Qualitative and quantitative analysis of Baltic phytoplankton pigments*

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Abstract

The paper presents the results of research into the pigment composition of seawater samples taken from various depths in the Gulf of Gdańsk in April and September 1999. Pigments were separated by RP–HPLC, and identification was confirmed by co-injection with reference standards and on-line diode array spectra. The following groups of pigments were identified: chlorophylls: a, b, $c_1 + c_2$; photosynthetic carotenoids (PSC) – peridinin, fucoxanthin, α-carotene; photoprotecting carotenoids (PPC) – diadinoxanthin, alloxanthin, zeaxanthin, lutein, neoxanthin, violaxanthin and β-carotene. Quantitative and qualitative diversity in pigment characteristics were observed in both seasons, though lutein and α-carotene were not identified in the September samples. The respective April and September concentrations of the functional groups of pigments were: total chlorophylls content 0.47–104.25 and 0.57–13.66 μg dm^{-3}; PSC 0.07–21.23 and 0.02–1.56 μg dm^{-3}; PPC 0.05–14.08 and 0.04–1.79 μg dm^{-3}. Within the PSC group, peridinin and fucoxanthin were dominant in the April samples, but only fucoxanthin

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in the September ones. Among the PPCs, diadinoxanthin and alloxanthin were dominant in April, zeaxanthin in September. Photosynthetic and photoprotecting carotenoids display a linear correlation with chlorophyll *a* content in all the samples.

1. Introduction

The pigments present in phytoplankton organisms include numerous groups of compounds with diverse physical and chemical properties. Fundamentally, plant pigments are divided into 3 groups: chlorophylls (a, b, c_1, c_2, c_3), carotenoids (carotenes and their oxygenated derivatives known as xanthophylls), and biliproteins (allophycocyanins, phycocyanins, phycoerythrins). All carotenoids are labile towards oxygen, light, heat and acid (Liaaen-Jensen 1978). They are present in cells of all autotrophic organisms, being a functional part of the granular and lamellar structures of chromatophores. However, in the prokaryotic cells of Cyanobacteria, the pigments are located within the thylakoid membrane (Fogg *et al.* 1973).

Up to the present, over 30 forms of chlorophylls active in the photosynthesis of organic matter and over 600 carotenoid pigments have been found in cyanobacteria, algae and fungi (Liaaen-Jensen & Andrewes 1985).

The distribution of accessory pigments in the algae groups is quite unique (Rowan 1989, Wright *et al.* 1991, Bianchi *et al.* 1997, Schluter & Havskum 1997). For this reason, they act as potential taxonomic biomarkers of phytoplankton organisms. For example, fucoxanthin is considered to be a marker of diatoms, zeaxanthin – a marker of Cyanobacteria, 19′-hexanoyloxyfucoxanthin – of Prymnesiophyceae, alloxanthin – of cryptophytes, prasinoxanthin – of prasinophytes, peridinin – of dinoflagellates, chlorophyll *b* and lutein – of chlorophytes (Rowan 1989, Wright *et al.* 1991, Johnsen *et al.* 1994, Andersen *et al.* 1996). It should be noted that the marker pigments are not exclusive to any one group of algae. The presence of carotenoids in individual taxa is characterised by a considerable variability in their quantitative and qualitative attributes.

In the natural environment, the pigment composition may well vary with different ambient nutrient conditions. The concentrations of photosynthetic pigments in the sea are primarily dependent on the species composition and the photo-adaptive state of the phytoplankton present. Moreover, the pigment content of phytoplankton cells changes, *e.g.* while the phytoplankton cells undergo photoacclimation (Falkowski & LaRoche 1991), or when they are exposed to stress conditions, *e.g.* limitation of light and/or iron (Leeuwe & Stefels 1998).

Pigment studies have also led to a better understanding of succession in phytoplankton blooms in the upper water column, transformation of phytoplankton in the ocean interior (Llewellyn & Mantoura 1996) and the physiological condition of algae (Hallegraeff & Jeffrey 1985, Klein & Sournia 1987, Barlow *et al.* 1993a).

Pigments can provide a trophic characterisation of natural waters: besides being good chemotaxonomic biomarkers (Jeffrey *et al.* 1975, Gieskes & Kraay 1983, Wright & Jeffrey 1987, Goericke & Repeta 1992), they are also useful for indicating the trophic status of phytoplankton (Barlow *et al.* 1997). Furthermore, a pigment's distribution can yield information about light acclimation responses (Demers *et al.* 1991) and on the fate of those cells (Welschmeyer & Lorenzen 1985, Klein & Sournia 1987, Barlow *et al.* 1995).

All pigments participate in the photosynthesis of organic matter. On account of their specific absorption properties (carotenoids absorb light within the 400–500 nm wave band), they can play a double role in photosynthesis. A functional classification of carotenoids into two groups of pigments – photosynthetic and non-photosynthetic (mainly photoprotecting) – is commonly used in the literature (Bidigare *et al.* 1990a, Bricaud *et al.* 1995, Allali *et al.* 1996, Babin *et al.* 1996, Sadoudi *et al.* 1996, Bricaud *et al.* 1998). Photosynthetic carotenoids (PSC), also referred to as antennae, can assist photosynthesis by the transfer of part of the absorbed energy onto chlorophyll molecules. This group consists of the following carotenoids: fucoxanthin, 19′-hexanoyloxyfucoxanthin, 19′-butanoyloxyfucoxanthin, peridinin, prasinoxanthin and α-carotene. A second group, the photoprotecting (non-photosynthetic) carotenoids (PPC), protect the photosynthetic centre against the destructive influence of singlet oxygen and harmful radiation. This group includes antheraxanthin, diadinoxanthin, alloxanthin, diatoxanthin, dinoxanthin, lutein, violaxanthin, neoxanthin, zeaxanthin and β-carotene. Their absorbent properties are known to change along with certain variations in their chemical structure, such as the number of double bonds and the degree of cyclisation. Despite these conformational variations, however, often brought about by changes in the environment conditions, some carotenoids can simultaneously exist in both conformations (Koyama & Mukai 1993).

Carotenoids are also a structural part of photosystems. Apart from chlorophylls, proteins, lipids and other components, PS I (photosystem I) consists predominantly of carotenes, and PS II (photosystem II) of xanthophylls (Govindjee 1975). However, carotenoids are not the dominant component of either photosystem. A knowledge of the concentrations of the major pigments instead of chlorophyll *a* can be used in methods for estimating

the absorption coefficient of phytoplankton (Bidigare *et al.* 1990, Hoepffner & Sathyendranath 1991, Ondrusek *et al.* 1991, Lazzara *et al.* 1996, Lutz *et al.* 1996, Woźniak *et al.* 1999).

There are numerous techniques enabling the concentrations of individual phytoplankton pigments to be specified, from simple spectrophotometric and fluorometric methods for calculating the concentrations of chlorophyll *a*, *b*, *c* and their derivatives (Strickland & Parsons 1972), to chromatographic methods (TLC – thin layer chromatography, liquid chromatography).

In recent years, high-performance liquid chromatography (HPLC) has become a technique universally applied in the separation of phytoplankton pigments, which permits a precise ($< 5\%$, Latasa *et al.* 1996) quantitative and qualitative analysis of the complex pigment mixtures found in natural waters even in trace amounts < 1 μg dm^{-3} (Mantoura & Llewellyn 1983, Wright & Shearer 1984, Bidigare *et al.* 1985, Wright *et al.* 1991, Heukelem *et al.* 1994, Andersen *et al.* 1996, Roy *et al.* 1996, Yacobi *et al.* 1996, Schluter & Havskum 1997), as well as the qualitative analysis of phytoplankton groups (Yacobi *et al.* 1996, Schluter & Havskum 1997, Latasa & Bidigare 1998).

The aim of this paper is to present the results of research into pigment composition in marine water samples taken from the Gulf of Gdańsk during two periods: April 1999 and September 1999.

The hydrological, environmental and trophic conditions of the Gulf of Gdańsk are quite characteristic. From the geographical point of view it is part of the southern Baltic, a relatively shallow shelf sea with a limited exchange of water with the world ocean and a massive inflow of water from the River Wisła (Vistula). The Gulf of Gdańsk is a highly dynamic area with a mean salinity of *ca* 8 PSU. The impact of anthropogenic nitrogen and phosphorus derives mainly from the Wisła, whose basin makes up 60% of the Baltic catchment area. Hence the advanced eutrophication of the Gdańsk Basin (Renk 1991, 1993, Pastuszak 1995, Trzosińska & Łysiak-Pastuszak 1996, Pliński & Jóźwiak 1999). This state of trophicity is reflected by the diversity of phytoplankton in the Gulf. This phytoplankton has been the subject of study for several decades (Ringer 1973, Pliński *et al.* 1985, Pliński & Picińska 1986, Pliński 1995). The dominant groups of algae identified there are dinoflagellates, diatoms, chlorophytes, cyanobacteria and other flagellates (Ringer 1990, Pliński 1995). The species composition of the phytoplankton fluctuates seasonally; some of the species recorded are typically freshwater organisms.

In the existing literature, the chlorophyll *a* content has usually been identified spectrophotometrically (Renk 1991, 1993, Ochocki *et al.* 1995, Niemkiewicz & Wrzołek 1998). Data concerning the identification of other

phytoplankton pigments in the Gulf of Gdańsk by chromatographic techniques are few in number, and refer to selected phytoplankton samples (Łotocka & Falkowski 1994) and samples of bottom sediments (Kowalewska *et al.* 1996, Kowalewska 1997, Łotocka 1998).

2. Materials and methods

2.1. Collection of samples

The study area encompassed the whole Gulf of Gdańsk. The positions of the measuring stations are shown in Fig. 1. During two cruises – from 20 to 29 April 1999 and from 4 to 15 September 1999 – when a series of hydrological and optical measurements were made, the water was also sampled for pigment content analysis. The samples (0.5–1.5 dm^3) were taken with an SBE 32 bathometer, usually from three depths – the surface, the maximum fluorescence depth, and below the euphotic zone – and were immediately filtered through Whatman GF/F glass-fibre filters ($\phi = 25$ mm) under a gentle vacuum (< 0.6 atm). The filtration time did not exceed one hour. The samples were stored in liquid nitrogen (–196°C) until laboratory analysis to improve extraction efficiency and minimise pigment alterations (Mantoura *et al.* 1997).

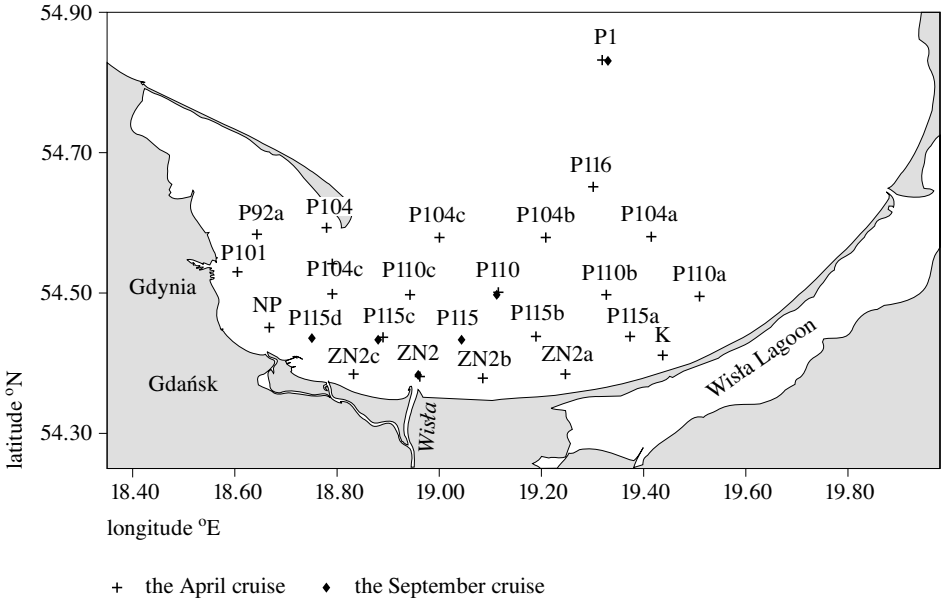


Fig. 1. The geographical position of the measuring stations in the Gulf of Gdańsk during the two cruises in April and September 1999

2.2. Extraction and chromatographic analysis

The frozen filters were placed in 3 ml of 90% acetone and extracted by grinding and sonication (20 min, 20 kHz) under darkness at 4°C for 2 hours, and finally centrifuged (20 min, temp. = 5°C, 4000 rpm) to remove cellular particle debris. The clarified extract was then subjected to chromatographic analysis.

Pigments were separated using the RP–HPLC technique. The system was equipped with an HP 1050 pump, an HP 1046 fluorescent detector, and an HP 1050 diode array detector, connected via a precolumn with a LichroCAR^{TM} Hypersil ODS analytical column (dimensions: 250 × 4 mm, particle size: 5 μm, Merck).

500 μl of clarified extract was mixed with 500 μl of ion-pairing reagent (1 M ammonium acetate – Mantoura & Llewellyn 1983, Wright *et al.* 1991) prior to injection. After 5 min mixing and equilibration, 100 μl of this solution was injected into the chromatographic column.

The pigments were separated in a gradient mixture of methanol, 1 M ammonium acetate and acetone. The composition of solvents varied from 100% A (80 : 20 methanol : 1 M ammonium acetate, v/v) to 100% B (60 : 40 methanol : acetone, v/v) along a 10 min linear gradient, followed by a 15 min 100% B isocratic hold with a 0.8 flow rate. After 25 min of analysis the solvent composition was returned to the initial conditions over 10 min, which allowed the system's equilibrium to be restored before the next sample injection. This gradient allowed for an adequate resolution of all dominant pigments (Mantoura & Llewellyn 1983, Barlow *et al.* 1993). The eluting pigments were detected using an absorbance detector set at 440 nm and a fluorescence detector set at $\lambda_{ex} = 431$ nm and $\lambda_{em} = 660$ nm to confirm the presence of chloropigments.

2.3. Qualification and quantification

The qualitative and quantitative analysis of the pigment content in natural samples was performed using commercially available pigment standards. High purity reference pigments isolated from reference monocultures were obtained from the International Agency for C^{14} Determination in Denmark (known until 1 January 2000 as VKI (Water Quality Institute)). The pigment standards (chlorophyll *a*, chlorophyll *b*, chlorophyll c_1, chlorophyll c_2, chlorophyll c_3, fucoxanthin, 19′-hexanoyloxyfucoxanthin, 19′-butanoyloxyfucoxanthin, diadinoxanthin, alloxanthin, antheraxanthin, aphanizophyll, echinenone, peridinin, neoxanthin, violaxanthin, prasinoxanthin, myxoxanthophyll, canthaxanthin, lutein, zeaxanthin, α-carotene, β-carotene) were subjected to chromatographic analysis in order to obtain calibration curves, detection limits and absorption spectra.

Qualitative analysis was based on a comparison of the retention times and the absorbance spectra of eluting peaks with those of the standards (Wright & Shearer 1984). Identification was confirmed by co-injection and on-line diode array spectra.

The quantitative characteristics of the pigments occurring in natural samples were based on the external standardisation eq. (1) (Mantoura & Repeta 1997).

$$C_p = \frac{A_p\, f_p\, \nu_{\text{ext}} 10^3}{\nu_{\text{inj}}\, \nu_{\text{filt}} B}. \tag{1}$$

This allows for a precise designation of the concentration of a particular pigment (C_p; ng dm^{-3}) with respect to the peak area (A_p; mAU) of the eluting pigment, the slope of the calibration curve (f_p; ng mAU^{-1}), the volume of filtered seawater (ν_{filt}; dm^3), the solvent used for the extraction (ν_{ext}; ml), the solvent injected into the chromatographic system (ν_{inj}; μl), and the buffer dilution factor B.

3. Results

3.1. Hydrological measurements

During the cruises in the Gulf of Gdańsk (20–29 April 1999 and 4–15 September 1999), water samples for pigment content analysis and the vertical distribution of temperature and salinity were taken simultaneously. The Gulf of Gdańsk has quite characteristic hydrological conditions: the influence of river water on temperature-salinity variability is very apparent. The average surface water temperature in April is 4–6°C. The minimum (3.82°C) was recorded at station P104c, which is under the distinct influence of open-sea water, while the maximum value of 11.37°C was measured at station ZN2b, situated just offshore near the Wisła mouth. In September, surface seawater temperatures are considerably higher, the upshot of high summer temperatures and prolonged exposure to light. The average temperature varied around 17°C. The influence of river water on salinity is particularly apparent: this falls to 0.5 PSU at station ZN2b, whereas the average salinity in the Gulf of Gdańsk lies between 6 and 8 PSU. Generally speaking, vertical mixing in both spring and autumn is responsible for the vertical homogeneity of the water masses at these seasons. Station ZN2 is an exception: the masses of freshwater flowing around it form a patch of warmer, less saline water at the surface.

3.2. Results of chromatographic analysis

Chromatographic analysis of the water samples revealed the presence of a wide range of pigments. The following groups were identified:

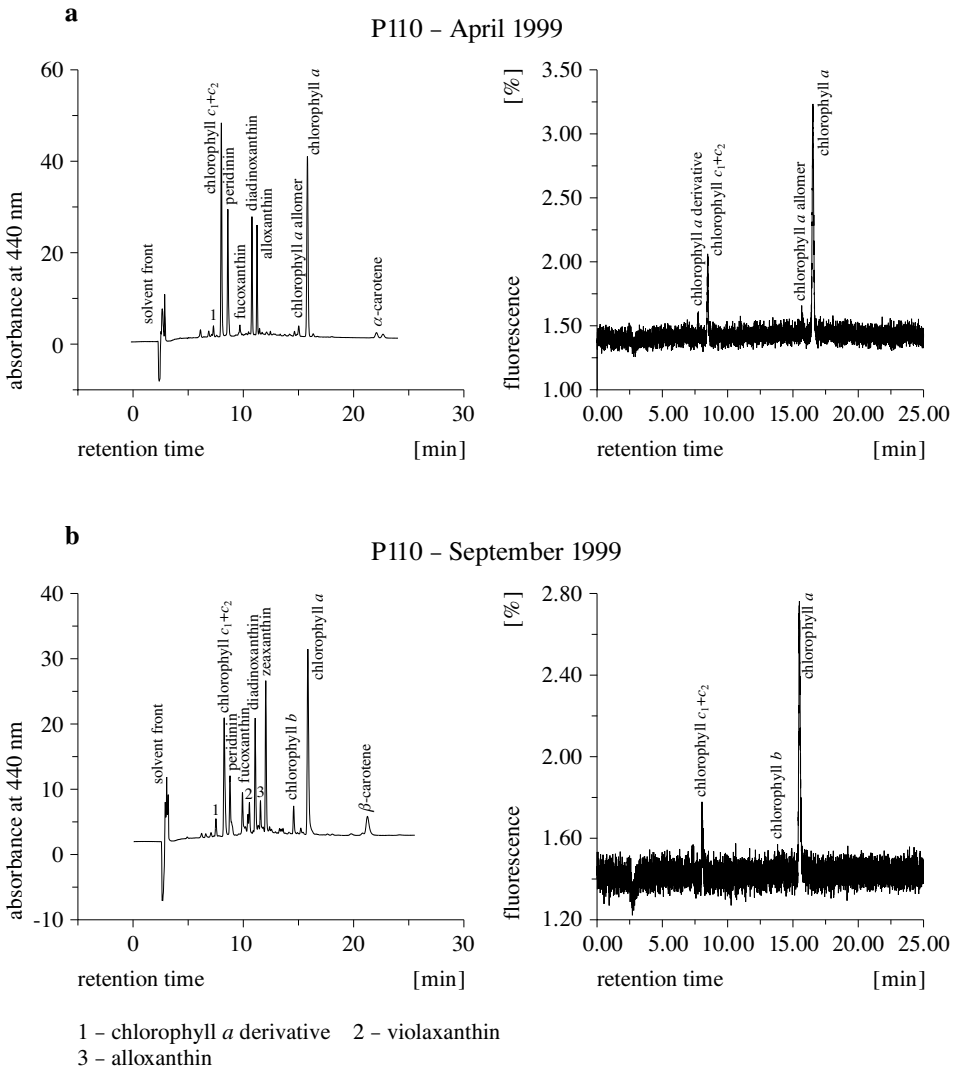


Fig. 2. Exemplary absorbance (λ = 440 nm) and fluorescence (λ_{ex} = 431 nm, λ_{em} = 660 nm) chromatograms obtained during HPLC resolution of a sample of surface seawater taken from station P110 in two periods: April 1999 (a), September 1999 (b). Chromatographic column: LichroCART^{TM} Hypersil ODS, dimensions: 250 × 4 mm, particle size 5 μm, Merck; mobile phase: 0 min 100% A linear gradient, 10 min 100% B isocratic hold, 25 min 100% B – final composition of mobile phase (solvent A – 80 : 20 methanol : 1 M ammonium acetate (v/v), solvent B – 60 : 40 methanol : acetone (v/v)); flow rate – 0.8 μl min^{-1}

chlorophylls a, $c_1 + c_2$, b; PSC – peridinin, fucoxanthin, α-carotene (detected only in the April samples); PPC – diadinoxanthin, alloxanthin, zeaxanthin, lutein (detected only in the April samples), neoxanthin, violaxanthin

and β-carotene. Exemplary chromatograms showing the resolution of the mixture of phytoplankton pigments from the samples collected at station P110 during both cruises are presented in Fig. 2. The results indicate that

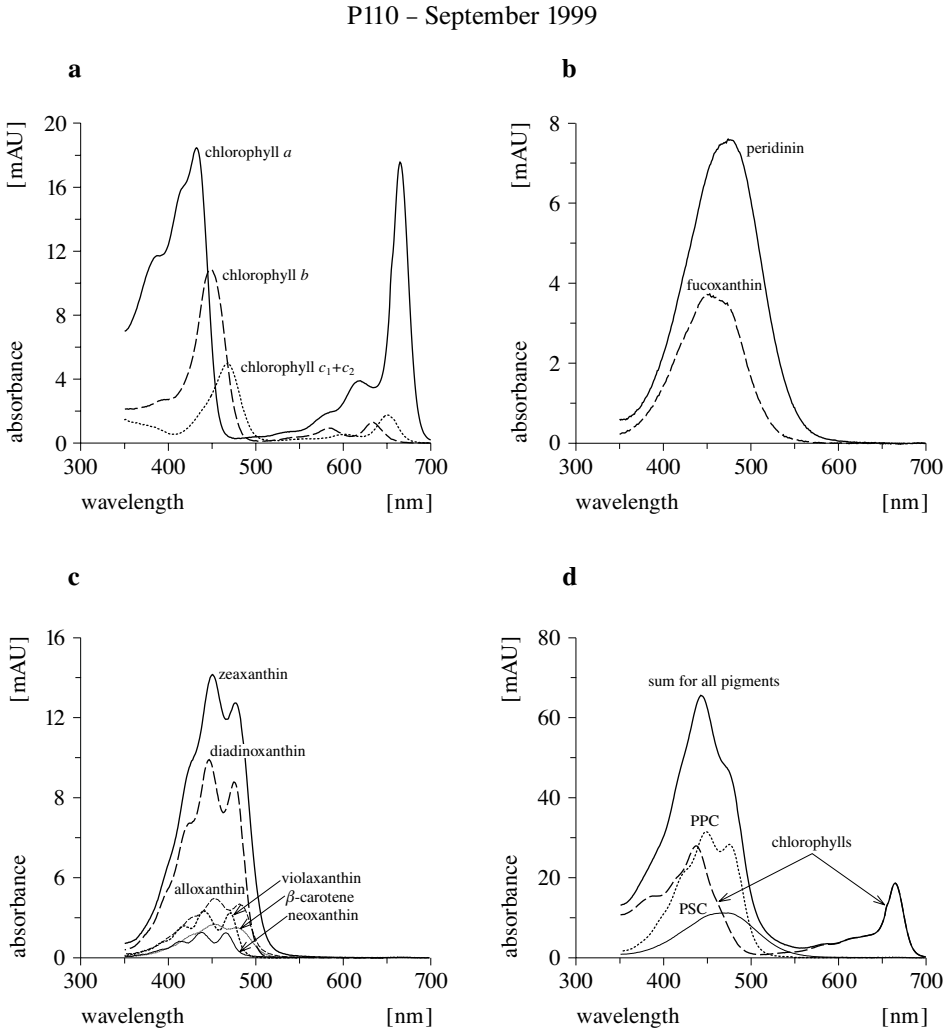


Fig. 3. Absorption spectra of phytoplankton pigments obtained by HPLC resolution of a sample of surface seawater from station P110 (September 1999) with the application of a diode array detector: absorption spectra of chlorophylls a, b, $c_1 + c_2$ (a), absorption spectra of photosynthetic carotenoids (peridinin and fucoxanthin) (b), absorption spectra of photoprotecting carotenoids (zeaxanthin, diadinoxanthin, violaxanthin, β-carotene, neoxanthin, alloxanthin) (c), a summary spectrum of all pigments present in the sample with specification of the various groups of component pigments (d)

chlorophylls c_1 and c_2 are eluted unresolved, as a single peak, as is the case with zeaxanthin and lutein. Complete resolution of these two pairs of pigments is possible only by using a polymer-packed chromatographic column (Heukelem *et al.* 1992). The chromatographic system used here cannot identify phycobilins, which are soluble in water (Lutz *et al.* 1996). The spectra of the pigments identified in these samples are shown in Fig. 3. As can be seen, the pigment spectra vary in the number of absorption bands, their positions and shapes.

The concentration of the identified pigments displayed a wide range of qualitative and quantitative changes in their composition, mainly related to the sampling depth. These relationships are presented in Fig. 4 for one station (P110) in both seasons. The range of changes in the concentration of all pigments occurring in natural samples of marine phytoplankton are summarised in Table 1.

Chlorophyll *a* was present in very much larger concentrations in comparison to the other pigments. The maximum chlorophyll *a* concentrations were recorded in the April samples, at intermediate sampling depths (5–10 m). A maximum concentration of 91.79 μg dm^{-3} was recorded in the surface layer at station K. During the September cruise, the largest concentrations were noted at the surface, and there was no deep chlorophyll maximum. The September maximum was 11.59 μg dm^{-3}, measured at the surface at station P110.

Chlorophylls c_1 + c_2 were identified in all samples. Concentrations of these pigments were about one order of magnitude less than the chlorophyll *a* concentrations: 0.04–11.79 μg dm^{-3} (April) and 0.02–0.88 μg dm^{-3} (September). Though present in considerably smaller amounts than the other chloropigments, chlorophyll *b* was not detected in all the samples: concentrations ranged from 0.06 to 1.68 μg dm^{-3} in spring and from 0.16 to 1.18 μg dm^{-3} in autumn. As chlorophyll *b* is regarded as a marker of Chlorophyceae, its occurrence is closely related to its presence in these species in natural phytoplankton populations. This pigment was found mainly in surface and intermediate water masses; it was absent below the euphotic zone in both seasons.

The photosynthetic carotenoids (peridinin, fucoxanthin and α-carotene) were present in a smaller range of concentrations, about one order of magnitude, than the total chlorophylls. Peridinin and fucoxanthin, taxonomical biomarkers of Dinoflagellates and Diatoms, were identified in most of the samples. In April, the concentrations detected included 0.015–6.31 μg dm^{-3} for fucoxanthin and 0.05–19.31 μg dm^{-3} for peridinin. α-Carotene, in concentrations from 0.06 to 0.58 μg dm^{-3}, was identified sporadically; it was not present in the September analysis. Fucoxanthin was identified in all samples

in concentrations from 0.02–0.43 μg dm^{-3}, unlike peridinin (0.11–1.13 μg dm^{-3}), which was not present in the samples from the deepest regions.

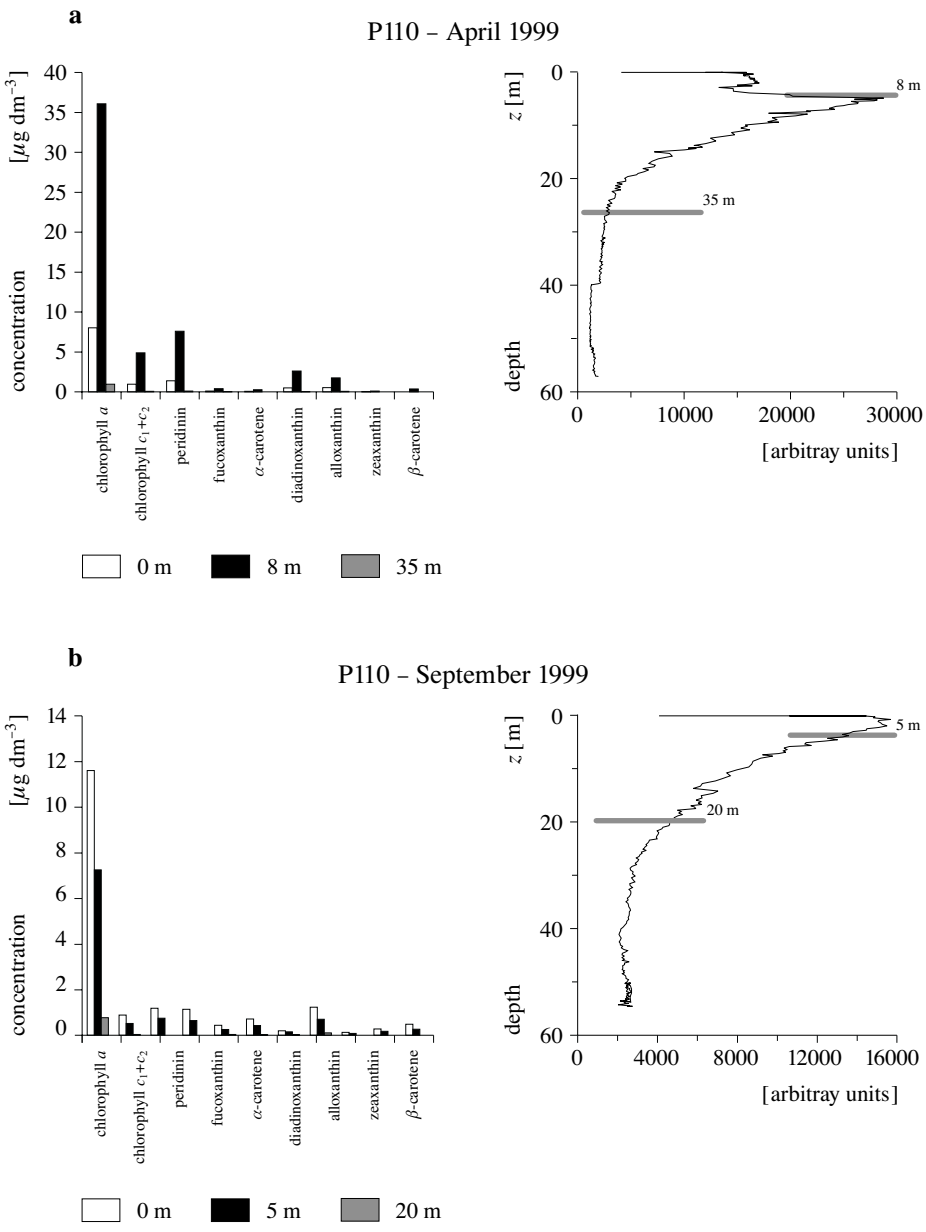


Fig. 4. Representative vertical distributions of concentrations of individual pigments identified in seawater samples from station P110 against a background of *in vivo* fluorescence profiles (fluorometer 'pump probe') in two seasons: April 1999 (a), September 1999 (b)

Table 1. The range of changes in concentration of pigments identified by HPLC in natural samples of marine phytoplankton for the two seasons

Pigment concentrations in natural samples [μg dm^{-3}]				
Pigment	April 20.04.1999–29.04.1999		September 04.09.1999–15.09.1999	
	Minimum	Maximum	Minimum	Maximum
Chlorophyll *a*	0.43 (30 m, P104b)	91.79 (0 m, K)	0.05 (20 m, P115)	11.59 (0 m, P110)
Chlorophyll $c_1 + c_2$	0.04 (30 m, P104b)	11.79 (0 m, K)	0.02 (20 m, P115)	0.88 (0 m, P110)
Chlorophyll *b*	0.06 (20 m, P110a)	1.68 (3 m, Zn2b)	0.16 (0 m, P116)	1.18 (0 m, P110)
Fucoxanthin	0.015 (30 m, P116)	6.31 (3 m, ZN2b)	0.02 (20 m, P115)	0.43 (0 m, P110)
Peridinin	0.05 (30 m, P104b)	19.31 (0 m, K)	0.11 (7 m, P116)	1.13 (0 m, P110)
α-Carotene	0.06 (10 m, P104c)	0.58 (0 m, K)		
Diadinoxanthin	0.022 (30 m, P116)	9.166 (0 m, K)	0.026 (20 m, P110)	0.71 (0 m, P110)
Alloxanthin	0.034 (40 m, P110b)	3.41 (0 m, K)	0.018 (20 m, P110)	0.198 (0 m, P110)
Zeaxanthin	0.02 (40 m, P110b)	0.24 (0 m, K)	0.04 (7 m, P116)	1.23 (0 m, P110)
β-Carotene	0.099 (10 m, P104c)	1.27 (0 m, K)	0.18 (5 m, P115)	0.49 (0 m, P110)
Lutein	0.07 (0 m, ZN2a)	0.93 (15 m, ZN2b)		
Neoxanthin	0.02 (0 m, P101)	0.21 (3 m, ZN2b)	0.038 (5 m, P115)	0.128 (0 m, P110)
Violaxanthin	0.09 (0 m, ZN2b)	0.51 (3 m, ZN2b)	0.07 (5 m, P115)	0.28 (0 m, P110)

The photoprotecting carotenoids are a diverse group consisting of the following pigments: diadinoxanthin (marker of Diatoms), alloxanthin (marker of Cryptophyceae), zeaxanthin (marker of Cyanobacteria), lutein (marker of Chlorophytes), neoxanthin and violaxanthin (identified in green algae) and β-carotene, which is present in many species. Diadinoxanthin, alloxanthin and zeaxanthin were widely distributed in the water and present in higher concentrations than the other pigments (see Table 1). Lutein, neoxanthin, violaxanthin and β-carotene were not identified in every sample. Violaxanthin and neoxanthin always appeared together. The two seasons were qualitatively distinct, owing to the presence of these pigments. Lutein was not detected in the September analysis.

The spatial distribution of the sum of all detected pigments in the surface waters of the Gulf of Gdańsk in April is presented in Fig. 5. As indicated in Table 1, the maximum concentrations of different pigments may be situated at various depths at different stations. The largest numbers of qualitative and quantitative results were obtained at station K in April and P110 in September. Uniquely, for the purposes of this research, station K lay

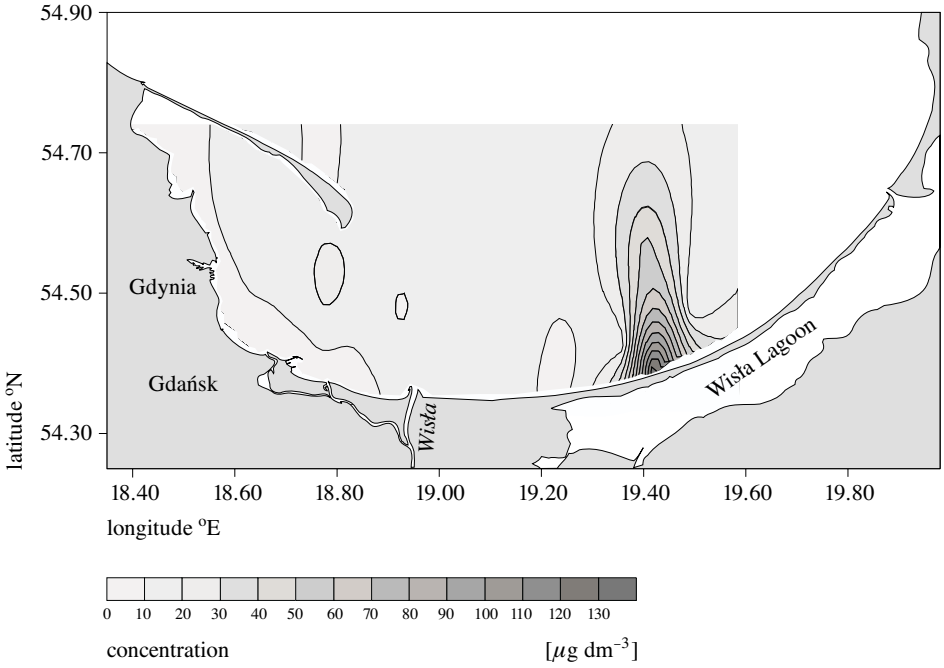


Fig. 5. The spatial distribution of the concentrations of all identified pigments at the sea surface during the cruise in April 1999

just off shore, in an area where the water masses are affected by human pressure. Such pressure may be the cause of the nutrient inflow into the marine environment.

Located in the central part of the Gulf, station P110 in September was noteworthy: despite the thermal stabilisation of autumn waters (∼17°C), the influence of the specific local circulation of brackish and fresh waters (Kowalik 1990) can affect the trophic interaction with phytoplankton around this station. Carrying a large nutrient content (mainly nitrate and phosphate), Wisła waters have a significant influence on the phytoplankton community (Trzosińska 1990).

4. Discussion

Photosynthetic carotenoids (PSC = peridinin + fucoxanthin + α-carotene) and photoprotecting carotenoids (PPC = diadinoxanthin + alloxanthin + zeaxanthin + lutein + neoxanthin + violaxanthin + β-carotene) were positively correlated with chlorophyll *a* content in all samples (Fig. 6). The April and September PSC pigment concentrations ranged from 0.07 to 21.23 μg dm^{-3} and from 0.02–1.56 μg dm^{-3} respectively, the relevant PPC values varied from 0.05 to 14.08 μg dm^{-3} and from 0.04 to 1.79 μg dm^{-3}. While there were no great qualitative variations among the pigments detected in the two seasons, the differences among the quantitative characteristics were significant. The April PSC and PPC concentrations assumed similar values. In September, however, PPCs were present in very much greater quantities than PSCs.

The fact that there is a considerable PPC concentration in phytoplankton cells suggests that pigments of this kind are involved in a mechanism serving to protect their genetic material from harmful UV radiation. The effect of non-photosynthetic pigments is expected to be restricted to wavelengths shorter than 550 nm. The composition of the auxiliary pigments and the relative importance of their absorption are variable. A relationship exists between the concentration of the major pigments and absorption at different wavelengths (Hoepffner & Sathyendranath 1991).

Phytoplankton has adapted to specific water types by evolving specific pigment combinations, which provide photoprotection and/or photosynthetically usable radiation for optimised growth rates. So, for example, the zeaxanthin found in the deeper water column may result from the vertical mixing of surface populations of cyanobacteria and/or the presence of deeper-living prochlorophytes.

There was a quantitative dissimilarity among the detected pigments in both cruises. In spite of the fairly small qualitative differences in the two measuring seasons (the September samples did not contain lutein

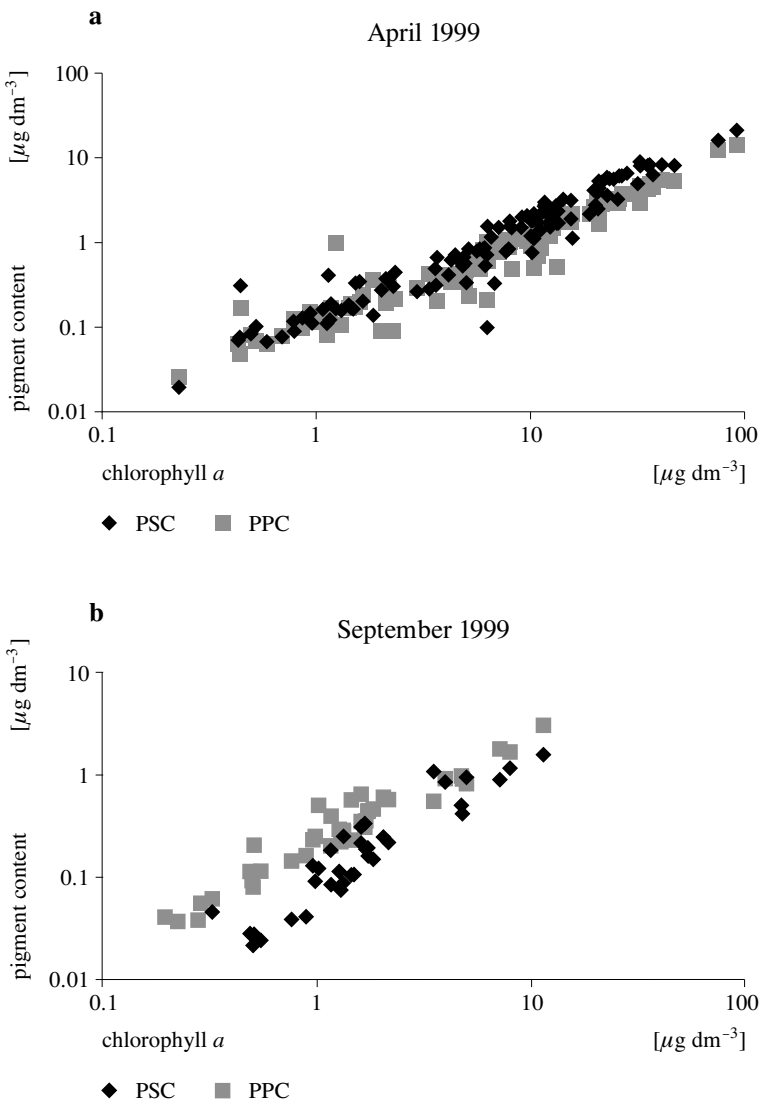
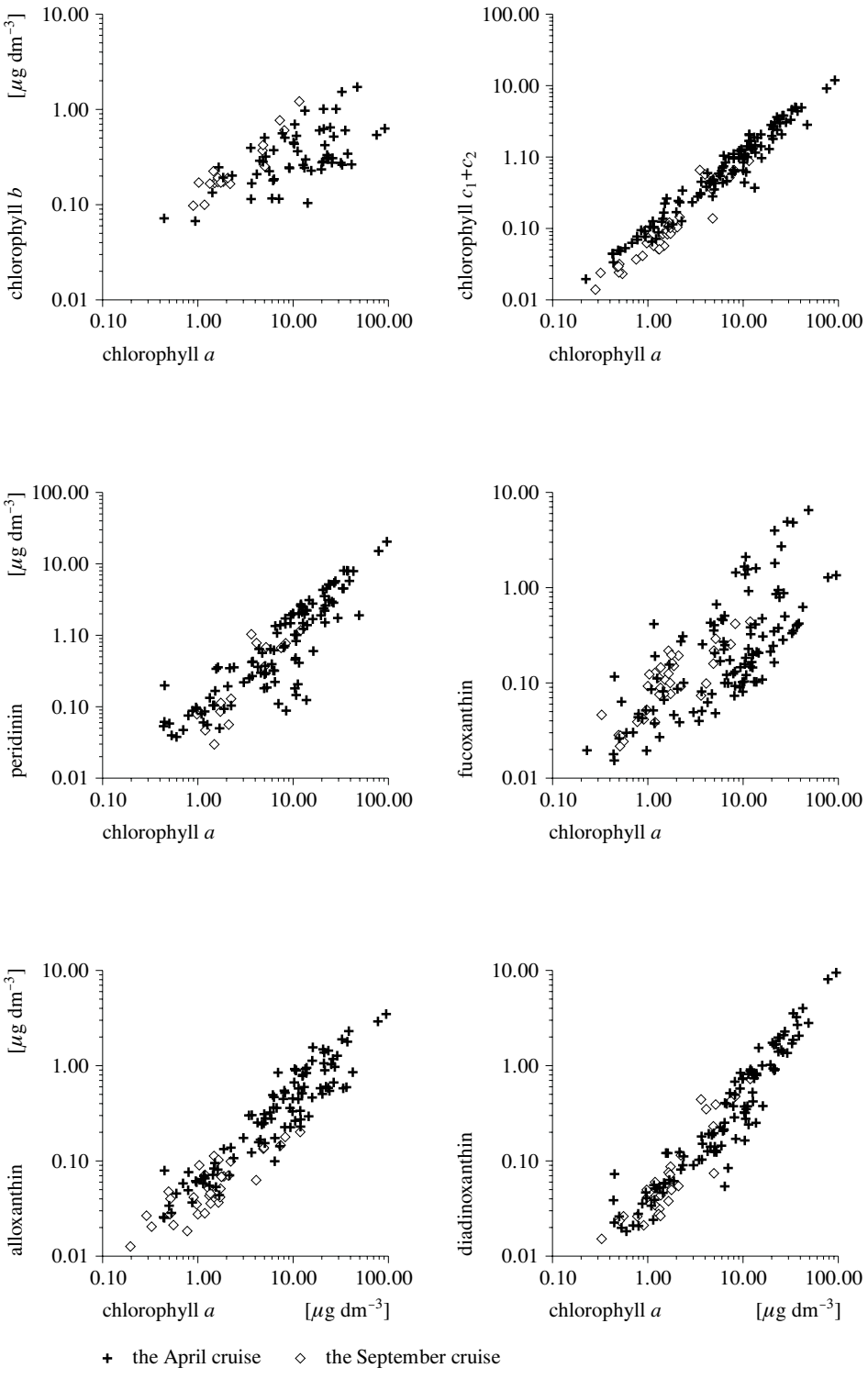


Fig. 6. Relationship between the concentrations of photosynthetic and photoprotecting carotenoid pigments and the concentration of chlorophyll *a* in samples taken during two cruises in the Gulf of Gdańsk: April 1999 (a), September 1999 (b)

or α-carotene), the pigment concentrations in April were one order of magnitude higher in comparison to the September samples. This suggests that a phytoplankton bloom could have occurred in the spring.

Furthermore, analysis of the phytoplankton pigment contents in natural water samples revealed the presence of different dominant carotenoid


chlorophyll b [μg dm−3]
chlorophyll a
chlorophyll c1+c2 [μg dm−3]
chlorophyll a
peridinin [μg dm−3]
chlorophyll a
fucoxanthin [μg dm−3]
chlorophyll a
alloxanthin [μg dm−3]
chlorophyll a [μg dm−3]
diadinoxanthin [μg dm−3]
chlorophyll a [μg dm−3]
0.01
0.10
1.00
1.10
10.00
100.00
+ the April cruise
◇ the September cruise

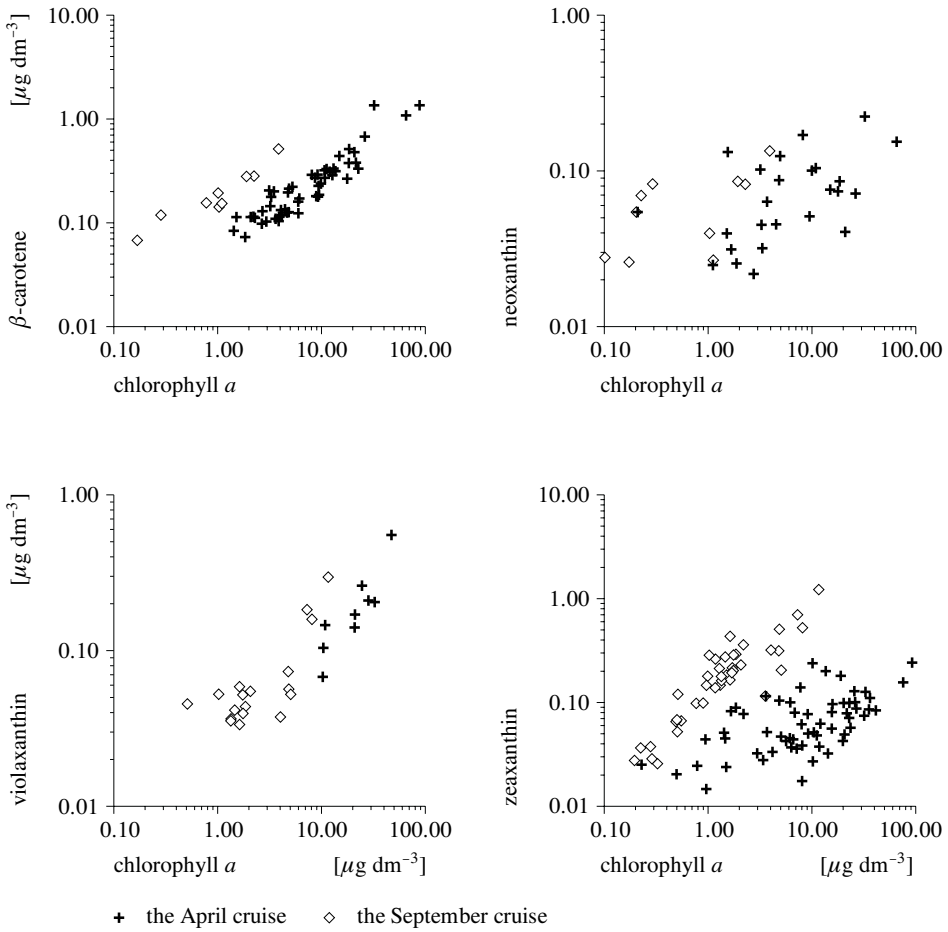


Fig. 7. Relationships between concentrations of pigments detected in samples from two seasons (April, September 1999) and chlorophyll *a* content

pigments in the two seasons. In April the dominant PSCs were peridinin (0.05–19.31 μg dm^{-3}) and fucoxanthin (0.02–6.31 μg dm^{-3}), whereas in September peridinin (0.11–1.13 μg dm^{-3}) was by far the most abundant pigment of this group. Within the PPC group, diadinoxanthin (0.02–9.17 μg dm^{-3}) and alloxanthin (0.03–3.41 μg dm^{-3}) were the principal pigments in April, while in September zeaxanthin (0.04–1.23 μg dm^{-3}) was dominant.

Dependences between pigment concentration and chlorophyll *a* concentration for the two periods are presented in Fig. 7. As can be seen, not all pigments show a linear correlation with chlorophyll *a*. Although the April chlorophyll *b* data displays considerable scatter, the September results are correlated linearly. The same applies to zeaxanthin. Relationships between neoxanthin and violaxanthin are characterised by an accidental

distribution of concentration towards the chlorophyll *a* content, whereas chlorophyll $c_1 + c_2$ and diadinoxanthin show a distinct linear correlation. The distribution of the β-carotene concentration is characteristic.

Further studies are required to establish the links between phytoplankton pigmentation and the availability of spectral irradiance, and how they relate to depth-dependent variations in phytoplankton growth rate. This would make it feasible to model the photosynthetic quantum yield from a knowledge of the light intensity and the given pigment distribution in the water column.

The absorption and scattering of light by phytoplankton are generally considered to be responsible for most of the variations in the optical properties of open seawaters. A knowledge of these inherent optical properties of phytoplankton is therefore a necessary prerequisite for any model of light penetration (Sathyendranath & Platt 1988) and light utilisation by plankton (Smith *et al.* 1989). Absorption of light by phytoplankton populations is an optical property involved in the prediction of oceanic (marine) primary production from light and pigment data. More recently, photosynthetic pigment distributions have been employed to identify different algae groups and to estimate primary production rates bio-optically (Bidigare *et al.* 1992). So for these reasons, the results presented in this paper can be used in further environmental analysis.

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