Variability of the specific fluorescence of chlorophyll in the ocean. Part 2. Fluorometric method of chlorophyll a determination*

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Abstract

Two methods of determining the chlorophyll a concentration in the sea have been formulated on the basis of artificially induced fluorescence measured with the aid of submersible fluorometers. The method of statistical correlation is founded on the empirical relationship between fluorescence and chlorophyll concentration. The theoretical model of fluorescence described in Part 1 of this paper (see Ostrowska *et al.* 2000, this volume) provides the basis of the other method, the physical method. This describes the dependence of the specific fluorescence of phytoplankton on the chlorophyll concentration, a diversity of photophysiological properties of phytoplankton and the optical characteristics of the fluorometer.

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In order to assess their practicability, the methods were subjected to empirical verification. This showed that the physical method yielded chlorophyll concentrations of far greater accuracy. The respective error factors of the estimated chlorophyll concentration were $x = 2.07$ for the correlation method and $x = 1.5$ for the physical method. This means that the statistical logarithmic error varies from -52 to $+107\%$ in the case of the former method but only from -33 to $+51\%$ in the case of the latter. Thus, modifying the methodology has much improved the accuracy of chlorophyll determinations.

1. Introduction

Part 1 of this paper (Ostrowska *et al.* 2000, this volume) described a theoretical model of artificially photoinduced phytoplankton fluorescence that takes into consideration the complex influence of three groups of factors on this phenomenon: the chlorophyll *a* concentration C_a, physiological characteristics of phytoplankton and the optical characteristics of the fluorometer. The range of variability of the specific fluorescence $F_0'^*$ with respect to seawater trophicity and depth were determined using this model. The practical significance of this is that when determining chlorophyll *a* concentrations from fluorescence measurements, one should bear such relationships in mind, since the measured fluorescence F_0' and chlorophyll *a* concentration are variously related in different trophic types of sea and at different depths. The objectives of Part 2 of this paper are thus:

(1) To derive a method of computing chlorophyll *a* concentrations from fluorescence measurements that accounts for the above-mentioned variability in specific fluorescence.

(2) To compare the accuracy of chlorophyll *a* determinations obtained with this method and with another that ignores the variability in specific fluorescence.

To achieve these objectives, two possible ways of determining chlorophyll *a* are formulated and verified on the basis of *in situ* phytoplankton fluorescence measured by means of submersible fluorometers. They are:

- the method of statistical correlation, which is based on simple statistical relationships between the chlorophyll *a* concentration C_a and the measured fluorescence F_0',

- the physical method, the foundation of which is the theoretical fluorescence model described in Part 1 (see Ostrowska *et al.* 2000, this volume).

For the empirical verification of these methods we used the database described in Part 1 (see section 3 in Ostrowska *et al.* 2000, this volume).

2. Method of statistical correlation

There is already sufficient evidence forthcoming that the principal factor influencing the intensity of artificially induced fluorescence in the sea is the concentration of chlorophyll a C_a (*e.g.* Karabashev 1987, Ostrowska 1990, Kolber & Falkowski 1993). The relationship between fluorescence and C_a with respect to our database is presented in Fig. 1a. As one can see, the measured fluorescence F_0' usually increases when C_a does so. However, the increase in fluorescence is not as striking as that of C_a. In our empirical data (Fig. 1a) the fluorescence varies over a range of about two orders of magnitude, whereas C_a varies over almost four orders. This becomes clear from a perusal of Fig. 1b, which illustrates the dependence of the slope F_0'/C_a *i.e.* the specific fluorescence $F_0'^*$, on C_a.
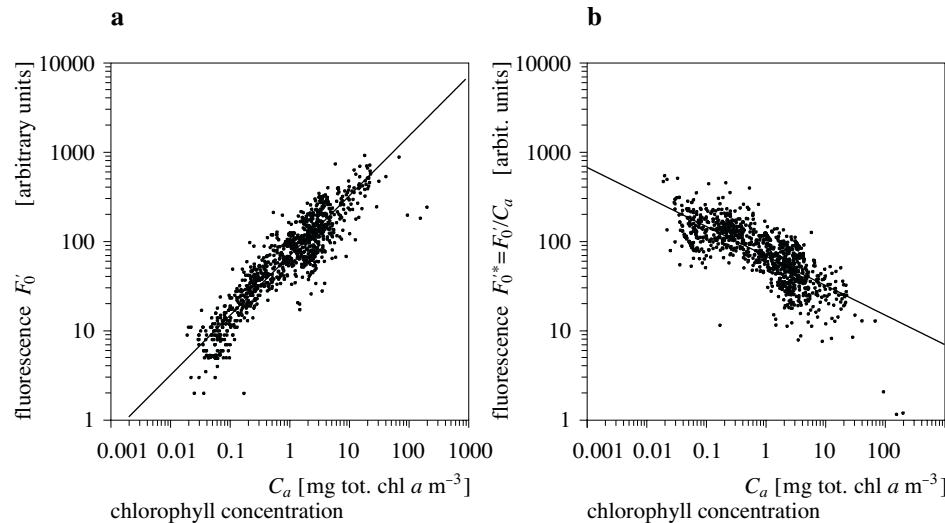

Fig. 1. The relationships between the measured fluorescence F_0' and chlorophyll a concentration C_a in the sea; the points correspond to measured data, the line corresponds to a regression curve according to the equation:
$\log F_0' = 0.6697 \log C_a + 1.8429$ (a), and the specific fluorescence $F_0'^*$ and chlorophyll a concentration C_a in the sea; the points correspond to measured data, the line corresponds to a regression curve according to the equation:
$F_0'^* = 69.65\, C_a^{-0.3303}$ (b)

It can be seen that with increasing water trophicity this specific fluorescence $F_0'^*$ decreases significantly in value. It is characteristic of these relationships that the experimental points are widely scattered. Nevertheless, using the least squares method, we can find relationships connecting

- the fluorescence F_0' with the chlorophyll a concentration C_a:

$$\log F_0' = 0.6697 \log C_a + 1.8429, \text{ or} \tag{1}$$

- the specific fluorescence $F_0'^*$ with the chlorophyll a concentration C_a:

$$F_0'^* = 69.65 \, C_a^{\,(0.6697-1)} = 69.65 \, C_a^{-0.3303}, \tag{1a}$$

where

C_a [mg tot. chl a m^{-3}] – chlorophyll a concentration (tot. chl a or chl a + pheo).

The correlation coefficient for these relationships $r = 0.84$.

By transforming eq. (1) we obtain a formula for the dependence of C_a on F_0':

$$C_a = 10^{\,[1.4932(\log F_0' - 1.8429)]}. \tag{2}$$

Formula (2) is the basis of the statistical correlation method for determining the chlorophyll a concentration from fluorescence measurements.

3. Physical method

In using the theoretical model of the phytoplankton fluorescence described in Part 1 (Ostrowska *et al.* 2000, this volume), we assume that the measured fluorescence is directly proportional to the theoretical value (given in Part 1 by eq. (11)). This can be written thus:

$$F_0'\,_{\text{measured}} \, [\text{arbitrary instrument units}] = \text{const} \, F_0'\,_{\text{theor.}} \, [\text{arbitrary units}] \tag{3}$$

where

$$F_0'\,_{\text{theor.}} = F_0'^*{}_{\text{theor.}} \, C_a \tag{3b}$$

and

$$F_0'^*{}_{\text{theor.}} = \langle a_{pl,\,PSP}^*(\lambda) \rangle_{I(\lambda)} \langle Q^*(\lambda) \rangle_{f_{fl}(\lambda)}, \tag{3a}$$

where

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

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

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

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

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

$Q^*(\lambda)$ – spectrum of the package effect function,

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

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

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

The value of const in eq. (3) depends on the properties of the fluorometer employed. In our experiments using the least squares method of approximating the relevant observed and theoretical fluorescences (see Fig. 2) it was found to be const $= 10^{3.84}$.
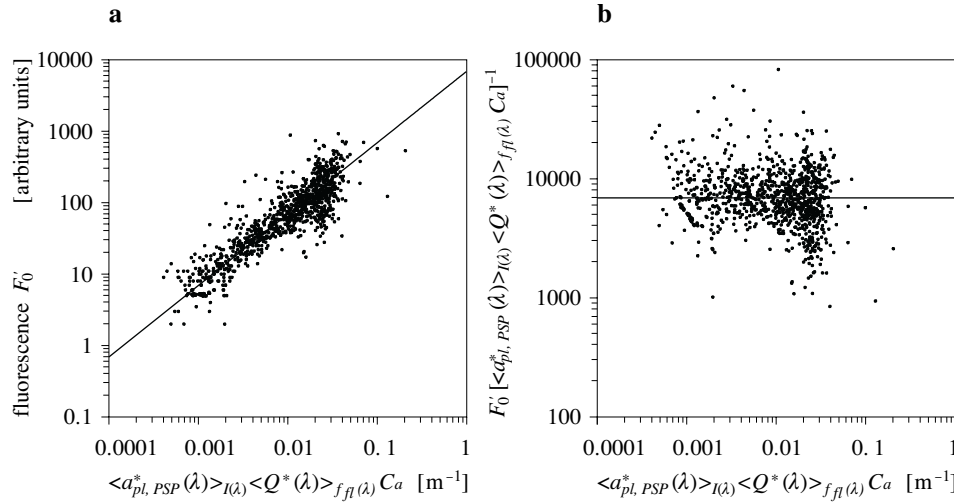

Fig. 2. The relationships between the measured fluorescence $F'_{0\text{ meas.}}$ and the theoretical fluorescence $F'_{0\text{ theor.}}$; the points correspond to measurements, the line corresponds to a regression curve according to the equation:

$\log F'_{0\text{ meas.}} = \langle a^*_{pl,\,PSP}(\lambda)\rangle_{I(\lambda)}\langle Q^*(\lambda)\rangle_{f_{fl}(\lambda)}\,C_a + 3.84$ (a), and the ratio of measured and theoretical fluorescence $F'_{0\text{ meas.}}/F'_{0\text{ theor.}}$ as a function of the theoretical fluorescence $F'_{0\text{ theor.}}$; the points correspond to measurements, the line corresponds to a regression curve according to the equation:

$10^{3.84} = \dfrac{F'_{0\text{ meas.}}}{C_a\,\langle a^*_{pl,\,PSP}(\lambda)\rangle_{I(\lambda)}\langle Q^*(\lambda)\rangle_{f_{fl}(\lambda)}}$ (b)

Hence, in our experiments, the relationships between the measured and theoretical (modelled) fluorescence take the form

$$F'_{0\text{ meas.}} = 10^{3.84}\,\underbrace{\langle a^*_{pl,\,PSP}(\lambda)\rangle_{I(\lambda)}\langle Q^*(\lambda)\rangle_{f_{fl}(\lambda)}\,C_a}_{F'_{0\text{ theor.}}},$$

and the formula describing the chlorophyll a concentration as a function of fluorescence is

$$C_a = \frac{F'_{0\,\text{meas.}}}{10^{3.84} \langle a^*_{pl,\,PSP}(\lambda) \rangle_{I(\lambda)} \langle Q^*(\lambda) \rangle_{f_{fl}(\lambda)}} .$$ (4)

Formula (4) is the foundation of the physical method of estimating the chlorophyll a concentration C_a. However, we also need to know the specific absorption of photosynthetic pigments $\langle a^*_{pl,\,PSP}(\lambda) \rangle_{I(\lambda)}$ and the package effect function $\langle Q^*(\lambda) \rangle_{f_{fl}(\lambda)}$ in phytoplankton cells. These quantities can be determined from a known or given optical depth τ and surface chlorophyll concentration $C_a(0)$ in the sea with the aid of the simplified polynomial formulae given in Part 1 (Ostrowska *et al.* 2000, this volume):

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

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

where the coefficients $A_{m,n}$ and $B_{m,n}$ of these polynomials are given in Tables 2 and 3 in Ostrowska *et al.* 2000, this volume, pp. 215, 216.

The optical depths τ in our experiments were measured simultaneously with fluorescence. But values of $C_a(0)$ are not known. Nevertheless, the latter can be estimated from the real and optical depth known for each profile using Woźniak's bio-optical classification of waters (Woźniak *et. al* 1992a and b).

Table 1. Values of $C_{m,n}$ in eq. (5)

a) for $0 < \tau < 1$

n/m	0	1	2	3	4
0	1.42008	−1.56264	−1.24215	8.6257	−12.3604
1	0.325625	0.168647	−14.5657	35.5867	−20.6688
2	−1.15793	2.95813	−33.1223	51.2958	−17.4024
3	1.7538	5.5338	−28.351	25.3579	−3.3401
4	0.876821	2.97397	−7.91305	1.82888	−0.282705

b) for $1 \le \tau < 10$

n/m	0	1	2	3	4
0	1.43248	−1.61865	−3.32293	−3.19523	8.29916
1	0.243892	4.74305	9.13519	−8.5247	−18.9029
2	−1.69258	−9.14493	−2.22602	22.195	10.0107
3	3.47131	1.19548	−3.23597	−9.73787	−2.03738
4	−1.95027	4.54015	−4.83875	4.95673	−0.773145

From this classification we can establish the relationship between the trophic index of the sea (which we assume to be $C_a(0)$), the optical depth τ and the real depth z. For the purpose of the present work, this relationship is described by the following approximate polynomial:

$$\log C_a(0) = \sum_{m=0}^{4} \left[\sum_{n=0}^{4} C_{m,n} (\log z)^n \right] \log \left(\left(\frac{z}{\tau} \right)^m \right). \tag{5}$$

The coefficients of this polynomial are set out in Table 1.

4. Empirical verification of methods

In order to assess their practicability, the two methods of determining the chlorophyll a concentration were verified empirically, that is to say, the respective measured data of $C_{a,M}$ are compared with those calculated from eq. (2) (statistical correlation method) or eqs. (4) and (5) (physical method).

Table 2. The relative errors in estimating chlorophyll a concentrations at different depths in the sea using the statistical correlation method (eq. (2)) and the physical method (eqs. (4) and (5))

	Arithmetic statistics		Logarithmic statistics			
	systematic	statistical	systematic	standard error factor	statistical	
	$\langle \varepsilon \rangle$ [%]	σ_ε [%]	$\langle \varepsilon \rangle_{\mathrm{g}}$ [%]	x	σ_- [%]	σ_+ [%]
method of statistical correlations	30.9	$\pm$ 89.8	3.6	2.07	−51.7	107
physical method	16.3	$\pm$ 5.0	1.5	1.5	−33.3	50.8

where

$\varepsilon = (C_{a,C} - C_{a,M})/C_{a,M}$ – errors,

$\langle \varepsilon \rangle$ – arithmetic mean of errors,

σ_ε – standard deviation of errors (statistical error),

$\langle \varepsilon \rangle_{\mathrm{g}} = 10^{[\langle \log(C_{a,C}/C_{a,M}) \rangle]} - 1$ – logarithmic mean of errors,

$\langle \log (C_{a,C}/C_{a,M}) \rangle$ – mean of $\log (C_{a,C}/C_{a,M})$, $\langle \log (C_{a,C}/C_{a,M}) \rangle$,

$\sigma_{\log}$ – standard deviation of $\log (C_{a,C}/C_{a,M})$,

$x = 10^{\sigma_{\log}}$ – standard error factor,

$\sigma_- = \frac{1}{x} - 1$ and
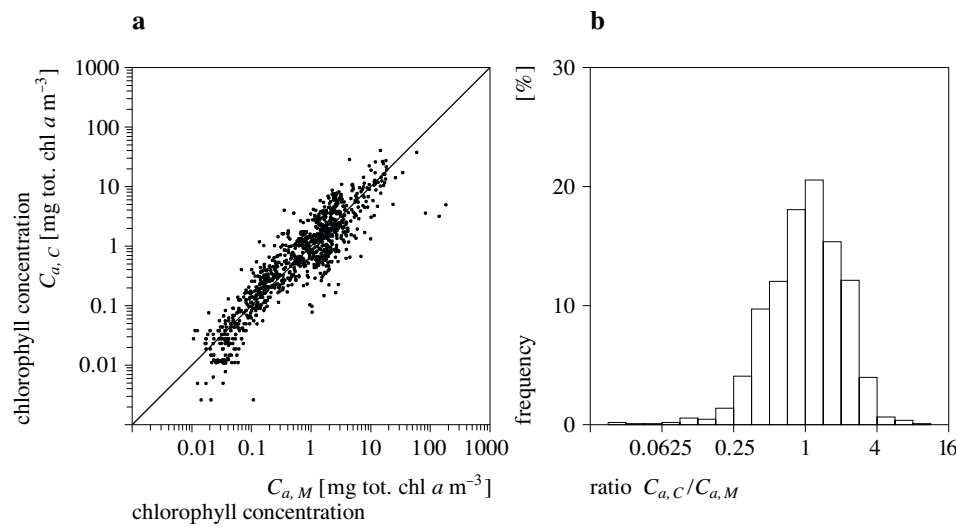
$\sigma_+ = x - 1$.

a

b

Fig. 3. Comparison between measured $C_{a,M}$ and calculated $C_{a,C}$ chlorophyll a concentrations using the method of statistical correlation (eq. (2)); comparison of calculated and measured chlorophyll a (a), probability density distribution of the ratio of calculated to measured chlorophyll a (b)
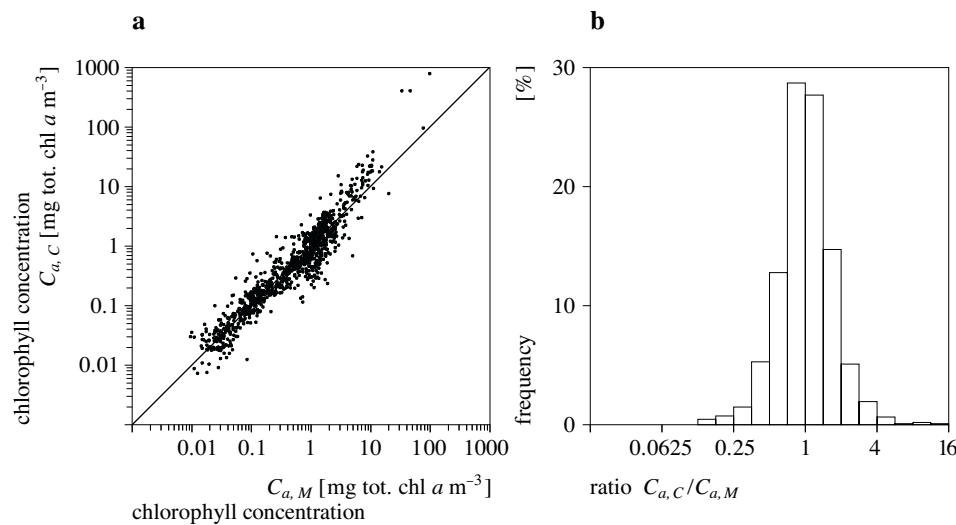
a

b

Fig. 4. Comparison between measured $C_{a,M}$ and calculated $C_{a,C}$ chlorophyll a concentration using the physical method (eq. (4) and (5)); comparison of calculated and measured chlorophyll a (a), probability density distribution of the ratio of calculated to measured chlorophyll a (b)

In the case of the statistical correlation method, actual values of $C_{a,C}$ were calculated from measurements of F_0'. In the case of the physical method, values of depths τ and z were used in addition. The results of the verifications are given in Figs. 3 and 4, and the errors of estimations are given in Table 2.

5. Conclusion

Clearly, the physical method yields significantly more accurate chlorophyll a concentrations than does the method of statistical correlation. The error factor of the estimated chlorophyll concentration $x = 1.5$ for the former method but $x = 2.07$ for the latter. Thus, the statistical logarithmic error of the former varies from -33% to $+51\%$, that of the latter from -52% to $+107\%$. Modifying the method of statistical correlation has thus brought about a highly desirable improvement in the accuracy of chlorophyll a determinations.

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