
Papers

Influence of underwater light fields on pigment characteristics in the Baltic Sea – results of statistical analysis*

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JOANNA STOŃ-EGIERT^{1,*}
ROMAN MAJCHROWSKI²
MIROSŁAW DARECKI¹
ALICJA KOSAKOWSKA¹
MIROSŁAWA OSTROWSKA¹

¹ Institute of Oceanology,
Polish Academy of Sciences,
Powstańców Warszawy 55, Sopot 81–712, Poland;

e-mail: aston@iopan.gda.pl

*corresponding author

² Institute of Physics,
Pomeranian University in Słupsk,
Arciszewskiego 22B, Słupsk 76–200, Poland

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Abstract

Changes in phytoplankton pigment concentrations in Case 2 waters (such as those of the Baltic Sea) were analysed in relation to the light intensity and its spectral dis-

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tribution in the water. The analyses were based on sets of empirical measurements containing two types of data: chlorophyll and carotenoid concentrations obtained by HPLC, and the distribution of underwater light fields measured with a MER 2040 spectrophotometer – collected during 27 research cruises on r/v ‘Oceania’ in 1999–2004. Statistical analysis yielded relationships between the total relative (to chlorophyll *a* concentrations) concentrations of major groups of phytoplankton pigments and optical depth τ , between the total relative concentrations of major groups of photosynthetic pigments (chlorophylls *b* ($C_{\text{chl } b \text{ tot}}/C_{\text{chl } a \text{ tot}}$), chlorophylls *c* ($C_{\text{chl } c \text{ tot}}/C_{\text{chl } a \text{ tot}}$) and photosynthetic carotenoids ($C_{\text{PSC tot}}/C_{\text{chl } a \text{ tot}}$) and the spectral fitting function (the ‘chromatic acclimation factor’), and between the total relative concentrations of photoprotective carotenoids ($C_{\text{PPC tot}}/C_{\text{chl } a \text{ tot}}$) in Baltic waters and the potentially destructive radiation (PDR), defined as the absolute amount of energy in the blue part of the spectrum (400–480 nm) absorbed by unit mass of chlorophyll *a*. The best approximations were obtained for the total chlorophyll *c* content, while the relative estimation errors were the smallest ($\sigma_- = 34.6\%$) for the approximation to optical depth and spectral fitting function. The largest errors related to the approximation of chlorophyll *b* concentrations: $\sigma_- = 56.7\%$ with respect to optical depth and 57.3% to the spectral fitting function.

A comparative analysis of the relative (to chlorophyll *a* content) concentrations of the main groups of pigments and the corresponding irradiance characteristics in ocean (Case 1) waters and Baltic waters (Case 2 waters) was also carried out. The distribution of $C_{\text{chl } b \text{ tot}}/C_{\text{chl } a \text{ tot}}$ ratios with respect to optical depth reveals a decreasing trend with increasing τ for Baltic data, which is characteristic of photoprotective pigments and the reverse of the trend in oceans. In the case of the $C_{\text{chl } c \text{ tot}}$ approximations, the logarithmic statistical error is lower for Baltic waters than for Case 1 waters: $\sigma_- = 34.6\%$ for Baltic data and $\sigma_- = 39.4\%$ for ocean data. In relation to photoprotective carotenoids (C_{PPC}), σ_- takes a value of 38.4% for Baltic waters and 36.1% for ocean waters. The relative errors of the approximated concentrations of different pigment groups are larger than those obtained for ocean waters. The only exception is chlorophyll *c*, for which the logarithmic statistical error is about 8.8% lower ($\sigma_- = 34.6\%$ for Baltic waters and 38.2% for ocean waters). Analysis of the errors resulting from the approximations of the photoprotective carotenoid content, depending on the energy characteristics of the underwater irradiance in the short-range part of PAR, showed that the relative errors are 1.3 times higher for Baltic waters than for ocean waters: $\sigma_- = 38.4\%$ for Baltic waters and 32.0% for ocean waters.

1. Introduction

The underwater light field is a major factor affecting the composition and quantitative characteristics of phytoplankton pigments in the environment. Changes in light intensity and its spectral distribution in the water body govern the physiological acclimation of phytoplankton cells (Harrison & Platt 1986, Falkowski & LaRoche 1991). These adjustments lead to morphological changes in algae cells, i.e. a change in volume and the number

of thylakoid membranes – by up to 50% (van Leeuwe & Stefels 1998), and a resizing of the different cellular structures (Sukenik et al. 1987). As a result, the contents of pigments and lipids and their composition in the cells of algae and cyanobacteria change (Berner et al. 1989, Falkowski & LaRoche 1991), which implies that the absorption characteristics of marine algae (Bricaud et al. 1983, Sathyendranath et al. 1987, Stramski et al. 2002), and by extension the quantum yield of photosynthesis (Morel et al. 1987) must have changed, too.

The nature of the underwater light field affects the intercellular content of the photosynthetic (PSP) and photoprotective (PPP) pigments by various types of photoadaptation, which enables organisms to achieve the most efficient absorption of light quanta for use in photosynthesis (Babin et al. 1996, Woźniak et al. 2003, Woźniak & Dera 2007, Dera & Woźniak 2010). These processes may occur as a result of the high intensity of blue light in the surface water layer, which would cause photooxidation of chlorophyll *a*, or of the presence of a narrow spectral irradiance at different depths, which prevents the chlorophyll *a* molecule from directly absorbing light quanta. In the first case, the cells produce larger amounts of protective pigments (intensity adaptation, also called photoadaptation), while in the second case, they support the production of additional pigments (antenna pigments), which permit the more efficient utilization of solar energy through photosynthesis (chromatic acclimation). In both cases the modifications affect not only the concentration of pigments in the cells, but also their relative content (i.e. the ratio $C_i/C_{\text{chl } a}$, where *i* denotes the relevant pigment), determining the vertical distributions of the relative content of PSP and PPP in the water body (Schlüter et al. 2000, Henriksen et al. 2002, Staehr et al. 2002).

Photoacclimation is a highly dynamic process. The increase in light intensity to the photosynthetic saturation level doubles the content of light harvesting complexes (LHC) in cells within 24 hours (Hoffmann & Senger 1988, Sukenik et al. 1990), and the changes in cellular pigment contents are measurable after 2 days (Berner et al. 1989, Staehr et al. 2002). With increasing light intensity, decreases are recorded in the cellular contents of chlorophyll *a* (even a 5-fold one, Goericke & Montoya 1998) and of diagnostic carotenoids of algae and cyanobacteria from different taxonomic groups (e.g. alloxanthin in *Rhodomonas marina* – *Cryptophyceae*, fucoxanthin in *Ditylum brightwellii* – *Bacillariophyceae*, chlorophyll *b* in *Brachiomonas* sp. – *Chlorophyceae*, Berner et al. 1989, Henriksen et al. 2002, Staehr et al. 2002). The relative contents of pigments also change, regardless of the growth phase of the phytoplankton cells (Henriksen et al. 2002). In organisms containing several pigment markers, their relative concentrations

respond differently to changes in light conditions (Mitchell & Kiefer 1988, Berner et al. 1989, Sosik & Mitchell 1991, Schlüter et al. 2000, Staehr et al. 2002). Summarizing, the ratio of pigment to chlorophyll concentrations decreases with increasing light intensity, indicating a parallel decrease of cellular pigments and chlorophyll content (Henriksen et al. 2002, Staehr et al. 2002). Changes in light intensity from low ($30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) to high ($300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) cause the ratio of e.g. zeaxanthin to chlorophyll *a* concentration to increase from 2- (*Synechococcus* sp. – *Nostocophyceae*) to 13-fold (*Pseudoscurfeldia marina* – *Prasinophyceae*) and that of lutein:chlorophyll *a* to increase from 1.6- (*Brachiomonas* sp. – *Chlorophyceae*) to 5-fold (*Pyramimonas disomata* – *Prasinophyceae*) (Henriksen et al. 2002). There are literature reports confirming the increase in the relative content of zeaxanthin (up to 100% in cells of *Synechococcus* sp., Schlüter et al. 2000). This is due to the photoprotective role of this pigment, involved in the cellular xanthophyll cycle (Demmig-Adams 1990, Demmig-Adams & Adams 1996), whose concentration may rise as a result of the deep oxidation of violaxanthin. In turn, the increase in lutein concentrations may be related to the ability of organisms to synthesize this pigment from α -carotene (Egeland et al. 1995, Niyogi et al. 1997). An increase in the relative content of alloxanthin was observed (approximately 2-fold for *Rhodomonas marina*), but this was just the result of a decrease in chlorophyll *a* concentration at a constant concentration of alloxanthin. The light harvesting role of this pigment is poorly known. Research confirms that there is a relative decline in its content with depth in Pacific phytoplankton (Mackey et al. 1998) and that its content rises with increasing light intensity to about 100% (Schlüter et al. 2000), which suggests that it plays a photoprotective role.

Certain regularities related to the vertical distributions of the relative pigment content have also been found (Majchrowski 2001, Woźniak & Dera 2007, Majchrowski & Ostrowska 2009). They are related to the characteristics of the light field in deep waters and are the result of mechanisms by which natural phytoplankton communities adapt to spectral irradiance in water bodies. The relative content of PSP increases with depth, while that of PPP decreases. The vertical distribution of pigment concentrations varies in different trophic types of water bodies (determined by the surface concentration of chlorophyll *a*). Oligotrophic waters, in which the shortwave part of the light spectrum is dominant at large depths, absorb mainly chlorophylls, because the absorption band of photosynthetic carotenoids (PSC) is outside that range. This means that $C_{\text{PSC}}/C_{\text{chl } a}$ ratios do not vary with depth, and even decrease in the deepest regions. In mesotrophic waters, where the light spectrum maximum in the water column shifts towards

long waves with increasing depth, PSC are dominant among the antenna pigments supporting photosynthesis. In eutrophic waters, the spectral distribution shows a red-shifted maximum, which can lead to a decline in the relative PSC concentration, and the part played by antennas in photosynthesis is taken over by other pigments, such as phycobilins. The vertical distributions of the relative content of photoprotective carotenoids (PPC) are also governed by the characteristics of light in different types of seas. In oligotrophic waters, there is deep penetration of blue light that would lead to photooxidation of the photosynthetic apparatus in phytoplankton cells, processes and thus the production of additional PPP. In eutrophic waters, however, the blue part of the irradiance spectrum is already absorbed at shallow depths, and phytoplankton therefore has no need for the additional production of protective pigments. Hence there is a rapid decrease in the concentrations of these compounds with depth.

The quantitative relationships between the concentrations and relative contents of different groups of pigments and the various optical characteristics of the natural light field relate mainly to oceanic waters (Case 1 waters), where light of wavelength $\lambda \approx 450$ nm can penetrate to the greatest depths; they have been investigated by many authors (Woźniak et al. 1997a,b, 2003, Majchrowski et al. 1998, Majchrowski & Ostrowska 1999, 2000, Majchrowski 2001). Similar relationships for Case 2 waters, which contain high concentrations of optically active, autogenous ingredients (other than phytoplankton), such as those of the Baltic Sea, where light of wavelength $\lambda \approx 550$ nm reaches the greatest depths, are difficult to establish and remain an unsolved problem.

The aim of this study was to determine the statistical relationships between the concentrations of the major groups of pigments ($C_{i\text{ tot}}$) and the various optical characteristics of the light fields in the waters of the southern Baltic Sea, such as optical depth τ (established empirically from measurements of spectral distributions of downward irradiance in the PAR region at different depths) and spectral distributions of underwater light and their absolute levels in Baltic Sea waters.

2. Material and methods

2.1. Empirical material

The analyses were based on a database of empirical measurements, including the chromatographic separation of pigments by RP-HPLC (Stoń & Kosakowska 2002, Stoń-Egiert & Kosakowska 2005) and distributions of underwater light fields measured with a MER 2040 spectrophotometer

during 27 research cruises on r/v ‘Oceania’ in different seasons in 1999–2004. Samples for pigment analysis were taken from the surface layer and different depths, the choice being dictated by the distribution of organic matter in the water column. The following groups of pigments were identified: chlorophylls (chlorophyll *a*, *b*, *c1* + *c2* and *c3*, chlorophyllide *a*), photosynthetic carotenoids – PSC (peridinin, fucoxanthin, α -carotene, 19′but-fucoxanthin, 19′hex-fucoxanthin, prasinoxanthin, echinenone, canthaxanthin), and photoprotective carotenoids – PPC (diadinoxanthin, alloxanthin, zeaxanthin, lutein, neoxanthin, violaxanthin, β -carotene, diatoxanthin, myxoxanthophyll, antheraxanthin). The study focused on southern Baltic ecosystems, including gulf waters (the Gulf of Gdańsk and the Pomeranian Bay) and open waters. The geographical positions of the measuring stations are given in Figure 1.

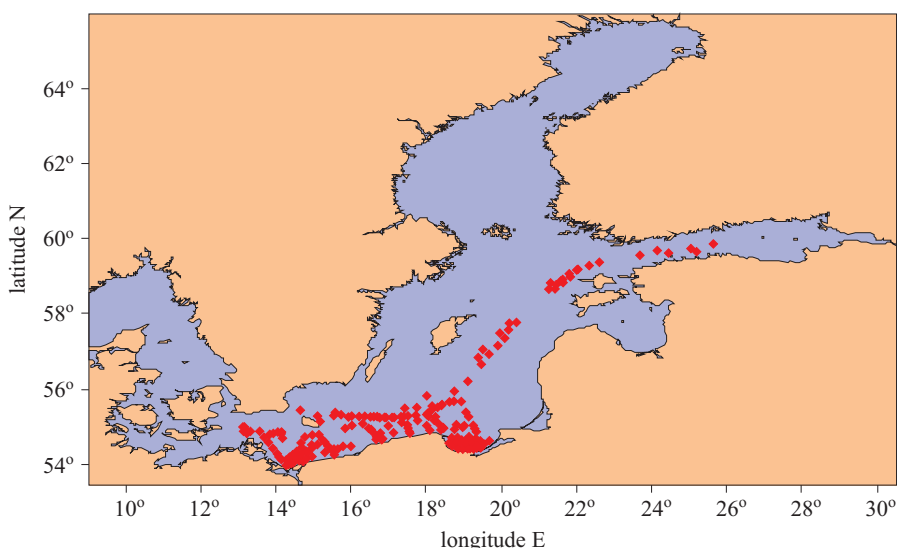


Figure 1. Spatial distribution of measuring stations in 1999–2004

2.2. Mathematical background

The relationships between the pigment concentrations and spectral distributions of the underwater light field in ocean waters are known and described in the literature (Babin et al. 1996, Majchrowski et al. 1998, Majchrowski 2001, Woźniak et al. 2003, Woźniak & Dera 2007). These authors have shown that spectral fitting functions, also known as chromatic acclimation factors (F_i), are quantities well correlated with the relative concentrations of particular groups of PSP, i.e. chlorophylls *b* and *c*, and

PSC. But in the case of the relative concentrations of PPP, such a function is the absolute amount of energy in the blue part of the spectrum (400–480 nm), identified as potentially destructive radiation (PDR). These values were used to obtain approximations of the relative contents of PSP and PPP in Baltic Sea waters. In both cases, the effects of water mixing in a 30 m thick layer were also taken into account, because the concentrations of the pigments in this layer must be a consequence of the history of movements of phytoplankton cells in the water column (Majchrowski 2001, Woźniak & Dera 2007).

The values used were defined as:

- the average chromatic acclimation factor in the mixing layer:

$$\langle F_i \rangle_{\Delta z = \pm 15 \text{ m}} = \frac{1}{z_2 - z_1} \int_{z_1}^{z_2} F_i(z) dz \quad (1)$$

$$z_2 = z + 15 \text{ m} \text{ and } z_1 = \begin{cases} 0 & \text{if } z < 15 \text{ m} \\ z - 15 \text{ m} & \text{if } z \geq 15 \text{ m} \end{cases} ,$$

where

$$F_i(z) = \frac{1}{a_{i,\max}^*} \int_{400 \text{ nm}}^{700 \text{ nm}} f(\lambda, z) a_i^*(\lambda) d\lambda \quad [\text{dimensionless}] \quad (2)$$

$f(\lambda, z) = \frac{E_d(\lambda, z)}{\text{PAR}(z)}$ – normalized spectral distribution of PAR irradiance at depth z [nm^{-1}],

$a_i^*(\lambda)$ – spectral mass-specific light absorption coefficient for the i th group of pigments [$\text{m}^2(\text{mg pigment})^{-1}$] (Ficek et al. 2004),

$a_{i,\max}^*$ – maximum mass-specific light absorption coefficient for the i th group of pigments [$\text{m}^2(\text{mg pigment})^{-1}$] (Ficek et al. 2004),

i – index denoting the main groups of pigments: chlorophylls a – chl a tot, chlorophylls b – chl b tot, chlorophylls c – chl c tot, photosynthetic carotenoids – PSC tot, and photoprotective carotenoids – PPC tot;

- the average PDR in the mixing layer:

$$\langle \text{PDR}^* \rangle_{\Delta z = \pm 15 \text{ m}} = \frac{1}{z_2 - z_1} \int_{z_1}^{z_2} \text{PDR}^*(z) dz \quad (3)$$

$$z_2 = z + 15 \text{ m} \text{ and } z_1 = \begin{cases} 0 & \text{if } z < 15 \text{ m} \\ z - 15 \text{ m} & \text{if } z \geq 15 \text{ m} \end{cases} ,$$

where

$$\text{PDR}^* = \int_{400 \text{ nm}}^{480 \text{ nm}} a_a^*(\lambda) \times \langle E_0(\lambda) \rangle_{\text{day}} d\lambda \quad (4)$$

PDR^* – potentially destructive radiation (per unit of chlorophyll *a* mass) [$\text{Ein} (\text{mg chl } a)^{-1} \text{ s}^{-1}$],

$\langle E_0(\lambda) \rangle_{\text{day}}$ – mean daily scalar irradiance in the sea [$\text{Ein m}^{-2} \text{ s}^{-1} \text{ nm}^{-1}$],

$a_a^*(\lambda)$ – mass-specific coefficient of light absorption by chlorophyll *a* [$\text{m}^2 (\text{mg chl } a)^{-1}$].

The functions F_i can vary in value from 0 to 1: the value is 0 when the spectrum of the pigment's absorption coefficient does not overlap the underwater light spectrum at any point, and 1 when the wavelength of the ambient irradiance coincides with that of the maximum absorption coefficient of the pigment group. The magnitude of F_i depends on relative rather than absolute spectral energies. In contrast, PDR^* is equal to the energy of blue-green light that can be absorbed by unit mass of chlorophyll *a* and which could cause the photooxidation of chlorophyll *a* (Majchrowski 2001).

3. Results and discussion

3.1. Analysis of the relationship between the concentration of pigment groups and optical depth

The statistical relationships were analysed between the relative concentrations of pigment groups ($C_{i \text{ tot}}/C_{\text{chl } a}$) identified in natural samples from the Baltic Sea and empirically established optical depths τ . The general form of the function approximating these values in the waters of the Baltic is analogous to that obtained for Case 1 waters (Majchrowski 2001):

$$C_{i, \text{ tot}}/C_{\text{chl } a \text{ tot}} = A_i \exp(B_i \times \tau), \quad (5)$$

where

$C_{i \text{ tot}}$ – concentration of *i*-pigment groups [$\mu\text{g dm}^{-3}$],

$C_{\text{chl } a \text{ tot}}$ – concentration of total chlorophyll *a* [$\mu\text{g dm}^{-3}$],

τ – optical depth, defined by the following formula: $\tau(z) = -\ln T(z)$, where T is the transmittance of PAR ($E_{\text{PAR}}(z)$) expressed by the ratio $T(z) = E_{\text{PAR}}(z)/E_{\text{PAR}}(z=0)$, and z is the actual depth, [dimensionless],

A_i , B_i – numerical values of coefficients for different pigments (Table 1).

The results of the verification of the approximating functions (eq. (5)) are shown in Table 2. The analysis was based on all sets of measurement

Table 1. Values of coefficients A_i and B_i used to approximate the relative total content of each group of photosynthetic pigments (chlorophylls b , chlorophylls c) and photosynthetic and photoprotective carotenoids in relation to optical depth τ (eq. (5)) in the Baltic Sea (N – number of empirical data)

Pigment	N	Estimated values of numerical coefficients	
		A_i	B_i
chlorophylls $b - C_{\text{chl } b \text{ tot}}/C_{\text{chl } a \text{ tot}}$	802	0.0695	-0.0477
chlorophylls $c - C_{\text{chl } c \text{ tot}}/C_{\text{chl } a \text{ tot}}$	1065	0.0829	0.0219
PSC – $C_{\text{PSC tot}}/C_{\text{chl } a \text{ tot}}$	1077	0.1375	0.0347
PPC – $C_{\text{PPC tot}}/C_{\text{chl } a \text{ tot}}$	1081	0.2175	-0.0220

Table 2. Relative errors in estimating the total concentrations of pigments from equation 5 on the basis of chlorophyll a concentrations, empirically established optical depth τ , and the numerical coefficients set out in Table 1

Pigment	Arithmetic statistics		Logarithmic statistics			
	Systematic error	Statistical error	Systematic error	Standard error factor	Statistical error	
	$\langle \varepsilon \rangle$ [%]	σ_ε [%]	$\langle \varepsilon \rangle_g$ [%]	x	σ_- [%]	σ_+ [%]
$C_{\text{chl } b \text{ tot}}$	53.1	196.9	0.1	2.31	-56.7	131.0
$C_{\text{chl } c \text{ tot}}$	7.9	49.1	-1.4	1.53	-34.6	52.9
$C_{\text{PSC tot}}$	16.9	81.2	-3.3	1.83	-45.3	82.9
$C_{\text{PPC tot}}$	10.8	61.7	-2.0	1.62	-38.4	62.4

where

$\langle \varepsilon \rangle = (C_{i, \text{calc}} - C_{i, \text{meas}})/C_{i, \text{meas}}$ – relative error.	$\langle \log(C_{i, \text{calc}}/C_{i, \text{meas}}) \rangle$ – mean of $\log(C_{i, \text{calc}}/C_{i, \text{meas}})$.
$C_{i, \text{meas}}, C_{i, \text{calc}}$ – concentrations of pigment groups measured and calculated using appropriate formulas (5)–(8).	σ_ε – standard deviation of errors (statistical error).
$\langle \varepsilon \rangle$ – arithmetic mean of errors.	σ_{\log} – standard deviation of $\log(C_{i, \text{calc}}/C_{i, \text{meas}})$.
$\langle \varepsilon \rangle_g$ – logarithmic mean of errors.	$x = 10^{\sigma_{\log}}$ – standard error factor.
$\langle \varepsilon \rangle_g = 10^{\langle \log(C_{i, \text{calc}}/C_{i, \text{meas}}) \rangle} - 1$.	$\sigma_+ = x - 1$ and $\sigma_- = \frac{1}{x} - 1$.

data obtained in 1999–2004 (value N in Table 1), when measurements were performed in different seasons, in different areas of the southern Baltic region and at various depths. The relative estimation errors are the smallest in the case of the total content of chlorophyll c ($\sigma_- = 34.6\%$), and the largest in the case of chlorophyll b ($\sigma_- = 56.7\%$).

A comparative analysis was also carried out to estimate the relative concentrations of the major groups of pigments – total chlorophylls b

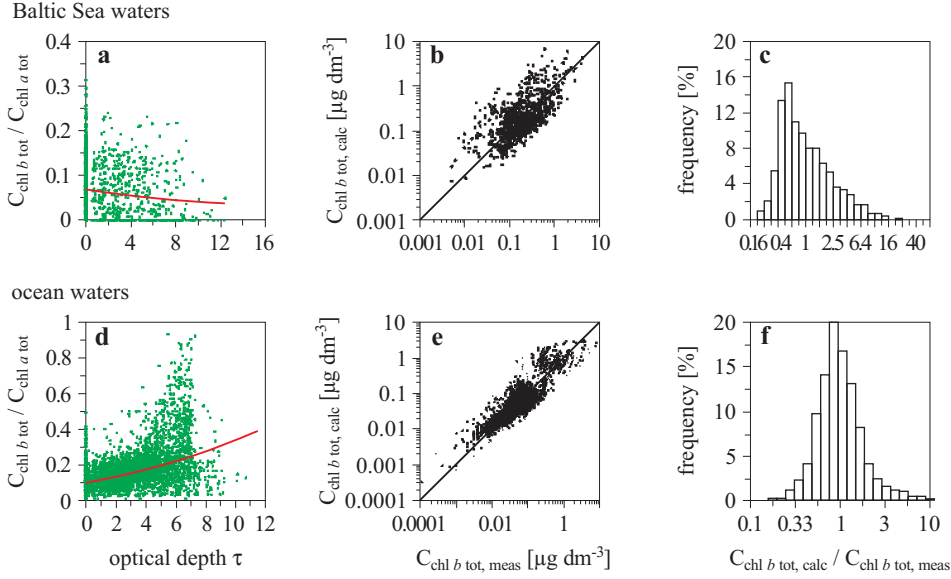


Figure 2. Comparison of the statistical relationships between the relative concentrations of chlorophylls b $C_{\text{chl } b \text{ tot}}/C_{\text{chl } a \text{ tot}}$ and optical depth τ (dots – experimental data, line – approximation of equation (5)) obtained for Baltic waters (a) (the results obtained in this study) and ocean waters (d) (Majchrowski 2001); comparison of empirical data of $C_{\text{chl } b \text{ tot, meas}}$ and $C_{\text{chl } b \text{ tot, calc}}$ approximated with the relevant equation (b, e); histograms of the ratios $C_{\text{chl } b \text{ tot, meas}}/C_{\text{chl } b \text{ tot, calc}}$ for Baltic and ocean data respectively (c, f)

($C_{\text{chl } b \text{ tot}}/C_{\text{chl } a \text{ tot}}$, where $C_{\text{chl } b \text{ tot}} = C_{\text{chl } b} + C_{\text{chl } b, nz}$, $C_{\text{chl } a \text{ tot}} = C_{\text{chl } a} + C_{\text{chl } ide} + C_{\text{chl } a, nz}$, nz – denotes unidentified pigments from groups whose content is roughly estimated on the basis of chromatographic characteristics), chlorophylls c ($C_{\text{chl } c \text{ tot}}/C_{\text{chl } a \text{ tot}}$, $C_{\text{chl } c \text{ tot}} = C_{\text{chl } c1+c2} + C_{\text{chl } c3} + C_{\text{chl } c, nz}$), the sum of photosynthetic carotenoids ($C_{\text{PSC } \text{ tot}}/C_{\text{chl } a \text{ tot}}$, $C_{\text{PSC } \text{ tot}} = C_{\text{PSC}} + C_{\text{PSC}, nz}$) and the sum of photoprotective carotenoids ($C_{\text{PPC } \text{ tot}}/C_{\text{chl } a \text{ tot}}$, $C_{\text{PPC}, \text{ tot}} = C_{\text{PPC}} + C_{\text{PPC}, nz}$) – with respect to the optical depth τ obtained for oceanic waters (Majchrowski 2001) and southern Baltic Sea waters (results obtained in this work). The results of these comparisons are presented in Figures 2–5 separately for each group of pigments. The distribution of the relationships $C_{\text{chl } b \text{ tot}}/C_{\text{chl } a \text{ tot}}$ with respect to optical depth reveals a decreasing trend with increasing τ for the Baltic data (Figure 2), which is characteristic of PPP (Figure 5) and the reverse of that in the oceans (Figure 2a,d). This may be due to the fact that the species diversity of phytoplankton groups at different depths in the sea has a greater impact on the relative amounts of a pigment in the water than acclimation to prevailing light conditions. Chlorophyll b

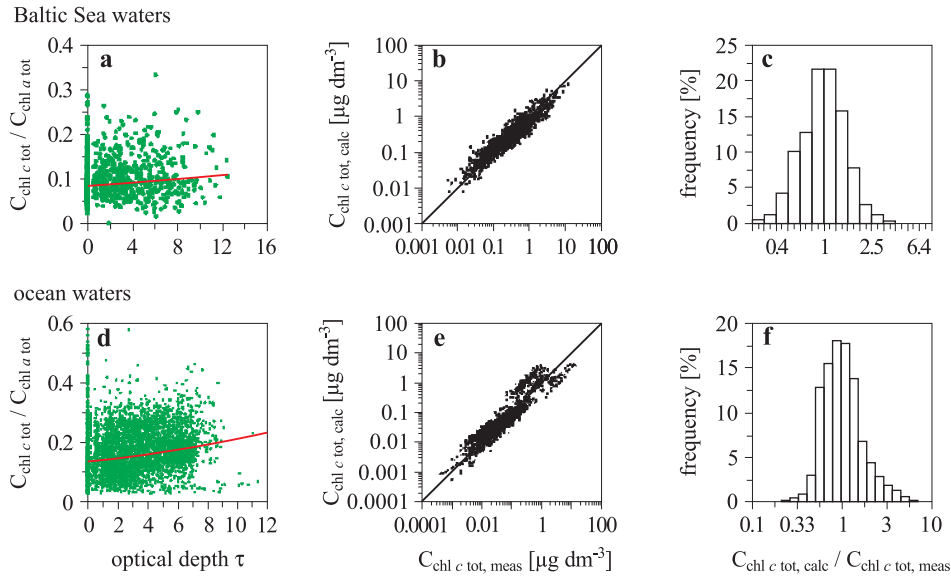


Figure 3. Comparison of the statistical relationships between the relative concentrations of chlorophylls c $C_{chl\ c\ tot}/C_{chl\ a\ tot}$ and the optical depth τ (dots – experimental data, line – approximation of equation (5)) obtained for Baltic waters (a) (the results obtained in this study) and ocean waters (d) (Majchrowski 2001); comparison of empirical data $C_{chl\ c\ tot, meas}$ and $C_{chl\ c\ tot, calc}$ approximated with the relevant equation (b, e); histograms of the ratios $C_{chl\ c\ tot, meas}/C_{chl\ c\ tot, calc}$ for Baltic and ocean data respectively (c, f)

is characteristic of green algae, prasinophytes and euglenophytes, whose optimum conditions for life, growth and development are found in the 0–5 m layer. The low $C_{chl\ b\ tot}/C_{chl\ a\ tot}$ ratios at large optical depths are due to the chlorophyll b concentrations, which are low in comparison to the concentration of chlorophyll a in the water. The trend with regard to the relative total content of chlorophylls c ($C_{chl\ c\ tot}/C_{chl\ a\ tot}$) and PSC ($C_{PSC}/C_{chl\ a\ tot}$) with increasing optical depth τ is an increasing one, as in ocean waters (Figures 3a, 4a), which indicates that photoacclimation is occurring in algal and cyanobacterial cells. Comparison of the estimation errors of the concentrations of photosynthetic ($C_{chl\ b\ tot}$, $C_{chl\ c\ tot}$, $C_{PSC\ tot}$) and photoprotective ($C_{PPC\ tot}$) pigments for Baltic waters (results obtained in this work) and oceanic regions (Majchrowski 2001) shows that in the case of the approximations for chlorophyll b and photosynthetic carotenoids, the formulas for Baltic waters are encumbered with a larger logarithmic statistical error ($\sigma_- = 56.7\%$ for $C_{chl\ b\ tot}$ and $\sigma_- = 41.3\%$ for $C_{PSC\ tot}$) than those for ocean waters ($\sigma_- = 42.2\%$ for $C_{chl\ b\ tot}$ and $\sigma_- = 25.7\%$ for $C_{PSC\ tot}$).

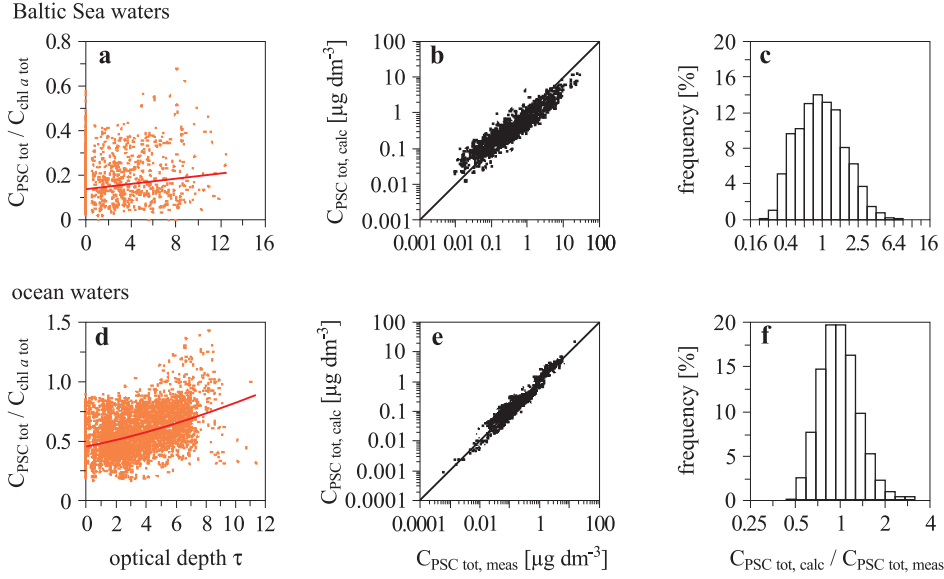


Figure 4. Comparison of the statistical relationships between the relative concentrations of photosynthetic carotenoids $C_{PSC\ tot}/C_{chl\ a\ tot}$ and the optical depth τ (dots – experimental data, line – approximation of equation (5)) obtained for Baltic waters (a) (the results obtained in this study) and ocean waters (d) (Majchrowski 2001); comparison of empirical data $C_{PSC\ tot,\ meas}$ and $C_{PSC\ tot,\ calc}$ approximated with the relevant equation (b, e); histograms of the ratios $C_{PSC\ tot,\ meas}/C_{PSC\ tot,\ calc}$ for Baltic and ocean data respectively (c, f)

The logarithmic statistical error of the approximations for $C_{chl\ c\ tot}$ is lower for Baltic waters than for Case 1 waters: $\sigma_- = 34.6\%$ (Baltic data) and $\sigma_- = 39.4\%$ (oceanic data). With respect to PPC (C_{PPC}), σ_- is 38.4% for Baltic waters and 36.1% for ocean waters.

3.2. Analysis of the relationships between the concentrations of identified pigment groups and underwater light characteristics

The statistical relationships were analysed between the relative total concentrations of the major groups of photosynthetic pigments in the Baltic Sea – chlorophylls *b* ($C_{chl\ b\ tot}/C_{chl\ a\ tot}$), chlorophylls *c* ($C_{chl\ c\ tot}/C_{chl\ a\ tot}$) and PSC ($C_{PSC\ tot}/C_{chl\ a\ tot}$) – and the spectral distribution of underwater irradiance (chromatic acclimation factor), as well as between the relative total concentrations of PPC ($C_{PPC\ tot}/C_{chl\ a\ tot}$) and the energy (PDR) distribution of the underwater light field. The following relationships were obtained from this statistical analysis:

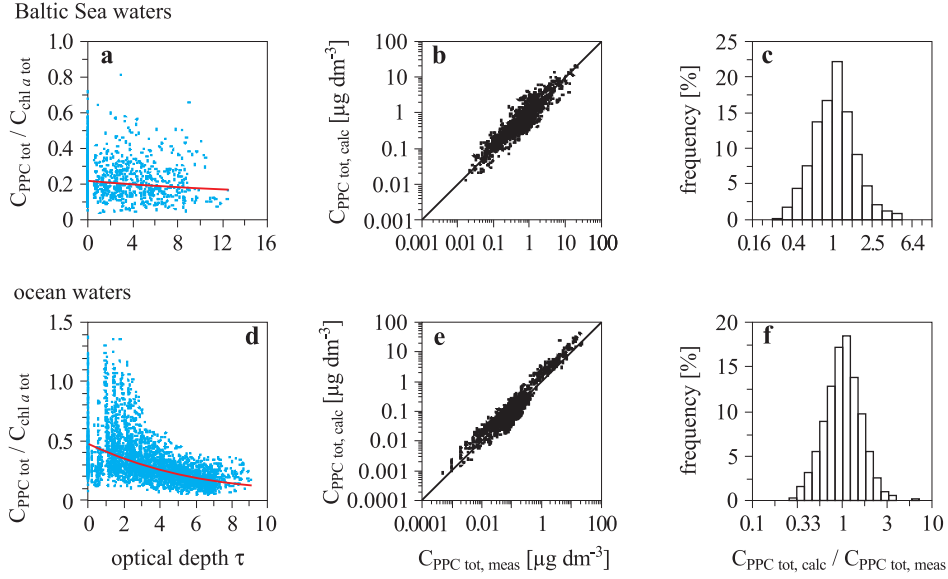


Figure 5. Comparison of the statistical relationships between the relative concentrations of photoprotective carotenoids $C_{PPC\,tot}/C_{chl\,a\,tot}$ and the optical depth τ (dots – experimental data, line – approximation of equation (5)) obtained for Baltic waters (a) (the results obtained in this study) and ocean waters (d) (Majchrowski 2001); comparison of empirical data $C_{PPC\,tot,\,meas}$ and $C_{PPC\,tot,\,calc}$ approximated with the relevant equation (b, e); histograms of the ratios $C_{PPC\,tot,\,meas}/C_{PPC\,tot,\,calc}$ for Baltic and ocean data respectively (c, f)

- for the relative total content of the major groups of PSP:
 - for chlorophylls *b*:

$$C_{chl\,b\,tot}/C_{chl\,a\,tot} = A_i < F_b >_{\Delta z = \pm 15\,m}^{C_i} + B_i \quad (6)$$

- for chlorophylls *c* and PSC:

$$C_{i\,tot}/C_{chl\,a\,tot} = A_i < F_i >_{\Delta z = \pm 15\,m} + B_i \quad (7)$$

- for the relative total content of PPC:

$$C_{PPC\,tot}/C_{chl\,a\,tot} = A_i < PDR^* >_{\Delta z = \pm 15\,m} + B_i, \quad (8)$$

where

A_i , B_i , C_i – numerical values of coefficients for different pigments (Table 3),

$< F_i >_{\Delta z = \pm 15\,m}$ – mean chromatic acclimation factor in a 30 m thick layer for the *i*th-group of pigments,

$< PDR^* >_{\Delta z = \pm 15\,m}$ – mean potentially destructive radiation in a 30 m thick layer.

Table 3. Values of coefficients A_i , B_i and C_i used to approximate the relative total content of each group of photosynthetic pigments: chlorophylls b – equation (6), chlorophylls c and photosynthetic carotenoids – equation (7), and photoprotective carotenoids – equation (8), depending on the spectral and energy characteristics of the underwater irradiance in the Baltic Sea (N – number of empirical data)

Pigment	N	Estimated values of coefficients		
		A_i	B_i	C_i
chlorophylls b (eq. (6))	626	90.01	0.0751	4.2825
chlorophylls c (eq. (7))	851	-0.2024	0.1110	
PSC (eq. (7))	861	-0.4810	0.3175	
PPC (eq. (8))	866	0.0623	0.2251	

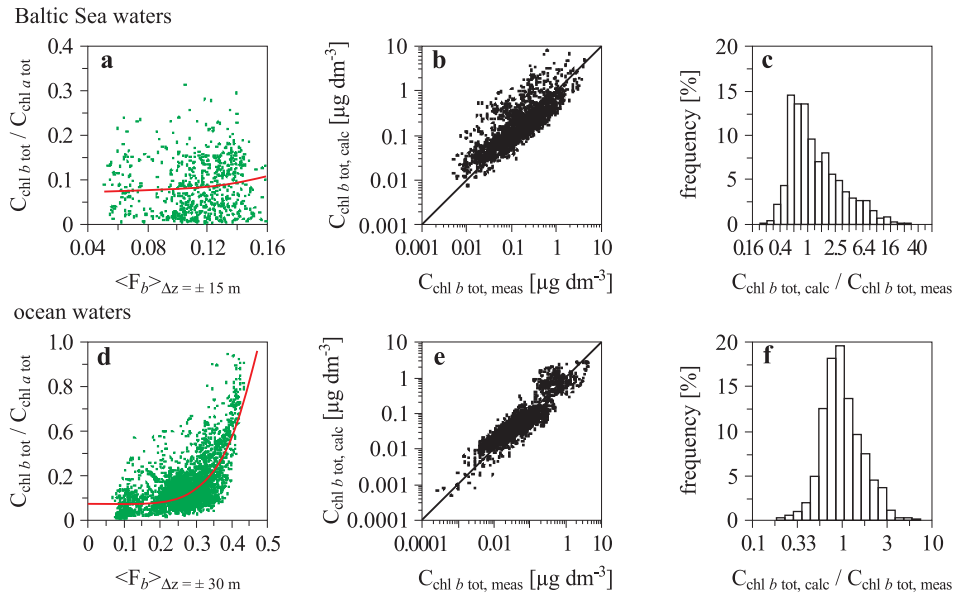


Figure 6. Comparison of the statistical relationships and the mean spectral fitting function $\langle F_b \rangle_{\Delta z = \pm 15 \text{ m}}$ in the mixing layer Δz (dots – experimental data, line – approximation of equation (6)) obtained for Baltic waters (a) (the results obtained in this study) and ocean waters (d) (Majchrowski 2001); comparison of empirical data of $C_{\text{chl } b \text{ tot, meas}}$ and $C_{\text{chl } b \text{ tot, calc}}$ approximated with the relevant equation (b, e); histograms of the ratios $C_{\text{chl } b \text{ tot, calc}}/C_{\text{chl } b \text{ tot, meas}}$ for Baltic and ocean data respectively (c, f)

The form of the functions is analogous to that obtained for ocean waters (Majchrowski 2001). The results of the validation of these approximations are presented in Table 4. The smallest estimation error refers to the total

Table 4. Relative errors in estimating the total concentrations of pigment groups – chlorophylls *b*, chlorophylls *c*, photosynthetic and photoprotective carotenoids – from equations (6)–(8)

Pigment	Arithmetic statistics		Logarithmic statistics			
	Systematic error	Statistical error	Systematic error	Standard error factor	Statistical error	
	$\langle \varepsilon \rangle$ [%]	σ_ε [%]	$\langle \varepsilon \rangle_g$ [%]	x	σ_- [%]	σ_+ [%]
$C_{chl\ b\ tot}$ (eq. (6))	99.3	263.1	27.6	2.34	-57.3	134.1
$C_{chl\ c\ tot}$ (eq. (7))	24.2	54.7	13.9	1.53	-34.6	52.9
$C_{PSC\ tot}$ (eq. (7))	66.2	121.3	36.8	1.83	-45.5	83.4
$C_{PPC\ tot}$ (eq. (8))	34.7	76.3	19.0	1.62	-38.4	62.4

Formulas used to calculate the errors are given in Table 2.

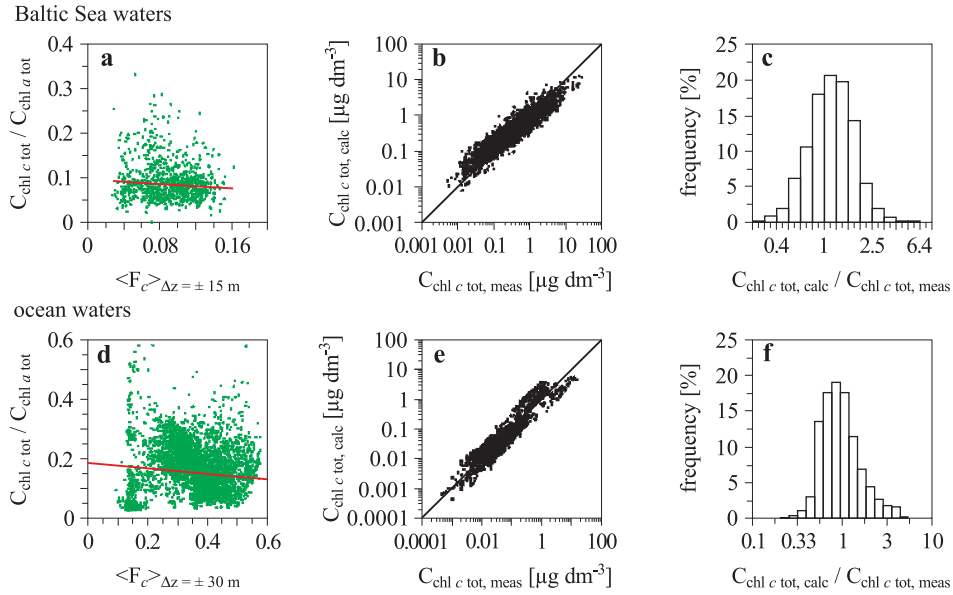


Figure 7. Comparison of the statistical relationships between the relative concentrations of chlorophylls *c* $C_{chl\ c\ tot}/C_{chl\ a\ tot}$ and the mean spectral fitting function $\langle F_c \rangle_{\Delta z = \pm 15\ m}$ in the mixing layer Δz (dots – experimental data, line – approximation of equation (7)) obtained for Baltic waters (a) (the results obtained in this study) and ocean waters (d) (Majchrowski 2001); comparison of empirical data $C_{chl\ c\ tot, meas}$ and $C_{chl\ c\ tot, calc}$ approximated with the relevant equation (b, e); histograms of the ratios $C_{chl\ c\ tot, meas}/C_{chl\ c\ tot, calc}$ for Baltic and ocean data respectively (c, f)

content of chlorophyll *c* ($\sigma_- = 34.6\%$), the largest to total chlorophyll *b* ($\sigma_- = 57.3\%$). The relative contents of the main groups of pigments and

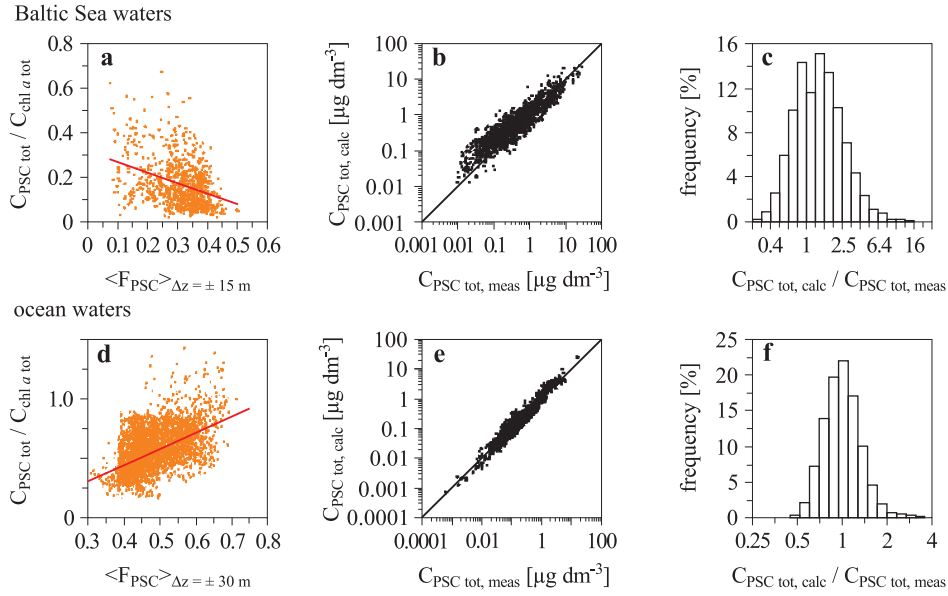


Figure 8. Comparison of the statistical relationships between the relative concentrations of photosynthetic carotenoids – $C_{PSC\ tot} / C_{chl\ a\ tot}$ and the mean spectral fitting function $\langle F_{PSC} \rangle_{\Delta z = \pm 15\ m}$ in the mixing layer Δz (dots – experimental data, line – approximation of equation (7)) obtained for Baltic waters (a) (the results obtained in this study) and ocean waters (d) (Majchrowski 2001); comparison of empirical data $C_{PSC\ tot,\ meas}$ and $C_{PSC\ tot,\ calc}$ approximated with the relevant equation (b, e); histograms of the ratios $C_{PSC\ tot,\ meas} / C_{PSC\ tot,\ calc}$ for Baltic and ocean data respectively (c, f)

the corresponding irradiance characteristics in ocean waters (Majchrowski 2001) and Baltic waters (the results obtained in this work) were compared in the next step of the analysis. Figures 6–9 present the results for each group of pigments.

The problem of the adaptation of phytoplankton cells to light conditions in the Baltic Sea is more complex than in Case 1 (ocean) waters. The relative errors of the approximated concentrations of different pigment groups are larger than for ocean waters. The only exception is chlorophyll *c*, for which the logarithmic statistical error was about 8.8% lower ($\sigma_- = 34.6\%$ for Baltic waters and 38.2% for ocean waters). Analysis of the approximated concentrations of other PSP groups, i.e. chlorophyll *b* and PSC, as a function of spectral fitting showed that the relative estimation errors were more than twice as large for the Baltic data than for the ocean data. This may have been due to the different distributions of the relative spectral irradiances at different depths in Case 1 and Case 2 waters. In the deeper regions of oligotrophic waters (such as ocean waters), light comes mainly from the

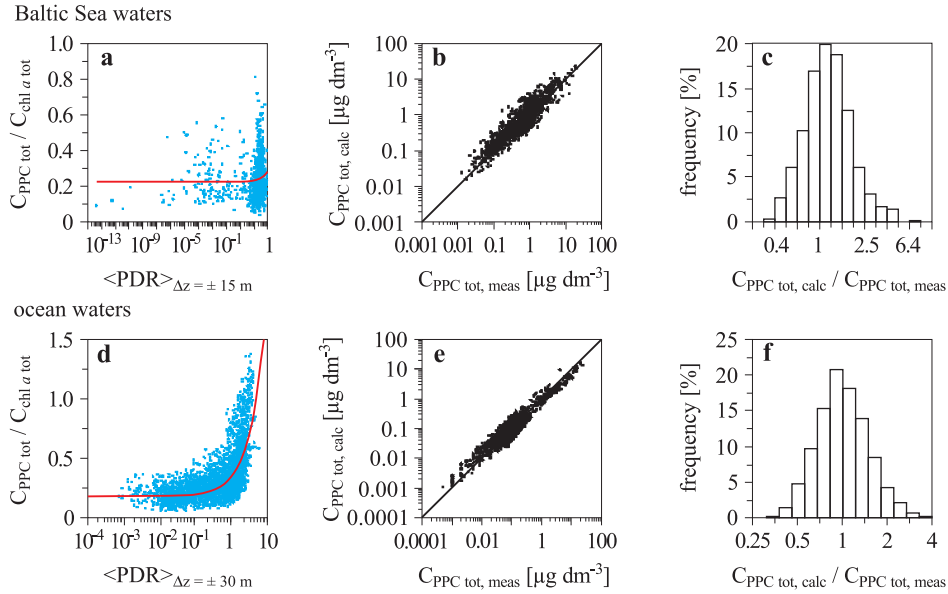


Figure 9. Comparison of the statistical relationships between the relative concentrations of photoprotective carotenoids $C_{PPC\ tot} / C_{chl\ a\ tot}$ and the potentially destructive radiation $\langle PDR \rangle_{\Delta z = \pm 15\ m}$ in the mixing layer Δz (dots – experimental data, line – approximation of equation (8)) obtained for Baltic waters (a) (the results obtained in this study) and ocean waters (d) (Majchrowski 2001); comparison of empirical data $C_{PPC\ tot, meas}$ and $C_{PPC\ tot, calc}$ approximated with the relevant equation (b, e); histograms of the ratios $C_{PPC\ tot, meas} / C_{PPC\ tot, calc}$ for Baltic and ocean data respectively (c, f)

blue-green part of the spectrum, whereas in eutrophic waters (such as Baltic waters), there is much less of this light. The chromatic acclimation factor gives a relatively good estimate of the concentrations of the major groups of PSP in ocean waters. But the large estimation errors in Baltic waters may be due to the phycobilin concentration modifying the light field spectrum in the Baltic, which is not taken into account in the analysis. Analysis of the errors resulting from the approximations of the PPC content, depending on the energy characteristics of the underwater irradiance in the short-range part of PAR (eq. (7)), showed that the relative errors are 1.3 times higher for Baltic waters than for ocean waters. The logarithmic statistical errors are $\sigma_- = 38.4\%$ for Baltic waters and 32.0% for ocean waters.

4. Conclusions

In summary, the problem of the adaptation and acclimation of phytoplankton cells to the irradiance conditions in Case 2 waters, such as those

of the Baltic Sea, appears to be more complex than in Case 1 (ocean) waters. Only in the case of certain pigments does the verification of the approximations of their concentrations or the environmentally dependent concentrations of pigment groups give lower estimation errors than those resulting from the approximations found for oceanic waters. This is the situation we are faced with when estimating the total content of chlorophylls c and PPC with respect to the optical depth and the total content of chlorophylls c with respect to chromatic adaptation factors. The spectral fitting function, i.e. the chromatic adaptation factor, approximates the content of the major groups of photosynthetic pigments in ocean waters fairly well. The large errors of estimation in Baltic waters may be due to the presence of phycobilins, not taken account of in this analysis, which significantly modify the light field spectrum in this sea.

This analysis of the composition of phytoplankton pigments and resources and their links with environmental parameters extends our knowledge of the acclimation of phytoplankton in different types of ecosystems. As mentioned earlier, most of the known relationships have been established for ocean waters (Case 1), where pigment concentrations are much lower than in Case 2 waters. Moreover, the distribution of environmental parameters (irradiance and its spectral distribution in the water, nutrient content, temperature and salinity) in the oceans and their variability in time and space are not subject to such dynamic fluctuations as in the eutrophic waters of the Baltic, where there are major inflows of river water supplying the environment with substances modifying the distribution of the environmental factors under scrutiny here.

The problems concerning the impact of environmental parameters on the composition and pigment content in samples of phytoplankton in different ecosystems are very complex. The results presented in this paper by no means exhaust this difficult subject, and further research and analysis of this problem are necessary.

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