Variability of the specific fluorescence of chlorophyll in the ocean. Part 1. Theory of classical *in situ* chlorophyll fluorometry*

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Abstract

The range of variability of the fluorescence properties of marine phytoplankton in different trophic types of seas and at different depths in the sea is analysed theoretically. An attempt is also made to interpret artificially induced *in situ* fluorescence measured with submersible fluorometers. To do this, earlier optical

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models of light absorption by phytoplankton (see Woźniak *et al.* 2000, this volume) and actual empirical data were applied. A straightforward theoretical model of artificially photoinduced phytoplankton fluorescence accounting for the complex influence of different photophysiological characteristics of phytoplankton and the optical characteristics of the instrument has been worked out. A physical method of determining chlorophyll *a* concentrations in seawater from fluorescence measured *in situ* with contact fluorometers can be based on this model.

1. Introduction

The standard techniques for measuring chlorophyll *a* concentrations in phytoplankton samples taken from the sea using traditional spectrophoto-metry or fluorometry (Lorenzen 1967, Strickland & Parsons 1968, Jeffrey & Humphrey 1975) are expensive, time-consuming and ineffective. Re-searchers have therefore been trying to find a method of determining the chlorophyll *a* concentration from *in situ* fluorescence measurements. These would cover not only fluorescence induced naturally by sunlight (Neville & Gower 1977, Grassl 1986, Babin *et al.* 1996, Ostrowska *et al.* 1997) but also that induced by artificial light sources (see, for example, Lorenzen 1966, Loftus & Seliger 1975, Slovacek & Hannan 1977, Karabashev 1987, Hundahl & Holck 1989, Shavykin & Ryzhov 1989, Ostrowska 1990, Shavykin 1990, Kolber & Falkowski 1993). Measurements of the latter are either contact measurements carried out *in situ* with submersible fluorometers or remote methods using lidars (see Fadeyev *et al.* 1979, Brown 1980, Bristov *et al.* 1981, Demidov *et al.* 1981, 1988, Vedernikov *et al.* 1990). The subject of this paper is the determination of chlorophyll *a* concentration using *in situ* measurements of artificially induced fluorescence.

Phytoplankton fluorescence is due to the emission by chlorophyll *a* of part of the energy, absorbed by all photosynthetic pigments, that the plant cannot utilise in photosynthesis. In line with many previous papers on this matter, we can assume that the phytoplankton *in situ* fluorescence intensity (F_0) is roughly proportional to the chlorophyll *a* concentration (C_a) in the seawater. This assumption leads to a simple method of determining chlorophyll *a* concentration using fluorescence *in situ* measurements:

$$C_a = \text{const } F_0, \tag{1}$$

where

const [arbitrary instrument unit] – constant of the particular submersible fluorometer which depends among other things on the exciting light intensity and the geometry of the instrument.

These methods (based on eq. (1)) or similar simple relationships) and others were applied by *e.g.* Lorenzen (1966), Loftus & Seliger (1975), Slovacek & Hannan (1977), Karabashev (1987), Hundahl & Holck (1989),

Shavykin & Ryzhov (1989), Ostrowska (1990), Shavykin (1990) and Kolber & Falkowski (1993). However, the results of determining the chlorophyll a concentration with this method are inaccurate because the intensity of fluorescence depends not only on the chlorophyll a concentration, but also on that of accessory photosynthetic pigments. The principal factor in this respect is the content of accessory photosynthetic pigments, which act as 'antennas' that absorb light energy and transfer it to chlorophyll a.

In this paper we attempt to establish how environmental factors affect fluorescence and the observed relationships between the intensity of fluorescence and chlorophyll a concentrations. The main aims of the paper are:

(1) To formulate a simple theoretical model of artificially photoinduced phytoplankton fluorescence which takes into account the complex influence of three groups of factors: the chlorophyll a concentration, the photophysiological characteristics of phytoplankton, and the optical characteristics of the instrument used.

(2) To apply this model to work out a physically justified method of determining chlorophyll a concentrations in seawater from *in situ* fluorescence measurements.

A further aim was to find a possible universal method of determining C_a, not just for one particular instrument, the lamps and optical filters of which have specific spectral characteristics, but for any instrument and modifications of it. This would require the development of objective means of calibrating the instruments.

The results of our analyses are presented in parts 1 and 2 of this paper (both in the present volume). This part, part 1, focuses on the first, theoretical aim. The practical objectives are discussed in part 2 (see Ostrowska *et al.* 2000, this volume).

2. Principles applied in the theoretical model of fluorescence

In order to achieve our objectives, we examined the phytoplankton *in situ* fluorescence measured following excitation of the photosynthetic apparatus with weak light pulses. Measuring instruments of two fundamentally different constructions were used for this purpose:

- fluorometers in which *in situ* excitation and measurement take place in the absence of ambient light,

- fluorometers in which *in situ* excitation and measurement take place in the presence of ambient light.

According to the convention proposed by Kolber & Falkowski (1993), the former, F_0', is the *in vivo* fluorescence yield induced by a weak probe

flash in the dark, measured in the ambient light-adapted state. The latter, F', is the *in vivo* fluorescence yield induced by a weak probe flash in the presence of ambient light, measured in the ambient light-adapted state.

Moreover, the latest results concerning adaptation processes and their influence on the light absorption capacities of phytoplankton were applied while the model relationships of fluorescence as a function of environmental and instrumental factors were being worked out (see Majchrowski & Ostrowska 2000, Majchrowski *et al.* 2000, Woźniak *et al.* 2000, this volume).

The chief aim of this section is to establish a formal relationship between the artificially induced fluorescence F'_0, the chlorophyll a concentration C_a, the physiological characteristics of phytoplankton, and the optical characteristics of the particular instrument used. Once it has been tested with actual empirical material, such a relationship could be useful in achieving the second aim, *i.e.* working out a practical method of fluorometrically determining the chlorophyll a concentration.

The power of artificially excited fluorescence per unit volume of water F'_0 depends on numerous factors. Generally speaking, this power is a function of the light energy absorbed by phytoplankton photosynthetic pigments, the efficiency with which this energy is converted into fluorescent light, *i.e.* the fluorescence quantum yield Φ_{fl}, and intercellular reabsorbance of fluorescent light (Mitchell & Kiefer 1988). This power of fluorescence also depends on the spectral characteristics of the exciting light. We can assume that the quantum yield of the chlorophyll fluorescence Φ_{fl} does not depend on the wavelength of light absorbed by the photosynthetic pigments. The expression for F'_0 can thus be given as the product:

$$
F'_0 = \overbrace{\left[C_a \int_{\lambda_{\min}}^{\lambda_{\max}} I(\lambda) a^*_{pl,\,PSP}(\lambda)\, d\lambda \right]}^{\text{absorbed energy}} \Phi_{fl} \underbrace{\int_{\Delta\lambda} Q^*(\lambda) f_{fl}(\lambda)\, d\lambda}_{1\,-\,\text{reabsorbance}}, \tag{2}
$$

where

C_a [mg tot. chl a m^{-3}] – chlorophyll a concentration,

$I(\lambda)$ [Ein m^{-2} nm^{-1} s^{-1}] – spectrum of the exciting light, which depends on the light source used by the instrument,

$\lambda_{\min}$, $\lambda_{\max}$ [nm] – the light wavelengths determining the spectral range of the exciting light,

$a^*_{pl,\,PSP}(\lambda)$ [m^2 (mg tot. chl $a)^{-1}$] – specific absorption coefficient of phytoplankton, photosynthetic, pigments,

Φ_{fl} [dimensionless] – quantum yield of fluorescence,

$Q^*(\lambda)$ [dimensionless] – spectrum of the package effect function (see, for example, van de Hulst 1981, Woźniak $et\ al.$ 1999),

$\Delta\lambda$ – wavelength range of the light emitted,

$f_{fl}(\lambda)$ [nm^{-1}] – relative spectral distribution of the fluorescent light emitted.

Eq. (2) can also be written as follows:

$$F_0' = I_c \langle a^*_{pl,\,PSP}(\lambda) \rangle_{I(\lambda)} \langle Q^*(\lambda) \rangle_{f_{fl}(\lambda)} \Phi_{fl} C_a, \tag{3}$$

where

I_c [Ein m^{-2} s^{-1}] – total intensity of excitation light:

$$I_c = \int\limits_{\lambda_{\min}}^{\lambda_{\max}} I(\lambda) d\lambda, \tag{4}$$

$\langle a^*_{pl,\,PSP}(\lambda) \rangle_{I(\lambda)}$ – mean specific absorption coefficient of photosynthetic phytoplankton pigments averaged with the weight of spectrum of exciting light:

$$\langle a^*_{pl,\,PSP}(\lambda) \rangle_{I(\lambda)} = I_c^{-1} \int\limits_{\lambda_{\min}}^{\lambda_{\max}} a^*_{pl,\,PSP}(\lambda)\, I(\lambda)\, d\lambda, \tag{5}$$

$\langle Q^*(\lambda) \rangle_{f_{fl}(\lambda)}$ – mean package effect function averaged with the weight of the spectrum of the fluorescent light emitted:

$$\langle Q^*(\lambda) \rangle_{f_{fl}(\lambda)} = \left[\int\limits_{\Delta\lambda} f_{fl}(\lambda)\, d\lambda \right]^{-1} \int\limits_{\Delta\lambda} Q^*(\lambda) f_{fl}(\lambda)\, d\lambda. \tag{6}$$

The spectral distribution of the emitted light $f_{fl}(\lambda)$ can be assumed roughly equal to the function describing the spectral distribution of light absorbed by chlorophyll a, $a_a^*(\lambda)$, in the red spectrum range, after the Stokes shift has been accounted for. The function $a_a^*(\lambda)$ can be described as a Gaussian function, (see Table 3 in Woźniak $et\ al.$ 1999, p. 194):

$$a_a^*(\lambda) = a_a^*(\lambda = 675)\, e^{-\frac{1}{2}\left(\frac{\lambda - 675}{\sigma}\right)^2},$$

where the dispersion $\sigma = 8.55$ nm. Therefore, after taking the Stokes shift into consideration, we assumed the following formula for $f_{fl}(\lambda)$:

$$f_{fl}(\lambda) = e^{-\frac{1}{2}\left(\frac{\lambda - 683}{8.55}\right)^2}. \tag{7}$$

Eq. (3) above describes the complex relationship between fluorescence F_0' and three groups of factors:

(1) The chlorophyll a concentration (C_a).

(2) The photophysiological properties of phytoplankton ($a_{pl,\,PSP}^*(\lambda)$, $Q^*(\lambda)$, $f_{fl}(\lambda)$, Φ_{fl}).

(3) The optical characteristics of the instrument ($I(\lambda)$).

The very clear division of expression (3) describing the fluorescence F_0' as a function of three groups of influential factors is of great significance for the solution of the problems under scrutiny here. First of all, it is evident that the measured phytoplankton fluorescence is not a simple function of chlorophyll a concentration, but is complicated by physiological factors and the characteristics of the measuring device. Secondly, eq. (3) enables different versions of instruments to be intercalibrated (see section 4.1).

3. Empirical data and methods

To achieve the aims set out in this paper, a suitable database containing the measured fluorescence, chlorophyll a concentration, light conditions and other factors describing environmental conditions in different seas is required. Our database contains the vertical profiles of the following physical parameters collected by teams from Sopot and Moscow during various cruises to the Baltic Sea, Norwegian Sea, Black Sea, Atlantic Ocean and Indian Ocean, or determined from model calculations:

A. The fluorescence F_0' or F' measured in arbitrary instrument units with three different fluorometers in different seas:

(1) IO PAS Pump Probe fluorometer (F_0') – measurements in the Baltic Sea, Norwegian Sea and Atlantic Ocean by the Sopot team, during r/v 'Oceania' cruises since 1993.

(2) IO PAS submarine 'classic' fluorometer (F') – measurements in the Baltic Sea, Norwegian Sea, Black Sea and Indian Ocean by the Sopot team during r/v 'Oceania' cruises since 1986 and r/v 'Vityaz' in 1988.

(3) Lomonosov University Pump Probe fluorometer (F_0') – measurements in the Black Sea by the Moscow team in August 1989.

In the case of the 'classic' fluorometer, only data from an optical depth τ below 1.5 were considered so as to eliminate the influence of sunlight on the measurement of artificial excited fluorescence. In this case $F'(\tau \geq 1.5)$ is practically the same as $F_0'(\tau \geq 1.5)$.

Moreover, the following parameters were measured along with the fluorescence:

B. Chlorophyll *a* concentration C_a [mg tot. chl *a* m^{-3}] measured with standard methods (Lorenzen 1967, Strickland & Parsons 1968, Jeffrey & Humphrey 1975).

C. Light conditions: spectra of the underwater scalar irradiance $E_0(\lambda, z)$ [Ein m^{-2} nm^{-1} s^{-1}], and the photosynthetically available radiation $PAR_0(z)$ [Ein m^{-2} s^{-1}] measured with techniques described by Woźniak & Montwiłł 1973, Woźniak *et al.* 1983.

D. Temperature *t* [°C] (Siwecki & Kućmierz 1985) and inorganic nitrogen concentration $N_{\mathrm{inorg.}}$ [μM] (the total nitrogen in nitrate, nitrite and ammonia) determined with a standard device (Wood *et al.* 1967, Raimbault *et al.* 1990).

All the parameters were measured at the same depths in the study areas. The number of measurements collected with each fluorometer is given in Table 1. Several optical characteristics of phytoplankton were also taken into consideration, such as the spectra of:

E. The light absorption coefficients of photosynthetic phytoplankton pigments $a^*_{pl, PSP}(\lambda)$.

F. The package effect function $Q^*(\lambda)$.

These last two characteristics were not measured directly but were calculated using the model described by Woźniak *et al.* (2000), this volume.

Table 1. Number of data measured with different types of fluorometers

Parameter	IO PAS Pump Probe fluorometer	IO PAS Submarine 'classical' fluorometer	Lomonosov University Pump Probe fluorometer
$F'_0(F')$, C_a	309	750, (440)*	331
$E_0(\lambda, z)$, $PAR_0(z)$	309	750, (440)*	331
t	203	347	331
$N_{\mathrm{inorg.}}$	203	280	302

* data only from $\tau \geq 1.5$.

4. Results

The analysis takes into consideration the elements of fluorescence theory presented in section 2 and the natural diversity of selected characteristics of phytoplankton photosynthesis (Woźniak *et. al.* 1999, and 2000, this volume, Majchrowski & Ostrowska 2000, this volume). It is additionally based on the

empirical material described in section 3. The separate steps of the analysis are now described.

4.1. Fluorescence quantum yield; intercalibration of instruments

As we are dealing with measurements done with three different fluorometers measuring fluorescence intensity in different arbitrary instrument units, it is not possible without prior intercalibration to combine these three sets of data into a single database and perform the statistical analyses. In order to combine readings from different fluorometers, a constant quantity independent of the environment has to be found, which permits comparison of the empirical data from different fluorometers. The quantum yield of fluorescence is such a quantity. This quantum yield is given by the equation

$$\Phi_{fl} = \frac{F_0'^*}{I_c \langle a_{pl,PSP}^*(\lambda)\rangle_{I(\lambda)} \langle Q^*(\lambda)\rangle_{f_{fl}}}, \tag{8}$$

where $F_0'^* = F_0'/C_a$.

To obtain the absolute value of the quantum yield of fluorescence requires I_c to be determined in absolute units, which is usually difficult. We therefore use the quantum yield of fluorescence expressed in arbitrary instrument units:

$$\Phi_{fl}\,[\text{arbitrary instrument units}] = \frac{F_0'^*}{\langle a_{pl,PSP}^*(\lambda)\rangle_{I(\lambda)} \langle Q^*(\lambda)\rangle_{f_{fl}}}, \tag{9}$$

where $[\text{arbitrary instrument units}] \equiv [I_c^{-1}]$.

First, the fluorescence quantum yields were determined in arbitrary instrument units for the three fluorometers from measured F_0', C_a, and $a_{pl,PSP}^*(\lambda)$, $Q^*(\lambda)$ calculated from the model. They were then compared with different environmental factors, in particular:

- the chlorophyll a concentration (C_a),

- the nitrogen concentration ($N_{\text{inorg.}}$),

- the temperature in the sea (t),

- the optical depth in the sea, determined from:

$$\tau = \ln[PAR_0\,(0^+)/PAR_0\,(z)].$$

Figures 1, 2, 3 and 4 show averaged relationships between the fluorescence quantum yield and the above-mentioned environmental factors determined for particular fluorometers. Thus, we can make the rough assumption that there is no clear relationship between the quantum yield of fluorescence and these environmental factors. From this we can draw the important conclusion that the quantum yield of chlorophyll fluorescence
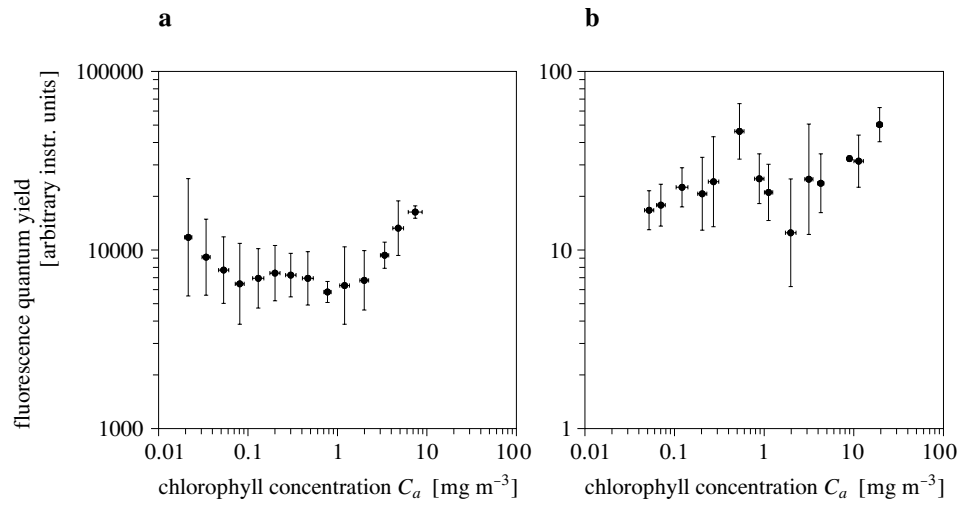
a

b

Fig. 1. Quantum yield of fluorescence Φ_{fl} as a function of chlorophyll a C_a concentration for: Lomonosov University – Pump Probe fluorometer, measurements by the Russian team (Black Sea) (a); IO PAS Pump Probe fluorometer, measurements by the Polish team (Baltic Sea, Norwegian Sea, Atlantic Ocean) (b)

a

b
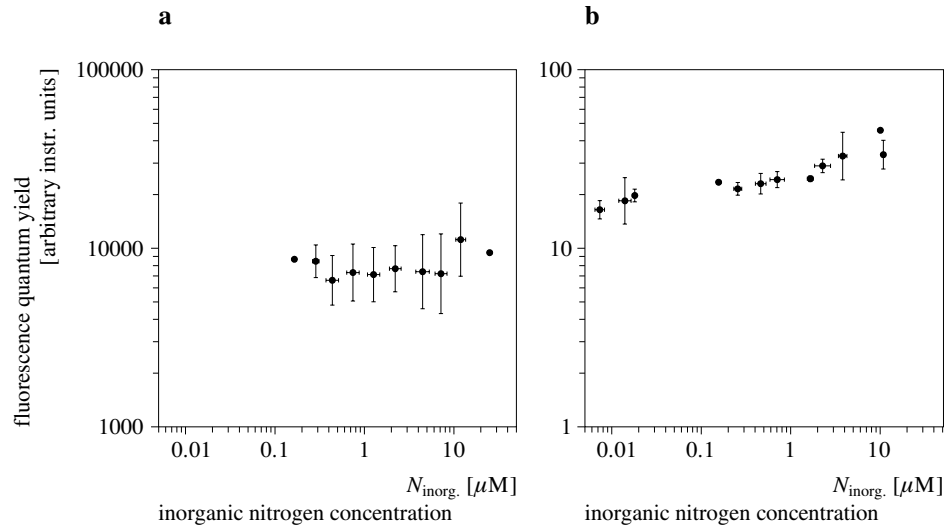
Fig. 2. Quantum yield of fluorescence Φ_{fl} as a function of inorganic nitrogen concentration $N_{inorg.}$ for: Lomonosov University – Pump Probe fluorometer, measurements by the Russian team (Black Sea) (a); IO PAS Pump Probe fluorometer, measurements by the Polish team (Baltic Sea, Norwegian Sea, Atlantic Ocean) (b)

a   **b**
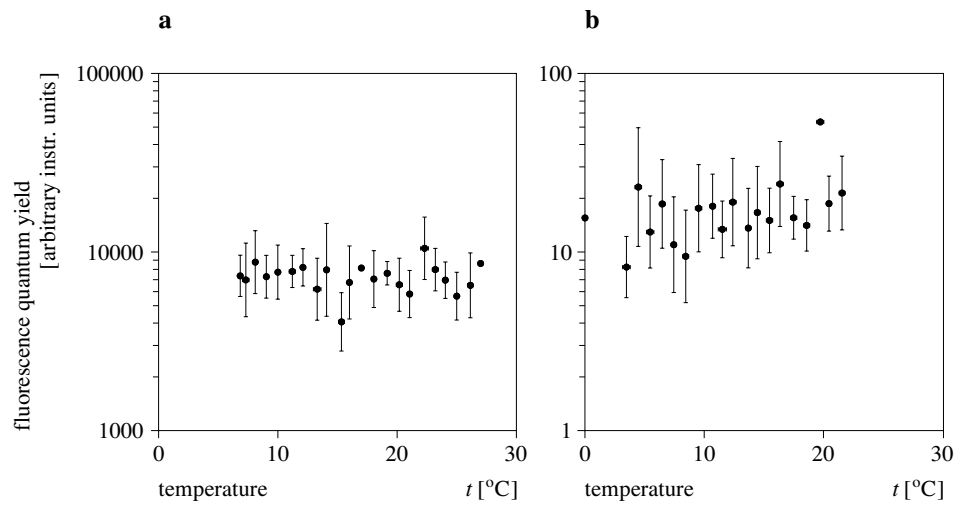
Fig. 3. Quantum yield of fluorescence Φ_{fl} as a function of temperature t for: Lomonosov University – Pump Probe fluorometer, measurements by the Russian team (Black Sea) (a); IO PAS Submarine 'classic' fluorometer, measurements by the Polish team (Baltic Sea, Norwegian Sea, Black Sea, Indian Ocean) (b)

a   **b**

Fig. 4. Quantum yield of fluorescence Φ_{fl} as a function of optical depth τ in the sea for: Lomonosov University – Pump Probe fluorometer, measurements by the Russian team (Black Sea) (a); IO PAS Pump Probe fluorometer, measurements by the Polish team (Baltic Sea, Norwegian Sea, Atlantic Ocean) (b)
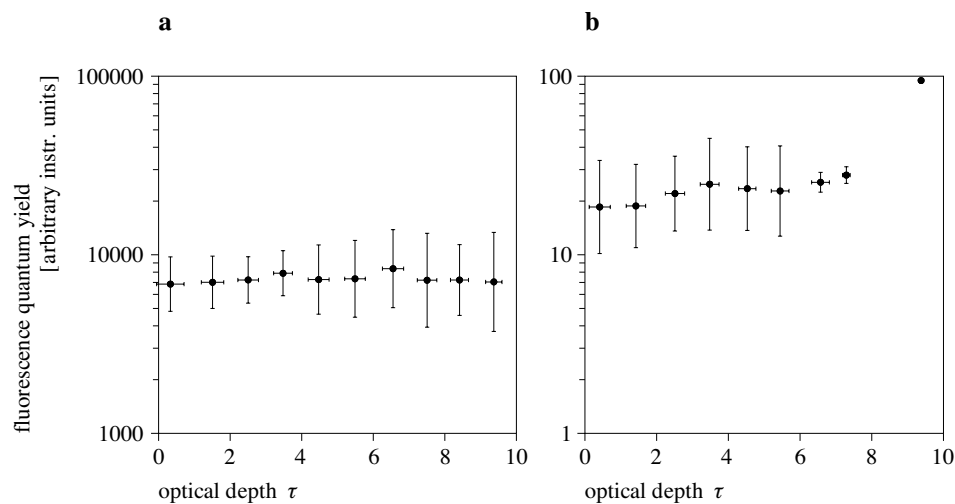
in vivo at sea is constant for exciting light over a relatively low intensity range, such as are used in fluorometers in practice. This significance stems from two facts:

- it can be used as a basis for intercalibrating different instruments,

- it enables the analysis of fluorescence properties, such as the specific fluorescence under the diverse environmental conditions existing in the World Ocean (see section 4.2).

We can carry out the intercalibration between the i–th and j–th fluorometers using the following relationship:

$$F'_{0\,i-\text{th fluor.}} = Calibr\ F'_{0\,j-\text{th fluor.}},\tag{10}$$

where $F'_{0\,i-\text{th fluor.}}$ and $F'_{0\,j-\text{th fluor.}}$ are determined for the same samples of water.

The coefficient *Calibr* is the ratio of the relevant quantum yields measured by the fluorometers

$$Calibr = \frac{\Phi_{fl\,i-\text{th fluor.}}}{\Phi_{fl\,j-\text{th fluor.}}} \frac{[\text{arbitrary instrument units for } i-\text{th fluor.}]}{[\text{arbitrary instrument units for } j-\text{th fluor.}]}\tag{10b}$$

The values of *Calibr* can be determined using arbitrary, uncorrelated readings from these three fluorometers.

In this work intercalibration was carried out with reference to the Moscow group's data. After this operation we obtained 1080 sets of data covering C_a, τ, and the fluorescence F'_0 in units of the Moscow group's fluorometer. These data were used in part 2 (see Ostrowska *et al.* 2000, this volume) to verify the methods of determining chlorophyll a concentrations.

4.2. The natural variability of the specific fluorescence of chlorophyll

The fact that the quantum yield of fluorescence is independent of environmental factors allows the relative range of natural variability of the specific fluorescence of chlorophyll in the oceans to be determined. Assuming $\Phi_{fl} = \text{const}$, eq. (3) can be rewritten to give an expression for the specific fluorescence ($F'^*_0 = F'_0/C_a$):

$$F'^*_0[\text{arbitrary units}] = \langle a^*_{pl,\,PSP}(\lambda)\rangle_{I(\lambda)}\langle Q^*(\lambda)\rangle_{f_{fl}(\lambda)},\tag{11}$$

where $[\text{arbitrary units}] \equiv [\text{m}^2\ (\text{mg tot. chl}\,a)^{-1}\ [I_c]\ [\Phi_{fl}]]$.

The characteristics of the specific fluorescence F'^*_0 for different trophic types of seas and for various depths can be determined using our model of absorption properties of phytoplankton (see Woźniak *et al.* 2000, this volume), which, among other things, enables the spectra of $a^*_{pl,\,PSP}(\lambda)$ and $Q^*(\lambda)$ to be determined. In addition, it is assumed that the spectra of the

light exciting fluorescence ($I(\lambda)/I_c$) have a certain fixed shape. Nevertheless, the spectra are similar for all the fluorometers actually used.

The vertical profiles of the specific fluorescence $F_0'^*(\tau)$ or $F_0'^*(z)$ for different trophic types of sea can be determined from the model of phytoplankton light absorption and eq. (11) (Fig. 5).

As one can see in this figure, the specific fluorescence generally falls with increasing water trophicity (we assume the surface chlorophyll a concentration, $C_a(0)$ to be the trophicity). The specific fluorescence also tends to increase with depth, especially in waters of low trophicity. Such behaviour is similar to that of the mean absorption coefficients of phytoplankton photosynthetic pigments (see Fig. 5b in Majchrowski *et al.* 2000, this volume, p. 198). However, the range of variability of the specific fluorescence recorded under natural conditions (about 50 times) is greater than that of the specific absorption coefficient (< 20 times). As eq. (11) clearly indicates, this is because the specific fluorescence depends not only on the specific absorption but also on the mean package effect function. This latter factor decreases with increasing chlorophyll a concentration and in different types of seas varies by about one order of magnitude.
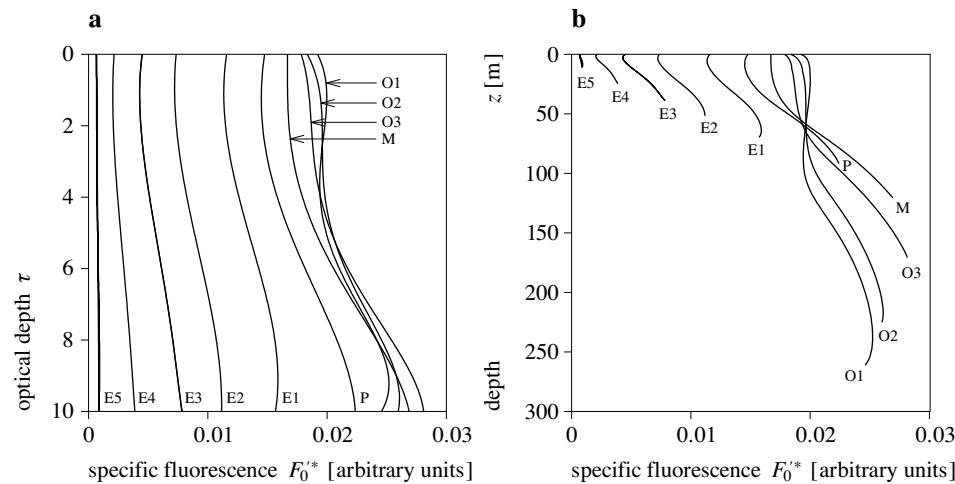
Fig. 5. Model vertical profiles of specific fluorescence $F_0'^*$ for optical depth τ (a) and for real depth z (b), determined for different trophic types of sea. The symbols of trophic types correspond to the surface chlorophyll a concentration $C_a(0)$: O1 – $C_a(0) = 0.035$ mg tot. chl a m^{-3}; O2 – $C_a(0) = 0.07$ mg tot. chl a m^{-3}; O3 – $C_a(0) = 0.15$ mg tot. chl a m^{-3}; M – $C_a(0) = 0.35$ mg tot. chl a m^{-3}; P – $C_a(0) = 0.7$ mg tot. chl a m^{-3}; E1 – $C_a(0) = 1.5$ mg tot. chl a m^{-3}; E2 – $C_a(0) = 3.5$ mg tot. chl a m^{-3}; E3 – $C_a(0) = 7$ mg tot. chl a m^{-3}; E4 – $C_a(0) = 15$ mg tot. chl a m^{-3}; E5 – $C_a(0) = 35$ mg tot. chl a m^{-3}

The relationships presented in Fig. 5 were obtained by performing the relevant calculations from eq. (11) and using the whole model mathematical apparatus (see Table 1 in Woźniak et $al.$ 2000, this volume, pp. 182–188) of phytoplankton optical properties $\langle a^*_{pl,\,PSP}(\lambda)\rangle_{I(\lambda)}$ and $\langle Q^*(\lambda)\rangle_{f_{fl}(\lambda)}$. The model calculations are very time-consuming. That is why for practical purposes we have worked out simplified polynomial approximations of the model results. This allows both factors occurring in eq. (11) to be estimated from the two following polynomial relationships between the $\langle a^*_{pl,\,PSP}(\lambda)\rangle_{I(\lambda)}$ and $\langle Q^*(\lambda)\rangle_{f_{fl}(\lambda)}$, trophic index of the sea $C_a(0)$ and optical depth τ in the sea:

$$\langle a^*_{pl,\,PSP}(\lambda)\rangle_{I(\lambda)} = \sum_{m=0}^{4}\left[\sum_{n=0}^{4} A_{m,\,n}(\log C_a(0))^n\right]\tau^m, \tag{12}$$

$$\langle Q^*(\lambda)\rangle_{f_{fl}(\lambda)} = \sum_{m=0}^{4}\left[\sum_{n=0}^{4} B_{m,\,n}(\log C_a(0))^n\right]\tau^m, \tag{13}$$

where the coefficients $A_{m,\,n}$ and $B_{m,\,n}$ of these polynomials are given in Tables 2 and 3. The estimated errors of these approximations do not exceed 3% of the values of $\langle a^*_{pl,\,PSP}(\lambda)\rangle$ and $\langle Q^*(\lambda)\rangle$ determined from the unabridged version of the model by Woźniak et $al.$ (2000) in this volume.

Formulae (12) and (13) to a significant extent simplify the determination of the chlorophyll a concentration using eq. (11). This fluorometric method of determining the chlorophyll a concentration is described in part 2 of this paper by Ostrowska et $al.$ (2000, this volume).

Table 2. Values of coefficient $A_{m,\,n}$ in eq. (12)

a) for $0.035 < C_a(0) < 1.5$

n / m	0	1	2	3	4
0	1.566×10^{-2}	-3.258×10^{-4}	1.840×10^{-4}	5.949×10^{-7}	-9.084×10^{-7}
1	-7.158×10^{-3}	-7.724×10^{-5}	3.924×10^{-5}	-2.930×10^{-5}	1.368×10^{-6}
2	-4.709×10^{-3}	1.912×10^{-3}	-6.868×10^{-4}	7.373×10^{-5}	-2.307×10^{-6}
3	4.181×10^{-4}	-1.604×10^{-4}	-2.463×10^{-5}	3.054×10^{-5}	-7.392×10^{-7}
4	1.068×10^{-3}	-7.220×10^{-4}	1.418×10^{-4}	4.622×10^{-6}	-3.326×10^{-7}

b) for $1.5 \leq C_a(0) < 70$

n / m	0	1	2	3	4
0	1.560×10^{-2}	-1.390×10^{-4}	1.075×10^{-4}	1.023×10^{-5}	-1.339×10^{-6}
1	-8.437×10^{-3}	1.933×10^{-3}	-8.726×10^{-4}	1.018×10^{-4}	-4.436×10^{-6}
2	-2.255×10^{-3}	-5.655×10^{-3}	2.611×10^{-3}	-3.748×10^{-4}	1.888×10^{-5}
3	1.849×10^{-3}	4.874×10^{-3}	-2.313×10^{-3}	3.424×10^{-4}	-1.753×10^{-5}
4	-2.572×10^{-4}	-1.276×10^{-3}	6.163×10^{-4}	-9.264×10^{-5}	4.781×10^{-6}

Table 3. Values of coefficient $B_{m,n}$ in eq. (13)

a) for $0.035 < C_a(0) < 1.5$

n/m	0	1	2	3	4
0	8.551×10^{-1}	-2.210×10^{-1}	-1.408×10^{-1}	-2.474×10^{-2}	5.512×10^{-3}
1	-1.441×10^{-2}	-1.289×10^{-2}	2.299×10^{-2}	-4.666×10^{-4}	-7.357×10^{-3}
2	3.927×10^{-3}	1.654×10^{-2}	-8.461×10^{-3}	-1.311×10^{-2}	3.961×10^{-3}
3	-2.847×10^{-4}	-2.761×10^{-3}	1.289×10^{-3}	2.754×10^{-3}	1.028×10^{-3}
4	4.384×10^{-6}	1.287×10^{-4}	-6.064×10^{-5}	-1.412×10^{-4}	-5.566×10^{-5}

b) for $1.5 \leq C_a(0) < 70$

n/m	0	1	2	3	4
0	8.494×10^{-1}	-2.455×10^{-1}	-2.961×10^{-2}	-1.322×10^{-1}	6.079×10^{-2}
1	5.923×10^{-4}	4.581×10^{-2}	-3.032×10^{-1}	3.154×10^{-1}	-8.937×10^{-2}
2	-2.895×10^{-3}	-1.325×10^{-2}	1.366×10^{-1}	-1.474×10^{-1}	4.219×10^{-2}
3	7.174×10^{-4}	1.773×10^{-3}	-1.885×10^{-2}	2.044×10^{-2}	-5.901×10^{-3}
4	-4.543×10^{-5}	-9.290×10^{-5}	8.950×10^{-4}	-9.613×10^{-4}	2.776×10^{-4}

5. Summary and conclusions

In this work the range of variability of phytoplankton fluorescence properties in different trophic types of water and at different depths in the sea have been analysed theoretically. We have also attempted to interpret the *in vivo* measurements of artificially induced fluorescence carried out with submersible fluorometers.

The most important achievement of this work has been to produce a simple theoretical model of fluorescence excited with artificial light that takes into account the complex influence of three groups of factors on this phenomenon: chlorophyll *a* concentrations, the various photophysiological characteristics of phytoplankton and the optical characteristics of the measuring instrument.

With this model the range of variability of the specific fluorescence $F_0'^*$ under natural conditions in the world's oceans have been characterised. The specific fluorescence varies over two orders of magnitude. Its values are lowest in eutrophic waters and increase with decreasing water trophicity. The specific fluorescence also varies with depth, usually increasing. These tendencies are characteristic, especially in oligotrophic waters.

The model of fluorescence presented here is a physically justified method determining chlorophyll *a* in seawater using fluorescence measured with contact fluorometers *in situ*. The application of this method is described in part 2 (Ostrowska *et al.* 2000, this volume).

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