Pigment composition in relation to phytoplankton community structure and nutrient content in the Baltic Sea*

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Abstract

The concentration of chlorophylls and carotenoids with respect to communities of characteristic phytoplankton species and hydrological parameters, such as temperature, salinity and nutrients were analysed. Samples from the southern Baltic were taken during three periods: spring 1999, autumn 1999 and 2000 during cruises of r/v 'Oceania' in this area. The seasonal differences between the phytoplankton species composition and pigmentation of samples (measured by HPLC) were noted. The total biomass of the spring phytoplankton population was 11–15 times greater than that of the autumn populations. However, the phytoplankton community was more diverse in the two autumns, whereas the spring population was almost mono-taxonomic: $>80\%$ of the total biomass consisted of dinophytes. The total content of chlorophylls (a, b, $c1+c2$) was about 20 times higher in spring. Moreover, in spring the concentrations of

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photosynthetic carotenoids (with dominant perdinin) were 2–4 times higher than those of the photoprotecting carotenoids (with dominant diadinoxanthin), whereas in the two autumns the situation was reversed: PPC concentrations (with dominant zeaxanthin and diadinoxanthin) exceeded those of PSC (with dominant fucoxanthin) by c. 3–10 times. Pigment markers have proved to be extremely useful biomarkers for elucidating the composition of phytoplankton populations in natural samples.

1. Introduction

The presence of pigments in water samples, as well as their qualitative and quantitative diversity, depends on the interaction of numerous environmental factors. In particular, pigment quality emerges from the species composition of phytoplankton occurring in a given geographical region and at a specific season.

Chlorophylls and carotenoids are widely distributed in nature and their concentrations are closely related to the presence of specific organisms in cells (Liaaen-Jensen & Andrewes 1985). The quantities and relative proportions of pigments in particular species are highly specific. For this reason, the pigment composition of plant cells is regarded as a taxonomic feature. A number of pigments, mainly xanthophylls, the dominant group of pigments in phytoplankton cells, are therefore used as taxonomic markers of groups of algae (Jeffrey & Vesk 1997, Meyer-Harms & von Bodungen 1997, Schluter & Havskum 1997). It should be noted that the marker pigments are not exclusive to any one group of algae, e.g. dinoflagellates may contain pigments characteristic of diatoms, prymnesiophytes may contain the same pigments as pelagophytes. Chlorophyll-containing pigments are also promising markers for some planktonic bacteria, such as purple and green phototrophic bacteria (Hurley & Watras 1991). In recent years, many attempts have been made to examine the phytoplankton community structure by using pigment fingerprints (Gieskes et al. 1988, Wright et al. 1996).

The carotenoid content in particular groups of algae, even between cells of the same organisms within a single group, is characterised by highly variable qualitative and quantitative parameters. This may be due to the influence of abiotic environmental factors, such as temperature and salinity, the nutrient content, and the underwater light field, biotic factors related genetically to the cells or to their state of development, cell size and physiological state, the phytoplankton species composition and their number. It is further recognised that physical processes affect the structure of phytoplankton communities. Water movements may cause vertical mixing, which can enhance nutrient transport from deeper waters to the surface layer, and introduce fresh nutrients to the euphotic layer, where

mixing and the hydrological conditions are highly dynamic. All these factors are expected to modify the composition of the phytoplankton community (Ahel et al. 1996, Obayashi et al. 2001, Vidussi et al. 2001).

For a given size of cell, the pigment concentration it contains depends on its physiological status, principally on its photoadaptive capabilities (Kim & Philpot 2000). A pigment's ability to absorb light over different wavelength ranges (chlorophylls absorb in the 430–450 and 600–690 nm ranges, carotenoids in the 400–500 nm range) and to adapt to the chromatic conditions and the light intensity can lead to modification of its pigment composition (Falkowski & LaRoche 1991).

The part that pigments play in the cells of autotrophic organisms is related to the process by which absorbed light is transferred and utilised. This can be done by way of photosynthesis or the light energy can be dissipated thermally by the photoprotecting carotenoids (Bidigare et al. 1990a, Demmig-Adams 1990, Allali et al. 1997). The accessory pigment system present in cells (photosynthetic carotenoids – PSC and photoprotecting carotenoids – PPC) enable the organism to extend its optical collection window, thereby improving absorption efficiencies and adaptation capabilities (Bidigare et al. 1990, Hoepffner & Sathyendranath 1991), which consequently screen the photosynthetic apparatus from excess light (Anning et al. 2000).

HPLC-based pigment analysis has become an increasingly routine method of characterising pico- and nano- plankton assemblages in stable open ocean systems, estuarine waters and sediments (Gieskes & Kraay 1983, Mantoura & Llewellyn 1983, Bianchi et al. 1996, Lazzara et al. 1996). This technique has provided oceanographers with a powerful tool for studying the processes affecting the phytoplankton pigment pool (Trees et al. 2000). This method is considered a precise (Latasa et al. 1996) and highly sophisticated tool for isolating even trace amounts of the pigments present in natural samples.

HPLC-based chlorophyll and carotenoid signatures are now routinely used as a taxonomic complement to cell identification (Andersen et al. 1996, Mackey et al. 1996, Roy et al. 1996). HPLC pigment analysis may be insufficient for understanding the finer scales of phytoplankton dynamics and cannot generally be used to make taxonomic distinctions within classes. Certainly, no single technique or methodology is ideal for resolving all the information relevant to the structure and dynamics of a phytoplankton community. Therefore, the role of HPLC pigment analysis in the quantitative assessment of phytoplankton composition should be considered complementary to, but not exclusively a replacement for, microscopic enumeration (Tester et al. 1995).

The aim of the present study was to compare the vertical distributions of particular pigments obtained by RP–HPLC with the distribution of individual phytoplankton groups against the background of changes in selected hydrological conditions. The study area was located in the southern Baltic, where discrete samples were collected in three seasons: spring 1999, and the autumns of 1999 and 2000. This provided an opportunity to seek regional and temporal dependencies and relationships. Moreover, an attempt was made to assess the proportional contribution of the various phytoplankton groups to the chlorophyll *a* biomass using pigment signatures.

The papers dealing with the pigmentation of Baltic phytoplankton are few in number. Previous studies of phytoplankton pigment concentrations in the Baltic Sea were based on the spectrophotometric designation mainly of chlorophylls (Renk 1993, Ochocki et al. 1995, Niemkiewicz & Wrzołek 1998). Only a few papers treat of pigments identified by the use of HPLC: in sediments (Kowalewska 1997, Łotocka 1998), or in macroalgae (Bianchi et al. 1997) and in phytoplankton (Łotocka & Falkowski 1994, Stoń & Kosakowska 2000). The results presented in the present paper have enabled us to extend our knowledge of Baltic phytoplankton pigments and to enrich the database of particular pigment concentrations, essential for verifying light absorption and primary production models.

2. Materials and methods

Investigation area and sample collection

The study focused on the southern Baltic Sea. Water samples and instrumental measurements were taken during three cruises of r/v 'Oceania' in different periods: spring 1999 (20–28 April 1999), autumn 1999 (4–14 September 1999) and autumn 2000 (20 September – 1 October 2000). The characteristic parameters of the measuring stations are given in Table 1, but their spatial distribution is presented in Fig. 1. While the series of hydrological and optical measurements were carried out as standard procedures, the water for analysis of the pigment content and phytoplankton composition was sampled at the same time. Guildline 87104 or Sea-Bird SBE9 probes were used for the CTD measurements, and an Ecomonitor 'pump-probe' fluorometer for the fluorimetric measurements. Nutrients were measured using the investigation procedure routinely used by the Institute of Meteorology and Water Management during their monitoring programmes in the southern Baltic (Grasshoff et al. 1974). Water for pigment and plankton analysis was taken with an SBE32 bathometer from three depths: the surface layer, the layer of maximum fluorescence (5–10 m) and the

layer below the euphotic zone (15–60 m). For analysis of the phytoplankton species composition – 250 ml of seawater was preserved with Lugol solution and stored in the dark at 4°C (Willen 1962).

Table 1. The geographical characteristics of measuring stations during three cruises on r/v 'Oceania' in the Southern Baltic

Station symbol	Latitude	Longitude	Date of sampling	Total depth [m]
P1	54°50.000 N	19°19.300 E	25.04.1999	106
P110c	54°29.882 N	18°56.103 E	26.04.1999	68
P110c	54°29.900 N	18°56.475 E	28.04.1999	69
P1	54°49.860 N	19°19.839 E	05.09.1999	105
P39	54°44.559 N	15°07.891 E	11.09.1999	66
P3	55°13.097 N	17°03.778 E	25.09.2000	92
IO7	55°08.147 N	17°44.796 E	29.09.2000	45

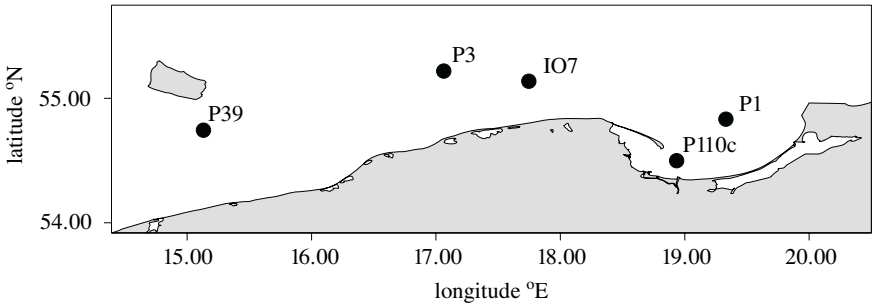

Fig. 1. The spatial distribution of the measuring stations during the sampling periods

The samples (0.5–2 dm^3) were filtered through Whatman GF/F glass-fibre filters (ϕ = 25 mm) under a gentle vacuum (< 0.6 atm) for no longer than one hour, and then immediately frozen with liquid nitrogen in a Dewar flask (–196°C). To minimise alterations to pigments and improve extraction efficiency, the filters were stored in liquid nitrogen and then in a deep-freeze until the laboratory analysis could be carried out (Mantoura et al. 1997).

Extraction and high-performance liquid chromatography

Pigments were extracted by grinding and sonication (5 min, 20 kHz, Cole Parmer, 4710 Series) in 3 cm^3 of 90% acetone as extraction solvent

at 4°C in the dark for 2 hours, after which the extracts were centrifuged (20 min, 5°C, 2150 g, Beckman, GS-6R), clarified and then subjected to chromatographic analysis.

The pigments were separated from the extract by RP–HPLC, in which the isolation procedure is based on interaction between the solvent mixture (mobile phase) and the chromatographic column filler (stationary phase). The procedure was introduced by Mantoura (Mantoura & Llewellyn 1983) and subsequently adopted by other researchers (Wright et al. 1991, Barlow et al. 1993, Stoń & Kosakowska 2002). The chromatographic system is equipped with an HP1050 pump, an HP1046 fluorescence detector, an HP1100 diode array detector, a Rheodyne injector (100 μl loop) connected via a precolumn with a LichroCART^{TM} Hypersil ODS (dimension: 250 $\times$ 4 mm, particle size 5 mm, MERCK) analytical column. Pigment detection is based on absorbance measurements at $\lambda = 440$ nm (in the absorbance spectrum of each pigment, chlorophylls and carotenoids absorb a certain amount of light of this wavelength). Parallel fluorescence measurements were also made with a detector set at $\lambda_{ex} = 431$ nm (excitation wavelength) and $\lambda_{em} = 660$ nm (emission) in order to confirm the presence of chloropigments in the samples.

The solvent composition varied from 100% of primary eluant A (A = 80:20 methanol:1M ammonium acetate, v/v) to 100% of secondary eluant B (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 ml min^{-1} flow rate. After 25 min of analysis the solvent composition was returned to the initial conditions for 10 min, which allowed the system equilibrium to be restored prior to the next sample injection.

Qualification and quantification analyses of all pigments isolated from natural samples were performed using commercially available pigment standards obtained from The International Agency for ^{14}C Determination, DHI Institute for Water and Environment, Denmark. All the calibration parameters (retention times, absorption spectra, calibration curves, detection limits) required to identify and quantify the concentrations of pigments isolated from natural sample solutions were obtained (Mantoura & Repeta 1997, Stoń & Kosakowska 2002).

Microscopic-based phytoplankton enumeration

For microscopic analysis, phytoplankton samples were preserved with Lugol solution. The major taxonomic groups were determined by using the inverted microscope (Axiovert M40) and Utermöhl's sedimentation techniques (Dybern et al. 1976).

3. Results

Hydrological background

The vertical profiles of temperature and salinity reveal different hydrodynamic conditions during the sampling periods. The springtime water masses in the Gulf of Gdańsk exhibited a below-surface (0–8 m) thermocline with a temperature gradient of 0.625 m^{-1}: the temperature dropped from >9 to 4°C and then remained stable from there to the bottom. The salinity increased downwards from the surface from 3.5 to 7.1 PSU, forming a halocline at the same depth (8 m). Such a situation is specific to the Gulf of Gdańsk region, the hydrodynamic characteristics of which are subject to the considerable influence of river water masses. The surface temperature and surface salinity at the open-sea station were respectively lower (5.6°C) and higher (6.9 PSU), and both a thermocline and a halocline developed at 40–60 m; the highest temperature and salinity were recorded at the bottom (6.5°C, 11.5 PSU).

The hydrological situations during the two autumn cruises (September 1999, September 2000) were comparable to each other. The thermoclines were present at about 40 m, the haloclines lay deeper (50–60 m). The water temperature at the surface was 18.9°C and 13.5°C in 1999 and 2000 respectively, and fell to c. 4.6°C in the deepest waters. The salinity profiles were also similar: 7.1–7.3 PSU from the surface down to 40–60 m, and increasing to almost 13 PSU at the bottom. Such a situation is typical of stratified waters.

The spring and autumn hydrology thus exhibited stratified situations, with a pycnocline separating the less saline (6–7 PSU) and warmer surface waters (especially after the summer warming-up) from the deeper, more saline (11–13 PSU) waters.

In addition to the hydrodynamic structure of the water masses, the nutrient contents were also examined. The concentrations of various nutrient components were measured and analysed: nitrogen – nitrates, nitrites and ammonium; phosphorus – mainly phosphates; silicon – principally silicate ions. In the spring samples, the vertical profiles of NO_3, PO_4 and SiO_4 all increased with depth (see Figs. 2a, d, e). The surface concentrations fell to a minimum (>0.13 μmol dm^{-3} of PO_4; $\sim$0.01 μmol dm^{-3} of NO_3; $\sim$0.03 μmol dm^{-3} of NO_2; <0.7 μmol dm^{-3} of NH_4, $\sim$9 μmol dm^{-3} of SiO_4) in April 1999; the maximum values were recorded in the deepest waters (1.19–3.96 μmol dm^{-3} of PO_4; $\sim$8 μmol dm^{-3} of NO_3; $\sim$0.5 μmol dm^{-3} of NO_2; $\sim$1 μmol dm^{-3} of NH_4 and 27.68–47.25 μmol dm^{-3} of SiO_4). Only the distribution of the NH_4 concentration fluctuates with depth (Fig. 2c). In the case of the open-sea samples, the distribution of nitrate demonstrates

a concentration near zero in the 0–30 m layer, after which it gradually increases with depth, whereas nitrite reaches a maximum concentration of 0.43 μmol dm^{-3} at 60 m. As a consequence of these vertical nitrate and nitrite distributions, the vertical distribution of total nitrogen is oscillatory in shape.

The surface readings in the two September analyses were lower than those in the April one. The nutrients took the following values: PO_4 < 0.05 μmol dm^{-3}, NO_3 and NO_2 c. 0 μmol dm^{-3}, NH_4 < 0.6 μmol dm^{-3} and SiO_4 < 6.5 μmol dm^{-3}. The vertical nutrient concentrations were homogenous from the surface down to 20 m, after which they increased towards the bottom. The profile of the variation of ion concentrations with sampling depths is presented in Fig. 2. The *in situ* fluorimetric measurements confirmed the presence of phytoplankton in the surface layer; their occurrence there contributed to the lower nutrient concentrations recorded.

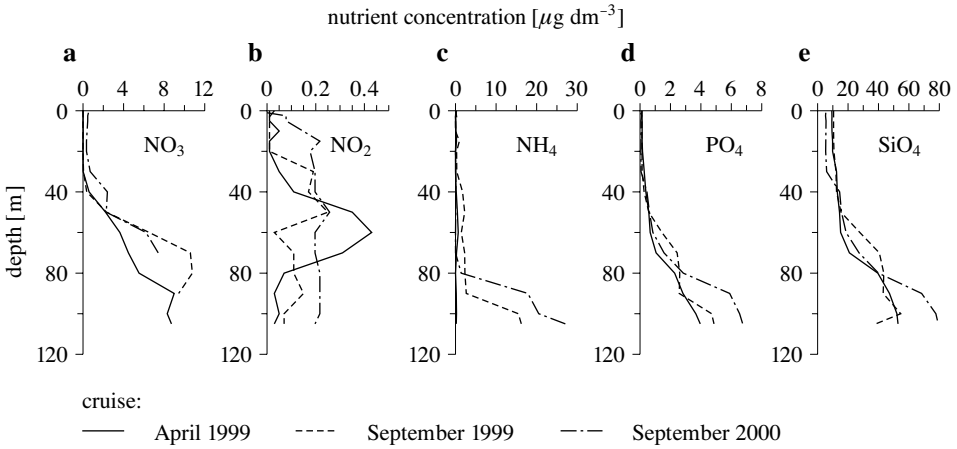

Fig. 2. Concentrations of nutrients [μg dm^{-3}] recorded at station P1 in three seasons: April 1999, September 1999, September 2000

Comparison of the nutrient situation in the three sampling periods leads us to assume that the nutrient deficit in the surface layer is the effect of phytoplankton growth and feeding activities. Nutrients are accumulated in winter and consumed in spring by the quickly growing algae. Diatoms are the only significant organisms that incorporate silicate and their growth results in a decreasing amount of silicate dissolved in water. Nitrogen compounds, especially NO_3, are the most important nutrient component in phytoplankton feeding processes. This is reflected in the vertical profiles of nitrates, which in the euphotic layer are almost entirely consumed by algae.

Pigment investigations

Pigments were isolated from natural samples, separated and detected by the use of the sensitive RP–HPLC method. Chlorophylls a, b, $c1 + c2$, the photosynthetic carotenoids (PSC) peridinin, fucoxanthin and α-carotene, and photoprotecting carotenoids (PPC) such as diadinoxanthin, alloxanthin, zeaxanthin, lutein, neoxanthin, violaxanthin and β-carotene were detected in the samples. The chlorophyll a content in springtime samples ranges from 23–41 μg dm^{-3} for surface samples and decreases with depth, reaching the smallest values (0.5–0.7 μg dm^{-3}) at the deepest sampling level. Peridinin was the dominant PSC in the April samples (range of concentrations 4.32–7.52 μg dm^{-3} for surface water). α-carotene – in concentrations of 0.04–0.14 μg dm^{-3} was only present at the open-sea station. Moreover, lutein and violaxanthin were not detected at station P1. Among the PPC, diadinoxanthin, was present in large quantities in a few species and was recorded in significant amounts (1.82–3.89 μg dm^{-3} at the surface). The vertical pigment variations show the highest concentration at the surface and a marked decrease below the euphotic layer to concentrations approaching detection thresholds (e.g. the vertical distribution in Fig. 3a). In the samples from the deepest waters, the respective concentrations of PSC and PPC were on average > 50 and > 28 times lower than at those found at the surface. Furthermore, *in vivo* fluorescence measurements decreased markedly from the surface to the bottom, and this was corroborated by the chlorophyll pigment pattern.

The concentration of pigments detected in the autumn samples of both years was 10–4 times smaller than in the April samples. The chlorophyll a concentration reached the highest values – 1.46–2.33 μg dm^{-3} – mostly in the layer of maximum fluorescence (7–12 m). α-carotene and lutein were entirely absent in these two seasons. Chlorophyll $c3$ was identified in one case, a sample taken at station P39, situated on the open sea near Bornholm. The dominant PSC in both years was fucoxanthin, a marker of diatoms, while zeaxanthin was the prevalent PPC in September 1999, but not in 2000, when diadinoxanthin was present in the largest amounts. The total PSC concentrations ranged from 0.02 to 0.27 μg dm^{-3}, those of PPC from 0.06 to 0.61; the minimum values are associated with the deepest sampling level. An example of the vertical distribution pattern of individual pigments recorded in autumn is shown in Figs. 3b, c.

Taxonomic group contributions

The contribution of the taxonomic groups to the phytoplankton community structure was studied microscopically. In the April samples, seven algae

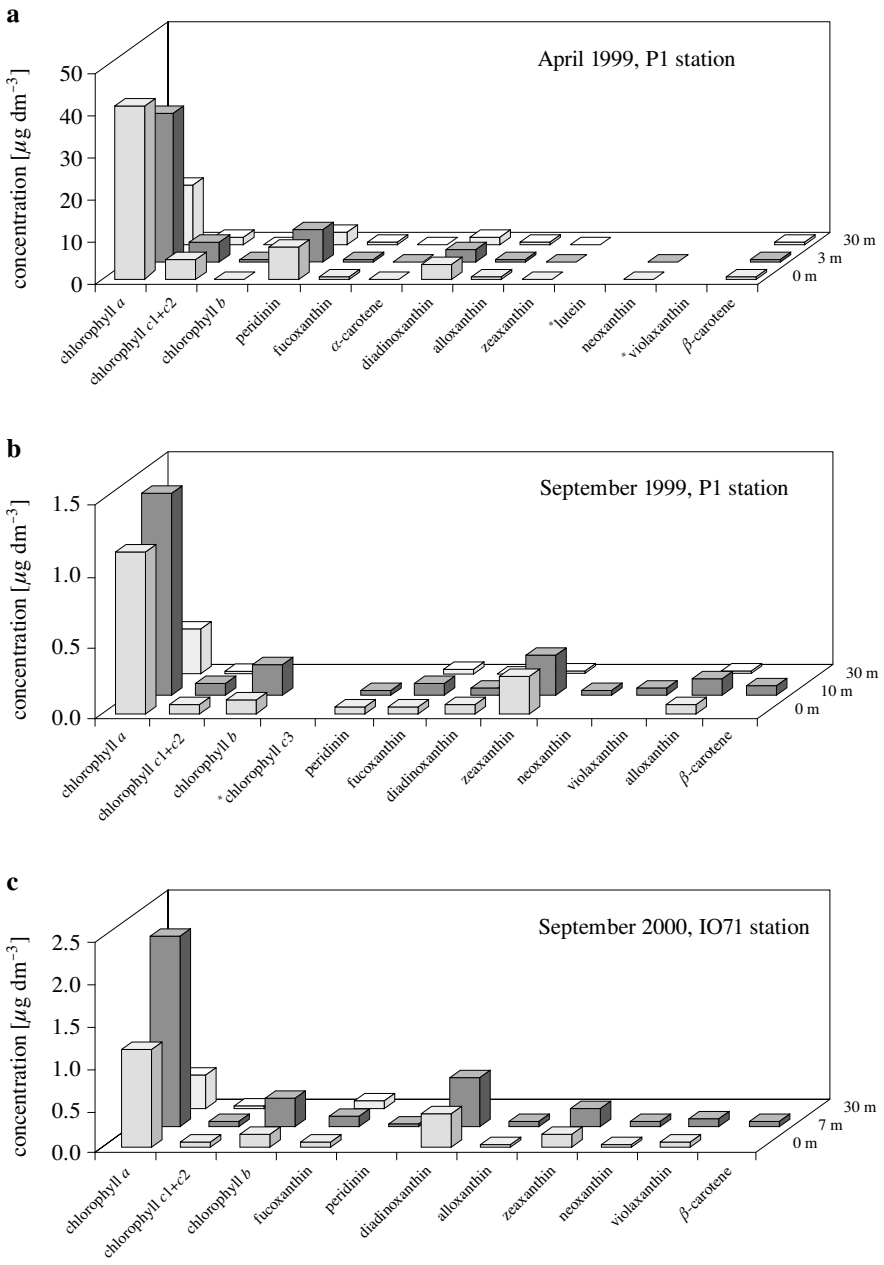

Fig. 3. Concentration of pigments [μg dm^{-3}] identified at different depths in three different seasons: April 1999 (a), September 1999 (b), September 2000 (c); (an asterisk ($*$) next to the pigment names indicates that the pigment in question was recorded during the measuring period but at a given station was below the detection level)

groups were identified: diatoms, chlorophytes, cryptophytes, cyanobacteria, dinophytes, euglenophytes and 'others' – organisms < 15 mm in size. In the spring phytoplankton community, dinophytes were predominant (81–97% of the total biomass of the surface population in the open Baltic and the Gulf of Gdańsk). This group was represented by *Peridiniella catenata*, the dominant species in the sample. It is thus regarded as a late-spring bloom consisting exclusively of dinophytes. Only in the sample from the layer at 7 m was the biomass of diatoms higher (56%) than that of dinophytes (35%). Diatoms were recorded only in the Gulf samples (1–55% of the total biomass) with the dominant species *Nitzschia* spp. and *Nitzschia delicatissima*. The percentage contribution of the other identified algae groups to the total biomass was relatively small (see Table 2): chlorophytes (0.75–3.6%) with *Monoraphidium griffithii*, *Monoraphidium* spp., *Pandorina* spp., *Scenedesmus quadricauda* as dominant species; cryptophytes (0.03–0.15%), but in the sample from the maximum fluorescence level the contribution was 0.6%; cyanobacteria (0.28–2.18%) represented mainly by *Aphanizomenon* spp. and *Merismopedia gluaca*.

The autumn phytoplankton community was more diverse than the almost mono-taxonomic one characteristic of April, different percentage ratios of taxonomic groups to total organic mass and different species being identified in the two autumn periods.

Dinophytes were present in both years (15.57–62.34% of the total surface water biomass), but *Heterocapsa triquetra* and *Prorocentrum minimum* dominated in September 1999. *Gyrodinium* spp. and *P. minimum* were the dominant dinophytes in the September 2000 samples. Chlorophyceae were present in large numbers in September 1999 (16.34–27.61% of total biomass), whereas in the 2000 samples they made up only a small part of the biomass (0.09–0.24%). In 1999 the Chlorophyceae were represented by *Carteria* spp., but in 2000 by the prevalent *Monoraphidium* spp. (also dominant in the spring samples) and *Oocystis* spp. The situation was reversed in the case of diatoms: large amounts were present in September 2000 (19.30–28.64%) and in both seasons were represented by *Cyclotella meneghiniana*. Furthermore, the 'other' species also made a substantial contribution – 12.45–29.78% in 1999 and 17.4–37.67% in 2000. This group was represented mainly by nanoplanktonic organisms (3–6 μm).

Cryptophytes and cyanobacteria were found in the samples, too. In general, the cyanobacteria were far outnumbered by cryptophytes, of which *Aphanizomenon* spp. were dominant in 1999 (as in the April samples), and *Gomphosphaeria* spp. and *Microcystis aeruginosa* in the September 2000 samples. Prasinophytes were present only in the autumn 2000 samples; represented by *Pyramimonas* spp.; like the Euglenophycea, they made a only

Table 2. Characterisation of phytoplankton species on the basis of microscopic numeration: number (N – [number of organisms × 10^4 × dm^{-3}]) of identified individuals within one species, total biomass (B – [mm^3 dm^{-3}]) and dominant representative of species (R)

Algae species		April 1999				September 1999			September 2000	
		P1/3 m	P110c/0 m	P110c/7 m	P110c/0 m	P1/0 m	P1/10 m	P39/1 m	P3/0 m	IO7/0 m
diatoms	N	–	115.9	76.6	10.2	0.75	0.12	0.52	0.96	3.72
	B	–	0.688	1.365	0.1	0.023	0.121	0.01	0.137	0.194
	R	–	*Nitzschia delicatissima*	*Nitzschia* spp.	*Nitzschia* spp.	*Cyclotella meneghiniana*	*Chaetoceros danicus*	*Cyclotella meneghiniana*	*Cyclotella meneghiniana*	*Cyclotella meneghiniana*
chloro-phytes	N	1.79	22.66	3.75	4.02	13.27	30.47	19.78	0.27	0.04
	B	0.065	0.132	0.088	0.075	0.067	0.158	0.099	0.002	0.001
	R	*Pandorina* spp.	*Monoraphidium griffithii*	*Scenedesmus quadricauda* *Monoraphidium griffithii*	*Monoraphidium* spp.	*Carteria* spp.	*Carteria* spp.	*Carteria* spp.	*Monoraphidium* spp.	*Oocystis* sp.
crypto-phytes	N	2.25	1.63	3.63	7.00	6.54	37.02	2.93	70.65	10.64
	B	0.006	0.002	0.015	0.015	0.013	0.070	0.006	0.193	0.036
	R	5–12 μm	5–12 μm	12–20 μm	5–12 μm	5–12 μm	5–12 μm	2–5 μm	5–10 μm	5–10 μm
cyano-bacteria	N	1.17	3.09	2.70	2.51	–	–	4.53	1.97	1.83
	B	0.023	0.018	0.053	0.029	–	–	0.191	0.024	0.060
	R	*Aphanizo-menon* spp.	*Merismopedia gluaca*	*Aphanizomenon* spp.	*Aphanizomenon* spp. *Merismopedia* sp.	–	–	*Aphanizomnon* spp.	*Gomphosphaeria* spp.	*Microcystis aeruginosa*
dino-phytes	N	105.14	62.09	11.74	116.57	15.05	1.65	8.77	48.77	8.88
	B	7.895	4.208	0.856	8.960	0.257	0.043	0.101	0.224	0.105
	R	*Peridiniella catenata;* and 6–10 μm	*Peridiniella catenata;* and 6–10 μm	*Peridiniella catenata;* and 6–10 μm	*Peridiniella catenata;* and 6–10 μm	*Heterocapsa triquetra;* and 6–10 μm	*Heterocapsa triquetra;* and 6–10 μm	*Prorocentrum minimum;* and 6–10 μm	*Gyrodinium* spp.; and 6–10 μm	*Prorocentrum minimum;* and 6–10 μm
eugleno-phytes	N	0.19	–	–	0.07	–	3.82	0.26	0.52	1.29
	B	0.003	–	–	0.001	–	0.057	0.004	0.005	0.013
	R		–	–		–				
prasino-phytes	N	–	–	–	–	–	–	–	0.52	5.19
	B	–	–	–	–	–	–	–	0.001	0.013
	R	–	–	–	–	–	–	–	*Pyramimonas* spp.	*Pyramimonas* spp.
other	N	142.0	211.0	15.0	491.5	86.7	226.2	286.7	242.5	492.2
	B	0.097	0.119	0.075	0.847	0.051	0.121	0.174	0.123	0.255
	R	3–6 μm	3–6 μm	3–6 μm	6–10 μm	3–6 μm	3–6 μm	3–6 μm	3–6 μm	3–6 μm

small contribution to the total biomass: 0.18–1.92%. The numbers and total biomass of phytoplankton in southern Baltic waters in 1999 and 2000 are given in Table 2.

4. Discussion

The phytoplankton pigment composition was significantly different in each of the three seasons. The pigment concentrations identified in the spring samples were considerably higher than those noted in the autumn samples. With respect to the total content of chlorophylls in the surface water, the April samples contained about 20 times more chlorophyll pigments than either of the two September samples. Moreover, there were distinct seasonal differences in the concentrations of the carotenoid groups. In April, the total PSC concentration (with peridinin dominant) exceeded the total PPC concentration (where diadinoxanthin was present in significant amounts) by an average of 2–4 times. The reverse situation is characteristic of autumn, and this was indeed the case in both years. The total PPC content (with different characteristic dominant markers: zeaxanthin in 1999, and diadinoxanthin in 2000) exceeded that of PSC (where fucoxanthin was predominant in both seasons) by a factor of 3–10 times. Additionally, as far as the percentage contribution of a particular group of carotenoids to the total quantity of identified pigments is concerned, there was also considerable seasonal differentiation. For example, PSC in April ranged from 13.8–17.8%, whereas in September 1999 the figure was 4.7–7.5% and in September a year later 4.2–3.2%. The seasonal relationships of PPC content to total pigment content were reversed: in spring PPC made up from 5.9 to 9.2% of the total pigmentation, but the autumn the percentages ranged from 18.5–21.7% in 1999 and 28.0–32.1% in 2000. The higher peridinin concentration in the spring samples suggests that a spring bloom of dinophytes took place. Represented by *P. catenata*, dinophytes constituted the largest proportion of the in spring phytoplankton population. Similar blooms of dinophytes in spring have been reported by e.g. Pliński (1995), Wasmund et al. (1998), Niemkiewicz (1999). Some authors reported a dinophyte bloom in summer with *H. triquetra* the dominant species (Łotocka & Falkowski 1994). The specific pigment composition and concentration in the two autumns were reflected in the plant abundance specific to these seasons. Our investigation confirmed that the autumn phytoplankton community was not homogenous and comprised mainly cryptophytes (containing alloxanthin), cyanobacteria (containing zeaxanthin), diatoms (containing fucoxanthin) (Pliński 1995, Niemkiewicz & Wrzołek 1998, Niemkiewicz 1999). An example of the percentage of marker carotenoids detected in a sample in relation to the percentage

biomass of particular identified groups of algae is presented in Fig. 4. This confirms that phytoplankton carotenoids can be used to predict the presence of certain phytoplankton species in natural waters with satisfactory precision (e.g. neoxanthin and violaxanthin (16%) vs chlorophytes (17%), zeaxanthin (27%) vs cyanobacteria (33%)) although some incompatibilities may be due to the presence of nano- and picoplankton (Henriksen et al. 2002). The small proportion of diatoms (2%) vs fucoxanthin (22%) suggests that fucoxanthin-containing cells may have made a contribution to the 'other' phytoplankton category.

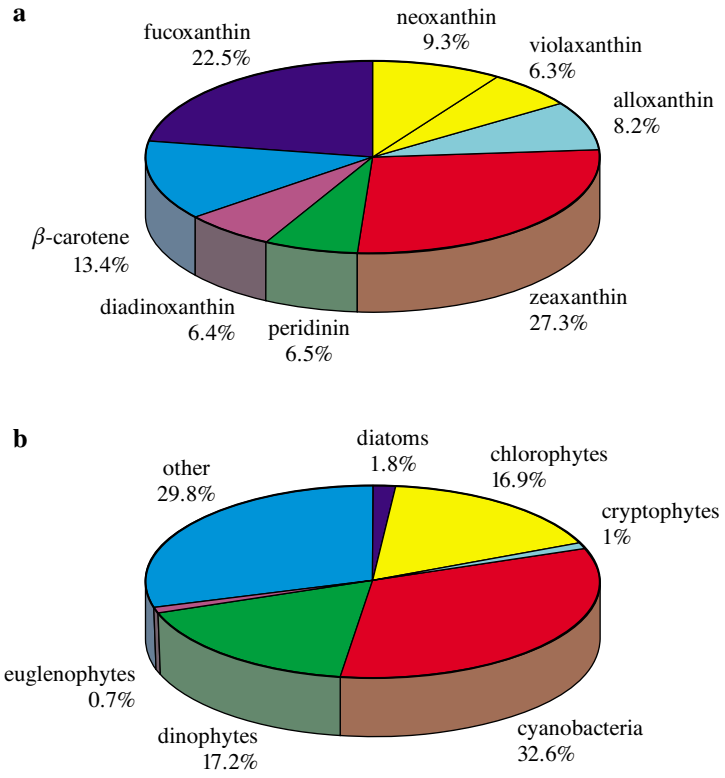

Fig. 4. Percentage contribution of detected carotenoids, used as marker pigments, in relation to the percentage of identified phytoplankton species in the total phytoplankton biomass exemplified by station P39 (September 2000). Individual colours correspond to the pigment marker – phytoplankton species relation

Furthermore, the higher concentration of PSC may have resulted from the chromatic and light-intensity adaptation of phytoplankton cells to specific light conditions. The reverse situation in autumn suggests that the light intensity and long-term exposure of plankton cells to light may

contribute to producing pigments with the ability to protect the reaction centre against photooxidation and damage (Anning et al. 2000, Eisner et al. 2000, Trees et al. 2000).

Of all the environmental factors affecting the changes in pigment quality and quantity in water samples, the phytoplankton species composition is the most important one. The large variation in chlorophyll *a* concentration in the present observations (< 50 μg dm^{-3} in spring and < 3 μg dm^{-3} in the autumns) may have been due to seasonal variations in the phytoplankton assemblage and the complex hydrographic conditions. The total biomass of the spring phytoplankton population was 11–15 times greater than that of the autumn populations.

The growth of algae requires certain preconditions: a seed population, nutrients in abundance, and hydrological and light conditions favourable to development. Temperature is one of the most important ecological parameters influencing the growth and biological activity of plankton cells. The differences in classes and species of algae identified in the three seasons may have been due to hydrological and meteorological parameters, which generate optimal conditions for the growth of particular classes of algae. The formation of the thermocline also commences in April, and the progressive stratification of the water column throughout the summer results in the formation of a deep chlorophyll maximum accompanied by low pigment levels at the surface. The erosion of the thermocline in autumn again induces nutrient replenishment of the upper layers (Barlow et al. 1997).

In our investigations, the nutrients, especially nitrates, were thought to be the factor limiting the plankton growth rate. By the end of April and in the Septembers, nitrate and ammonium salts were almost completely exhausted. While the diatoms did make a contribution to the phytoplankton composition, they were never present in dominant quantities – this is indicated by the residual silicate pool (≈ 10 μg dm^{-3}). The silicate and nitrate concentrations were similar to those in previous years and are well documented (Pastuszak 1995, Wasmund et al. 1998, Łysiak-Pastuszak 2000).

Nutrient depletion reduced the cellular content of chlorophylls and carotenoids and affected all pigment ratios. The most pronounced effects of nutrient depletion related to N-depletion. The trend is reversed with regard to pigment ratios (the marker xanthophyll) as a consequence of reduced growth rates within both phytoplankton groups (Goericke & Montoya 1998). In addition to non-systematic changes in pigment ratios resulting from nutrient-depletion or from changes in growth rates, the magnitude of pigment ratio changes due to nutrient-depletion varies considerably between phytoplankton groups.

It has been demonstrated that variations in environmental conditions can change the ratio of the logarithm of total accessory pigments to the logarithm of the total chlorophyll *a* content in a given phytoplankton species composition. For example, in Trees et al. (2000), this ratio reached a remarkably constant value of 0.93, whereas our investigations in discrete samples yielded 0.45. This could be the result of other environmental factors, not investigated in this work, affecting the composition of the detected pigments. Finding the relationships between the changes in the light field, other biotic factors, and pigment abundance in the water samples require further analysis.

5. Conclusions

- The combination of cell taxonomy and pigment analysis provided more detailed information about the physiological state of phytoplankton.
- Chlorophyll *a* can be used to estimate the phytoplankton biomass, but only microscopic analysis resolves questions of species composition or morphological variation.
- The seasonal differences between sample pigmentation and phytoplankton species composition are shown up by this work. Moreover, a number of xanthophylls have proved to be extremely useful biomarkers for elucidating the composition of phytoplankton populations.
- Phytoplankton size structure, species and pigment composition and temporal dynamics are important determinants of trophic interactions in marine ecosystems.

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