyll *a* estimated by us are higher, especially for great depths, than concentrations of the active chlorophyll which takes part in the induced fluorescence of the phytoplankton. That is why the specific fluorescences calculated from equation (6) are smaller than their real values. Moreover, the mentioned above differences of the observed and true specific fluorescences increase with depth in sea.

To summarize, the generalizations introduced in this paper correspond to statistical correlations of phytoplankton fluorescence with the total dependence of chlorophyll *a*, not with its active part. The same concerns the shown below fluorometric equations for determination of the chlorophyll *a* concentration. The approximate values of the total concentration Ba, related to active and non-active molecules, can be determined using these equations.

3.3. Fluorometric formulas for the determination of chlorophyll *a* concentration

In practice the knowledge of the functional dependences of phytoplankton fluorescence on the chlorophyll *a* concentration (eqs. (5), (9), (12)), enables approximate determination of this compound Ba on the basis of fluorescence ϕ measured "in situ" using a fluorometer. For this purpose one can use the following fluorometric formulas:

– after transformation of equation (5)

$$Ba = 4.35 \cdot 10^{-2}\phi - 7.91, \tag{17}$$

– after transformation of equation (9)

$$Ba = [4.79 \cdot 10^{-3}\phi]^{3.125}, \tag{18}$$

– after transformation of equation (11)

$$Ba = \left[9.09 \cdot 10^{-3} \exp\left(-0.515 ln\frac{10}{T} \cdot exp(0.4343 lnT)\right)\phi\right]^{3.125} \tag{19}$$

The illustration of the course of these equations and the comparison of these curves with the location of experimental points on the diagram of the Ba versus ϕ relation is given in Figure 10.

As one can see from Figure 10, the first of the three shown fluorometric formulas (17), based on the linear dependence of phytoplankton fluorescence on chlorophyll *a* concentration is not precise and inaccurate, particularly for small values of ϕ and Ba. For example, for $\phi < 182$, the concentrations Ba determined from equation (17) have no physical sense, since they have negative values. Utilization of this formula is not recommended.

Fluorometric formula (18), although having physical sense (does not yield negative values of the chlorophyll *a* concentration) is also not inaccurate due to a large scatter of experimental points around curve 2 in Figure 10. Hence this formula can be used for very approximate estimation of chlorophyll concentration, in cases when the optical depth T of phytoplankton occurence can not be determined.

The most accurate formula for the determination of the concentration Ba is fluorometric formula (18). According to this formula the chlorophyll concentration depends on fluorescence and depth T, which has to be determined additionally. However, owing to this one can determine chlorophyll concentration with good accuracy, which is testified by good

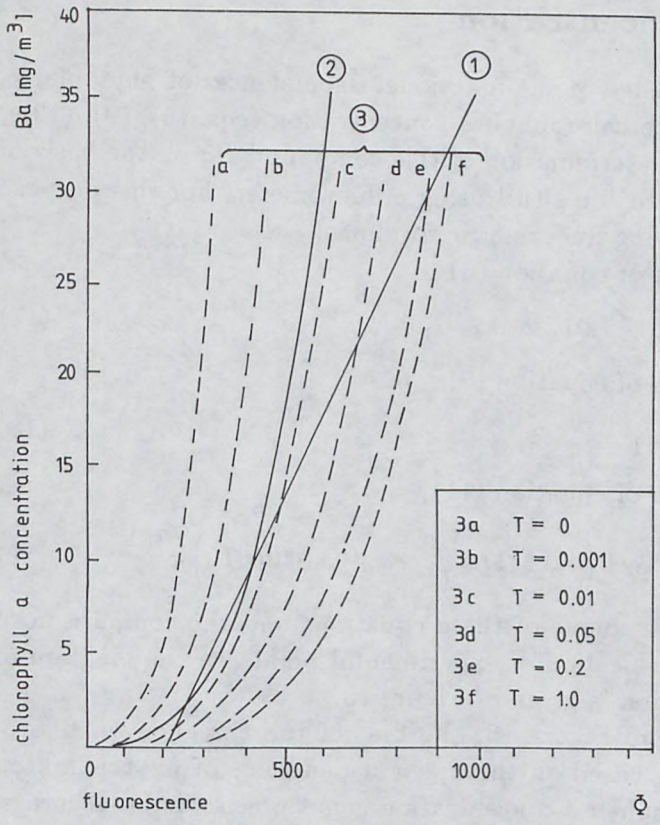


Figure 10: Comparison of three methods of chlorophyll *a* concentration determination on the basis of fluorescence: 1 – linear, fluorometric formula (eq. (17)), 2 – fluorometric formula, neglecting the influence of the optical depth T (eq. (18)), 3 – fluorometric formula with optical depth T (eq. (19)). Dots denote experimental points location

overlapping of the family of curves 3 in Figure 10 with the area occupied by the experimental points. The results of the experimental verification presented below also testify the high degree of accuracy of the fluorometric formula (19).

3.4. Experimental verification of the fluorometric formulas

Fluorometric formulas (18) and (19) for chlorophyll *a* concentration determination were experimentally verified. In order to do it, the results

of direct determinations of chlorophyll *a* concentrations Ba^{measur} at various depths in the Baltic were compared with the calculated values of these concentrations, Ba^{calc}. The calculations of the Ba^{calc} were carroed out on the basis of the measured values of the fluorescence ϕ – in the case of formula (18), and the measured fluorescence ϕ and transmission T – in the case of equation (19). The total number of points used for the verification was 80. They covered the range of chlorophyll *a* variations from about 0.5 to 40 mg/m^3. Hence, the verification concerns eutrophic and neso-eutrophis basins.

Statistically elaborated results of verification are illustrated by the diagrams in Figure 11. They show the probability distributions of the occurrence of certain ratios X of the calculated chlorophyll *a* concentration to the measured concentration:

$$X = \frac{Ba^{calc}}{Ba^{measur}}. \tag{20}$$

The ratios X are connected with a relative error E of the estimated chlorophyll *a* concentration, in the following way:

$$E[\%] = 100\frac{Ba^{calc} - Ba^{measur}}{Ba^{measur}} = 100(X - 1). \tag{21}$$

It follows from diagrams in Figure 11 that probability distributions $f(\log X)$ are similar to a normal, Gaussian distribution. Mean values of these distributions are:

- for the formula (18) (fluorescence method):
  $< \log X >= -6.0206 \cdot 10^{-3}$, which relates to geometric mean,
  $< X > g = 0.986$, and mean error about:
  $< E >= -1.4\%$;
- for the formula (19) case (depth-fluorescence method):
  $< \log X >= 1.300 \cdot 10^{-3}$, which relates to geometric mean,
  $< X > g = 1.003$, and mean error about:
  $< E >= +0.3\%$.

Mean values $< E >$ have a sense of a systematic error of determination of the chlorophyll *a* concentration using the analyzed fluorometric formulas. It is noticeable in both cases that mean values of $< E >$ are low. This means that the above listed methods are not biased with a systematic error.

A different situation occurs in case of the systematic error of the discussed methods of fluorometric determination of the chlorophyll concentration. As a measure of the statistical error one can take the standard

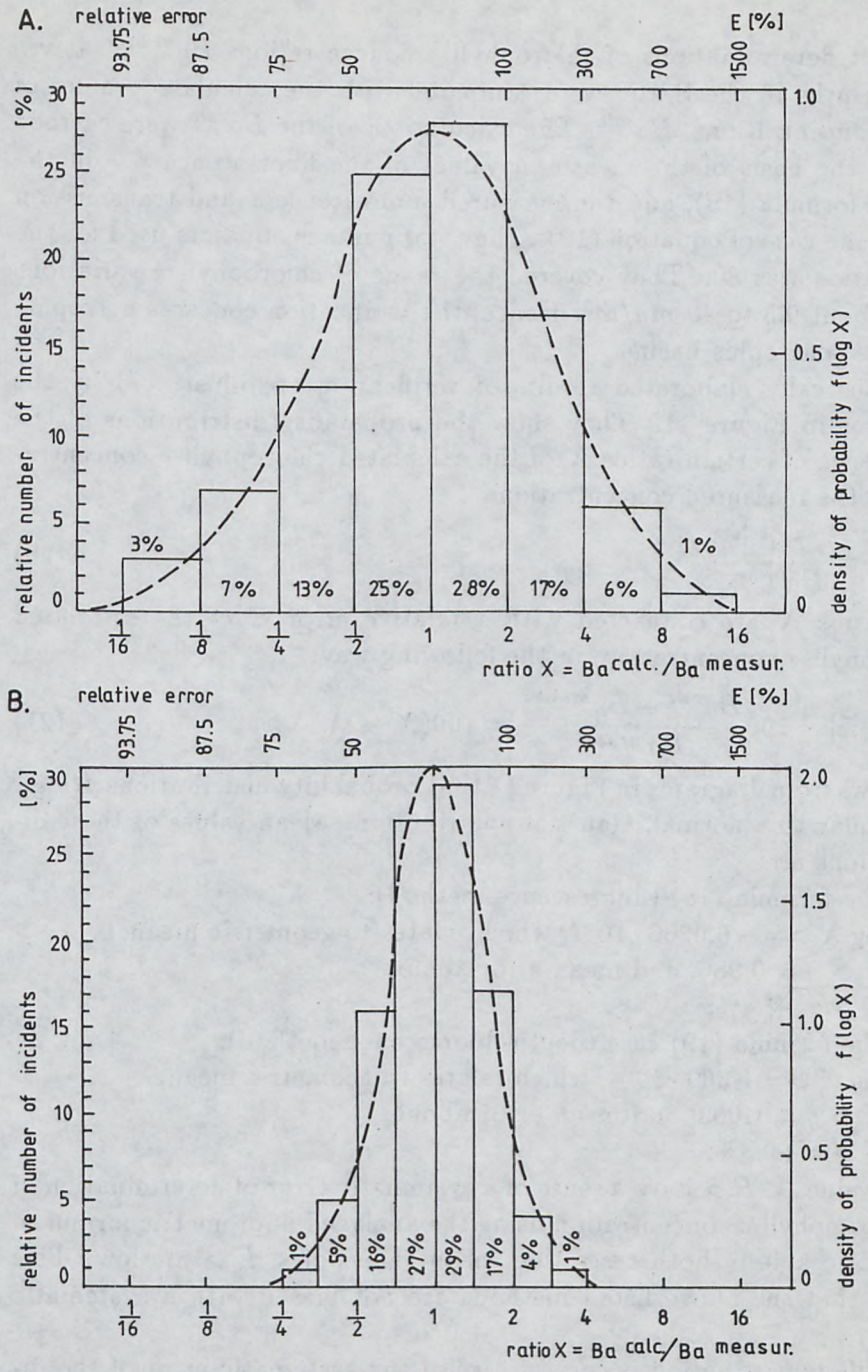


Figure 11: Statistical verification of fluorometric formulas: A – fluorescence method, B – depth-fluorescence method (description in the paper)

deviation $\sigma_{\log X}$ of the distributions $f(\log X)$. The values of these deviations are:

- for fluorescence method:

  $\sigma_{\log X} = \pm 0.4372$, which corresponds to variability range from $Ba^{calc} = 0.36 Ba^{measur}$ to $Ba^{calc} = 2.7 Ba^{measur}$, or to error in the range from $E = -64\%$ to $E = +170\%$;

- for depth-fluorescence method:

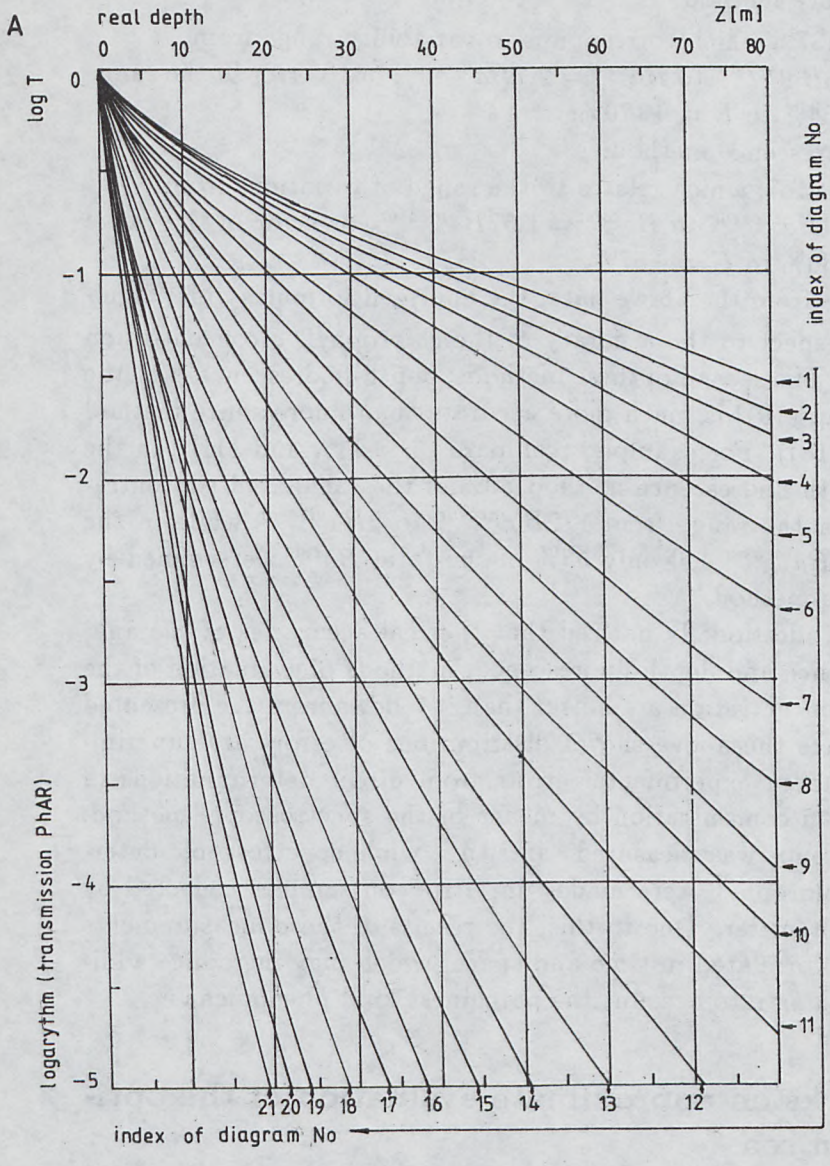
  $\sigma_{\log X} = \pm 0.19567$, which relates to the range of variations from $Ba^{calc} = 0.64 Ba^{measur}$ to $Ba^{calc} = 1.57 Ba^{measur}$, or error in the range from $E = -36\%$ to $E = +57\%$.

As one can see from the above data, the analyzed formulas differ significantly with respect to the accuracy of the chlorophyll *a* concentration determination. The second of these methods, depth-fluorescence (*i.e.* the one based on eq. (19)) is much more accurate than fluorescence method (based on eq. (18)). For example, (compare Figs. 11A and 11B), in the case of the depth-fluorescence method 89% of the calculated concentrations fell within the range from $1/2 Ba^{measur}$ to $2 Ba^{measur}$ while in the same range of Ba^{measur} fell only 53% of the total Ba^{calc} determined by the fluorescence method.

• It should be additionally noticed that the real accuracies of the analyzed (fluorescence and depth-fluorescence) methods of evaluation of the chlorophyll *a* concentration are better than it follows from the presented verification, since the above shown distributions of errors are superimposed with random experimental errors from direct determinations of the chlorophyll *a* concentration by means of the spectroscopic method. Beside, fluorescence was measured "in situ", while spectroscopic determinations of chlorophyll were made "in vitro" on samples collected by means of a bathometer. Due to this, the results of these measurements may be poorly correlated in time and space, which may introduce additional errors taking into account the patchiness kind phenomena.

3.5. Remarks on approximate evaluation of the optical depth in sea

As it follows from the verification presented above, from all the analyzed indirect fluorometric methods of determination of the chlorophyll *a* concentration the depth-fluorometric method, based on formula (19), yields


A
real depth
Z[m]
0 10 20 30 40 50 60 70 80
log T
0
-1
-2
-3
-4
-5
logarythm (transmission PhAR)
index of diagram No
1 2 3 4 5 6 7 8 9 10 11
21 20 19 18 17 16 15 14 13 12
index of diagram No

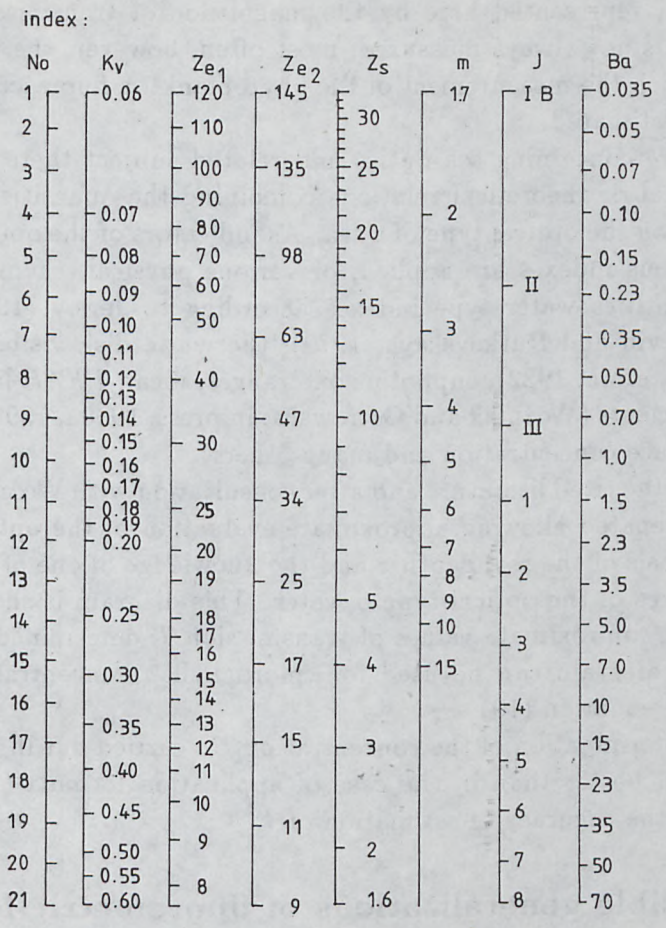


Figure 12: Diagram for approximate determination of $PhAR$ transmission magnitude at a particular depth in sea, on the basis of a known, optical indicator of the type of sea. $K_V[m^{-1}]$ – mean attenuation coefficient of $PhAR$ $(400 - 750\ nm)$ in a $0 - 30\ m$ layer; $Z_{e1}[m]$ – depth of 1% $PhAR$ irradiance; $Z_{e2}[m]$ – depth of 1% monochromatic irradiance in a band of wave-lenght corresponding to min. irradiance attenuation coefficient; $Z_s[m]$ – visibility range for a white disk; m – water type index according to Pelevin; J – water type index according to Jerlov; $Ba[mg\,Chl\,a/m^3]$ – chlorophyll a concentration in the surface layer

Instruction: 1) On the basis of known, arbitrary optical indicator of the sea type, using Figure B, determine the index of the diagram (N_0); 2) On the basis of the determined index N_0, using Figure A, determine $\log T$ for the particular real depth z.

the best results. In order to utilize it, however, it is neccessary to know the optical depth, represented here by the magnitude of transmission T. This quantity is not always measured; most often, however, the real depth z[m], at which the measurement of the phytoplankton fluorescence is carried out, is estimated.

In the literature concerning sea optics and related subject there are numerous empirical or theoretical relations combining the quantities T and z, depending on the optical type of a sea. As indicators of the optical type a basin various indexes are applied, or various physical, chemical or biological quantities water type indexes according to Jerlov (1968) and Pelevin (Pelevin and Rutkovskaya, 1979), the white disk visibility range (Shemshura *et al.*, 1982), euphotic zone ranges, mean $PhAR$ light attenuation coefficients (Woźniak and Ostrowska, in press, 1990a, 1990b), chlorophyll a surface concentration and many others).

On the basis of the cited literature and after consultation with Woźniak a diagram was prepared allowing approximate evaluation of the optical depth T on the basis of the real depth z and the knowledge of one of the above listed indexes of the optical type o water. This diagram is shown in Figure 12. The approximate values of transmission T determined on the basis of this diagram can be used for chlorophyll a concentration calculations using equation (19).

Accuracy of determination of the concentration Ba carried out in this way is most often better than in the case of application formula (18), independently of the accuracy of estimation of T.

3.6. On possible generalizations of fluorometric formulas

The presented in this paper quantitative, statistical results concerning the dependence of phytoplankton fluorescence on the chlorophyll concentration, hence also the derived fluorometric formulas, can be applied only for one type of fluorometer. It is impossible to make direct comparisons of the results obtained from the measurements made by different fluorometers, unless intercalibration is carried out. However, intercalibration can be difficult or even impossible.

There are two reasons for that:

1. differences in spectral characteristic of emission sources and fluorescence light detectors used in the fluorometer,

2. difference in sensitivity and calibration scales of the measured photocurrents.

In the first case, when the devices differ by spectral characteristics, intercalibration is impossible. It is possible, however, when the devices have similar spectral characteristics and differences concern only sensitivities and units of the measured currents. In this case many parameters of empiric correlations between the ϕ_M, ϕ and Ba quantities are preserved. For example, the slopes of the $\log \phi_M = f(logBa)$ (*i.e.* terms a in eq. (7)) dependence, as well as the exponents at chlorophyll concentrations Ba in equations (9), (12), (16) and the exponents for fluorescences in equations (18) and (19) remain constant.

The remaining constants of these empiric relations can be determined by calibration. For instance, the general shape of fluorometric formulas (18) and (19) is:

$$Ba = C_1 \phi^{3.125}, \tag{22}$$

$$Ba = C_2 \exp\left[-0.515 ln\left(\frac{10}{T}\right) \exp(0.4343 lnT)\right] \phi^{3.125}. \tag{23}$$

where: C_1, C_2 – constants which should be determined from one or, in order to increase the accuracy, from many measurements in environments of known chlorophyll concentration.

4. Conclusions

1. Phytoplankton fluorescence in the red region of the light spectrum is caused by chlorophyll *a*, however, this pigment luminates energy absorbed by itself and the energy from other pigments transferred to it. Phytoplankton fluorescence in the red region of spectrum is therefore the induced chlorophyll *a* fluorescence.

2. Phytoplankton fluorescence ϕ increases with the increase of chlorophyll *a* concentration Ba (compare *e.g.* Fig. 3 and eqs. (5) and (9)). However, the experimental relationship $\phi = f(Ba)$ reveals large scatter of experimental points, due to differences in composition of phytoplankton pigments in various biological types of basins and at various depths in sea.

3. Main factor determining the magnitude of phytoplankton fluorescence ϕ_M is the chlorophyll concentration Ba. Generally, the values of ϕ_M decrease with an increase in Ba in the basin (see Fig. 4 and eq. (8)). It is caused by a lower a relative content of additional pigments compared to chlorophyll *a* in photosynthetic apparatus of the phytoplankton in the basins rich in chlorophyll *a* compared to lean basins.

4. A factor influencing the fluorescence ϕ and the specific fluorescence ϕ_M magnitudes is also the optical depth at which the particular phytoplankton occurs. For constant chlorophyll *a* concentrations the magnitudes of ϕ are quite explicitly correlated with transmission function T and they increase with an increase in T (see Figs. 8, 9 and relations (11)–(16)). Specific compositions of pigments also influence such a character of the fluorescence properties.

5. Existence of certain relationships in a set of values of ϕ (or ϕ_M), Ba and T allows to derive fluorometric formulas (see eqs. (17)–(19)) for chlorophyll *a* concentration. These formulas form a basis for indirect methods of determination of the Ba concetration. The paper presents two such methods:

   - fluorescence method, which allows to estimate Ba on the basis of "in situ" phytoplankton fluorescence measurements (see eq. (18)),
   - depth-fluorescence method, which requires additionally the knowledge of the optical depths of the measured fluorescences represented by the magnitude of the transmission T (see eq. (19)), in order to determine Ba.

6. The performed experimental verification (see Fig. 11) showed a significant superiority of the depth-fluorescence method over the fluorescence method for chlorophyll concentration estimation. For example, the width of the standard interval of error of Ba determination is for the fluorescence method $\Delta E = 234\%$ (from -64% to $+170\%$), while for the depth-fluorescence method it drops about 2.2 times and is $\Delta E = 93\%$ (-36% to 57%).

7. Because of significantly lower accurracies of estimations of chlorophyll *a* concentration by means of the fluorescence method compared to the depth-fluorescence method, application of this second method is recommended. Therefore if the fluorescence measurements are not accompanied by optical measurements leading to determination of transmission T, this latter quantity can be approximatelly estimated using the diagram in Figure 11, on the basis of known arbitrary optical indicator of the water type.

To finish with, let us remind the restrictions of the results presented herein. Because of the choice of the experimental material – compare section 2.1., all the quantitative conclusions and the fluorometric formulas are related to cold (2–12^{o}C temperature range), mean and highly productive sea regions (with chlorophyll *a* concentrations in a range 0.2 to 40 mg/m^3).

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