# The influence of biotic factors on phytoplankton pigment composition and resources in Baltic ecosystems: new analytical results*

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**Abstract**

Mathematical expressions were derived describing the distribution and concentration of individual phytoplankton pigments with respect to biotic factors in the southern Baltic. Relationships were established between the chlorophyll *a* concentration and the total phytoplankton biomass (represented by the organic carbon content), as well as between the concentration of marker pigments and the biomasses of the corresponding phytoplankton classes. Knowledge of chlorophyll *a* concentrations allows the phytoplankton biomass to be estimated with a precision characterised by relative statistical errors according to logarithmic statistics of $\sigma_- =$ ca 56%. The best approximation was obtained for the dependence of the *Bacillariophyceae* biomass on the fucoxanthin concentration ($\sigma_- = 60\%$), *Chlorophyceae* on the lutein concentration ($\sigma_- = 48\%$), and the total biomass of *Dinophyceae*, *Bacillariophyceae* and *Euglenophyceae* on the concentration of

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diadinoxanthin, the main carotenoid pigment present in cells of species from these classes ($\sigma_{-} = 60\%$).

## 1. Introduction

The pigments present in the natural environment are a large group of compounds differing in their chemical structures and properties. Their research history goes back to the beginning of the 19th century. Widely distributed in nature, they have been found in the cells of higher plants, algae, fungi, bacteria and some animals. These coloured compounds can be divided into three main groups: chlorophylls – over 50 different kinds have been discovered so far (Scheer 1991); carotenoids – more than 750 are known (Britton et al. 2003), and over 100 of these have been isolated from marine organisms (Liaaen-Jensen 1978); phycobilins (biliproteins) – about 40 different types have been identified in cryptophytes, red algae and cyanobacteria (Toole & Allnutt 2003). In recent years, a fourth group of pigments with distinct properties, the so-called mycosporins, has been distinguished (Carreto et al. 1990). The ability to absorb visible light has determined the main role of these pigments: the utilisation of this energy in photosynthesis (Woźniak & Dera 2007). As is well-known, chlorophyll *a* is the pigment basic to this process, while other pigments fulfil accessory functions, such as assisting – by transferring part of the absorbed energy to the chlorophyll *a* molecule (these are the 'antenna' pigments, i.e. the other chlorophylls, photosynthetic carotenoids (PSC) and phycobilins), and protecting – by preventing the destruction and photo-oxidation of chlorophyll *a* (photoprotecting carotenoids (PPC) and mycosporins). The pigments are not restricted to these functions, however; under certain conditions of stress, PSC and PPC pigments can fulfil both functions interchangeably.

The pigment compositions of phytoplankton cells and their mutual proportions are characteristic of different classes of algae and of cyanobacteria. Despite being a unique taxonomic feature of phytoplankton (Rowan 1989, Wright et al. 1991), the qualitative and quantitative proportions of pigments have been shown experimentally to differ even in the cells of organisms belonging to the same class. Some carotenoids that are distinctly dominant quantitatively are regarded as taxonomic markers of phytoplankton. For example, fucoxanthin is considered to be the marker of diatoms (*Bacillariophyceae*); likewise, zeaxanthin is regarded as the marker of blue-green algae (cyanobacteria), alloxanthin that of cryptophytes (*Cryptophyceae*), 19'hex-fucoxanthin that of prymnesiophytes (*Prymnesiophyceae*), prasinoxanthin that of prasinophytes (*Prasinophyceae*), peridinin that of dinophytes (*Dinophyceae*), and chlorophyll *b*, neoxanthin and lutein

are the markers of green algae (*Chlorophyceae*) (Andersen et al. 1996, Jeffrey & Vesk 1997). It has become common practice to determine the phytoplankton community structure on the basis of pigment compositions and concentrations (Latelier et al. 1993, Mackey et al. 1996, Rodriguez et al. 2002).

The occurrence of pigments in the marine environment is determined by both biotic and abiotic factors. As already stated, pigments are components of phytoplankton cells, and their compositions and concentrations are intimately related to the taxon to which they belong. In addition, the physiological preferences of phytoplankton organisms and their biological adaptation to migration, region of phytoplankton growth, season and period of day, can all modify the pigment content. Abiotic factors, such as underwater light yields (governing the intensity and chromatic adaptation of phytoplankton cells), nutrient content and hydrological parameters (temperature, salinity gradients, density stratification), not to mention the water dynamics, affect qualitative and quantitative pigment compositions in phytoplankton cells. These factors are all responsible for the wide spectrum of pigment concentrations in the marine environment. Measurements have shown that concentrations of pigments identified in different regions of the World Ocean can vary by as much as four orders of magnitude, as in the case of chlorophyll *a* (Woźniak et al. 2003).

The influence of environmental parameters on the main pigment groups has been discussed extensively in the literature, although most papers describe dependences on biotic and abiotic factors on the basis of data derived from experiments carried out under controlled laboratory conditions (Schlüter et al. 2000, Henriksen et al. 2002, Staehr et al. 2002) or for case 1 waters (Majchrowski 2001, Bouman et al. 2005). The problems concerning the changes taking place in qualitative and quantitative pigment compositions as a result of the reaction of phytoplankton cells to variable environmental factors are poorly understood as far as case 2 waters, such as those in the Baltic Sea, are concerned.

The occurrence, and the qualitative and quantitative regularities of different chlorophylls and carotenoids measured by reverse-phase high performance liquid chromatography (RP-HPLC) have been investigated in the southern Baltic in recent years (Łotocka & Falkowski 1994, Ooms 1996, Stoń & Kosakowska 2000, 2002a, Stoń et al. 2002, Jodłowska & Latała 2003, Schlüter et al. 2004, Eker-Develi et al. 2008).

The main aim of the present research was to derive, for the southern Baltic Sea, a sequence of relationships describing the distribution and concentration of individual phytoplankton pigments and also the biotic factors (the presence of individual algal classes) affecting their

characteristics. In other words, the objective was to find mathematical expressions and statistical relationships describing the total phytoplankton biomass (represented by the carbon content) from known chlorophyll *a* concentrations, and the biomasses of particular phytoplankton classes based on marker pigment concentrations.

## 2. Material and methods

### 2.1. Study area

The study focused on the southern Baltic Sea. The experimental material used to the measurements of chlorophyll and carotenoid contents, and assessments of the phytoplankton species composition, were performed during 12 cruises on r/v 'Oceania' in 1999–2005. Figure 1 shows the geographical positions of the measurement stations in different seasons and Table 1 specifies the material collected during each cruise.

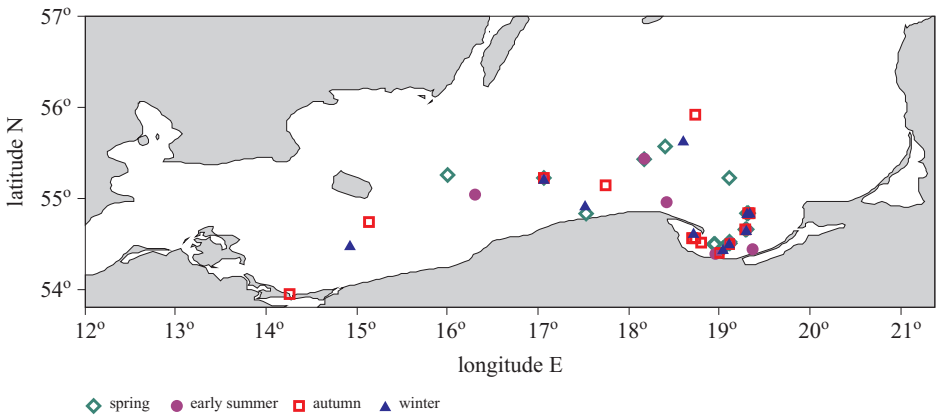


**Figure 1.** Spatial distribution of measuring stations in 1999–2005 in the southern Baltic

### 2.2. Methodology of pigment analysis

Sea water was sampled from the surface layer and different depths with an SBE32 bathometer. The samples (0.2–2 $dm^3$) were passed through glass-fibre filters (Whatman GF/F; $\phi = 25$ mm) under a gentle vacuum ($> 40$ kPa); the filtration time did not exceed one hour. The filters were immediately frozen in liquid nitrogen ($-196$°C) and stored in a deep-freeze ($-80$°C) until the laboratory experiments could be carried out (Mantoura et al. 1997).

Pigments were extracted from phytoplankton cells with 90% acetone

**Table 1.** Specification of empirical material collected in different seasons in the southern Baltic in 1999–2005

| Season | Sampling month | Number of samples collected for pigment and species indentification | Total |
|---|---|---|---|
| spring | April 1999 | 4 | 19 |
| | April 2003 | 3 | |
| | April 2004 | 12 | |
| early summer | May 2004 | 11 | 11 |
| autumn | September 1999 | 3 | 15 |
| | September 2000 | 2 | |
| | November 2003 | 2 | |
| | October 2004 | 4 | |
| | November 2004 | 4 | |
| winter | March 1999 | 5 | 12 |
| | February 2001 | 2 | |
| | January 2005 | 5 | |

solution (Parsons et al. 1984), and by mechanical grinding and sonication (2 min., 20 kHz, Ultrasonic Homogenizer 4710 Series, Cole Parmer Instrument) in the dark at 4°C for 2 hours. The extract was then centrifuged (20 min., 5°C, 4000 rpm, Beckman, GS-6R) to remove the filters and cell debris.

The chromatographic system used to isolate and separate the pigments consisted of a pump (HP1050), a diode array absorbance detector ('dad' HP1100), a fluorescence detector (HP1046) and a Rheodyne injection cell with a 100 $\mu$l loop. Two kinds of $C_{18}$ analytical column (dimensions $250 \times 4$ mm; particle size 5 $\mu$m; pore size 100 Å) were used to separate the pigments: a LichroCART$^{TM}$ Hypersil ODS (Merck) column to separate the samples collected in 1999–2001, and a LichroCART$^{TM}$ LiChrospher$^{TM}$ 100 RP18e (Merck) column to separate the 2002–05 samples. The analytical column was connected to the chromatographic system via a precolumn. Pigment detection was based on absorbance measurements at $\lambda = 440$ nm. The fluorescence measurements with extinction at $\lambda_{ex} = 431$ nm and emission at $\lambda_{em} = 660$ nm were taken at the same time as the absorbance measurements in order to confirm the presence of chloropigments in the sample. The clarified extract was mixed with an ion-pairing reagent (1M ammonium acetate; 1:1) 5 min. before injection to minimise dissociation of the compounds isolated (Mantoura & Llewellyn 1983). Filtered and degassed organic solvents (acetone, methanol) and buffer (1M

ammonium acetate) were used as the mobile phase. Introduced by Mantoura & Llewellyn (1983), this method of pigment isolation and separation was adapted and modified in later years by other researchers (Barlow et al. 1993, Stoń & Kosakowska 2002b, Stoń-Egiert & Kosakowska 2005). The chromatographic system was calibrated using a commercially available set of chlorophylls and carotenoids (The International Agency for $^{14}$C Determination, DHI Institute for Water and Environment, Denmark). Qualitative analysis was based on a comparison of the retention times and absorbance spectra of eluting peaks with those of the standards. Identification was confirmed by co-injection with a standard and on-line diode array spectra. The quantitative characteristics of the pigments occurring in the samples were based on an external standardisation equation (Mantoura & Repeta 1997). With the use of both types of analytical columns, complete separation even of trace amounts ($< 0.1$ $\mu$g dm$^{-3}$) of the chlorophylls and carotenoids present in the samples was possible during a single run with a precision of $2.9\% \pm 1.5\%$ and a recurrence error of $9.7\% \pm 6.4\%$ (Stoń-Egiert 2007). The phycobilins present in the cells of cryptophytes, red algae and cyanobacteria were not determined.

### 2.3. Microscopic phytoplankton count

The sea water samples (250 cm$^3$) collected for analysing the phytoplankton species composition were preserved in Lugol's solution and stored in the dark at 4°C. The species of the major taxonomic groups were determined using Utermöhl's sedimentation techniques (Willen 1962) and an inverted microscope (Axiovert M35, Carl Zeiss, Germany) fitted with phase contrast and differential interference contrast. Phytoplankton counts were carried out in accordance with the COMBINE programme of HELCOM (1997). The volume of each cell was calculated by measuring the appropriate morphometric characteristics. Volumes were converted to biomass, assuming 1 $\mu$m$^3$ to be equivalent to 1 pg (Edler (ed.) 1979). The carbon contents in phytoplankton samples were calculated according to Menden-Deuer & Lessard (2000) from the general formula Carbon [pgC cell$^{-1}$] = $0.216 \times$ cell volume$^{0.939}$, and in diatoms, because of their lower specific carbon content, from Carbon [pgC cell$^{-1}$] $= 0.288 \times$ cell volume$^{0.811}$, where cell volumes are given $\mu$m$^{-3}$.

## 3. Results

### 3.1. Phytoplankton species composition, biomass and carbon content

During several years of investigations into the phytoplankton species

composition in Baltic ecosystems, the following classes were identified in the southern Baltic: cyanobacteria, *Bacillariophyceae*, *Dinophyceae*, *Cryptophyceae*, *Chlorophyceae*, *Prasinophyceae*, *Chrysophyceae*, *Euglenophyceae*, as well as a group of unidentified nanoplankton (with cell sizes ranging from 3 to 15 $\mu$m).

That research confirmed that *Dinophyceae* and *Bacillariophyceae* are the main components of Baltic Sea phytocenoses. The proportion of *Dinophyceae* in the total biomass (Figure 2) ranged from 7.5% in autumn in the gulfs (represented mainly by large numbers of *Gyrodinium* spp.) to 59.2% in early summer in the open Baltic (represented by *Diplopsalis* sp., *Gyrodinium* sp., *Heterocapsa rotundata* and *Torodinium* sp. – the dominant species). The contribution of *Bacillariophyceae* to the total phytoplankton biomass varied from 23.5% in early summer in open waters (dominant species: *Achnanthes* cf. *taeniata*, *Chaetoceros similis* and *Skeletonema marinoi*) to 83.1% in winter in the same region (represented mainly by *Cyclotella meneghiniana*, *Chaetoceros* spp. and *Skeletonema marinoi*). With respect to the carbon content (Figure 3), *Dinophyceae* made up $>70\%$ of the phytoplankton community in both regions in spring and early summer, whereas *Bacillariophyceae* were dominant in winter (14.8% in the gulfs and 25.9% in the open Baltic).

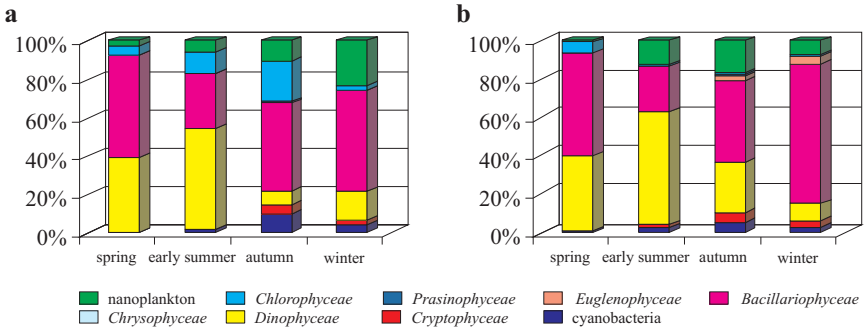


**Figure 2.** Mean relative biomass (percentage of total biomass) of individual phytoplankton communities identified in spring, early summer, autumn and winter in (a) the Gulf of Gdańsk and the Pomeranian Bay including the river mouths, and (b) open Baltic waters

The carbon content of *Cryptophyceae* (Figure 3) reached its highest value in both regions in autumn ($1.77 \pm 20.02$ $\mu$gC dm$^{-3}$ in the gulfs and $2.47 \pm 2.54$ $\mu$gC dm$^{-3}$ in open waters), the contributions to the total phytoplankton biomass in both regions being similar (4.6 and 5.3% respectively) (Figure 2). The lowest values for cryptophytes were noted in open waters in spring

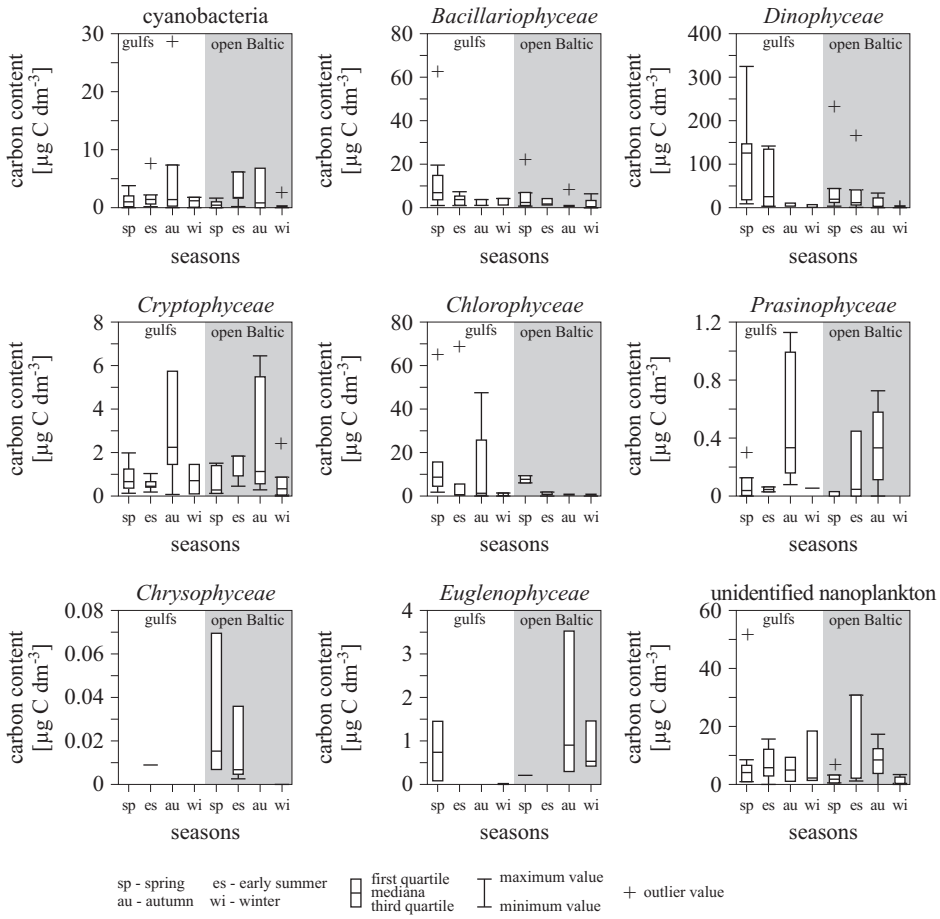


**Figure 3.** Ranges of carbon content in individual phytoplankton classes and cyanobacteria with respect to measuring seasons and regions

(mean $0.57 \pm 0.57$ $\mu$gC dm$^{-3}$) and in the gulfs in early summer (mean $0.53 \pm 0.31$ $\mu$gC dm$^{-3}$).

Figure 2 shows that the main contribution of cyanobacteria (mainly *Aphanizomenon* sp. and *Nodularia spumigena*) to the total biomass was recorded in the gulfs in autumn (mean $10.5 \pm 19.0\%$); in the open Baltic the proportions ranged from 0.5% in spring to 4.9% in autumn. This group of organisms was diverse in terms of the taxa identified in a given season as well as their cell size. Coenobia, colonies or filaments of cyanobacteria identified in the autumn and spring samples were mostly larger (but present in smaller numbers) than those in the early summer and winter samples. The carbon content of cyanobacteria (Figure 3) ranged from $0.56 \pm 0.93$ $\mu$gC dm$^{-3}$ in open waters in winter to $5.80 \pm 10.38$ $\mu$gC dm$^{-3}$ in the gulfs in autumn.

*Chlorophyceae* were present mostly in gulf waters (from 2.0% of the total phytoplankton biomass in winter to 22.1% in autumn), whereas *Euglenophyceae* were to be found more commonly in open Baltic waters (from 0.1% in spring to 4.4% in winter). Most of the unidentified nanoplankton inhabited the phytoplankton community of the gulfs in autumn and winter, and the open water community in early summer and autumn. *Prasinophyceae* and *Chrysophyceae* made no contribution of significance to the total phytoplankton biomass ($< 1\%$) in either region.

### 3.2. Qualitative and quantitative results of phytoplankton pigment analysis

Laboratory analysis of the phytoplankton pigment composition in different regions in 1999–2005 identified three groups of compounds: chlorophylls (chlorophyll *a*, chlorophyllide *a*, divinyl chlorophyll *a*, phaeophytin *a*, chlorophyll *b*, chlorophyll $c1 + c2$, chlorophyll $c3$), photosynthetic carotenoids (peridinin, fucoxanthin, $\alpha$-carotene, 19'hex-fucoxanthin, prasinoxanthin, canthaxanthin, echinenone, 19'but-fucoxanthin) and photoprotecting carotenoids (diadinoxanthin, alloxanthin, zeaxanthin, lutein, neoxanthin, violaxanthin, $\beta$-carotene, diatoxanthin, myxoxanthophyll, antheraxanthin). Figure 4 illustrates the seasonal and regional differences for some of the main taxonomically important pigments.

Chlorophyll *a* was present in every sample in the highest concentration in both regions in spring: mean $11.67 \pm 11.72$ $\mu$g dm$^{-3}$ (range: 1.20–32.48 $\mu$g dm$^{-3}$) in the gulfs, and mean $8.16 \pm 11.09$ $\mu$g dm$^{-3}$ (range: 1.17–35.27 $\mu$g dm$^{-3}$) in the open Baltic. High chlorophyll *a* concentrations were also noted in the gulfs in early summer (mean $9.19 \pm 9.10$ $\mu$g dm$^{-3}$; range: 2.76–22.59 $\mu$g dm$^{-3}$), whereas in open waters during this season they were approximately four times smaller (Figure 4a). Chlorophyllide *a*, present in phytoplankton cells with high chlorophyllase enzyme activity and in older cells (Takamiya et al. 2000), was also noted in each season in amounts from ca 1% to 8% of the chlorophyll *a* content. The highest mean chlorophyllide *a* values were noted in spring, both in the gulfs (mean $0.91 \pm 0.86$ $\mu$g dm$^{-3}$) and in open waters (mean $0.15 \pm 0.14$ $\mu$g dm$^{-3}$). Chlorophyll *b* and chlorophyll $c1 + c2$ were noted in both regions, but their distributions differed depending on the season (Figure 4b,c). Chlorophyll *b* concentrations in the gulfs were highest in early summer (mean $0.41 \pm 0.48$ $\mu$g dm$^{-3}$), but in the open waters in autumn (mean $0.19 \pm 0.08$ $\mu$g dm$^{-3}$). In the case of chlorophyll $c1 + c2$, the highest abundance of this pigment was found in spring samples (mean $2.15 \pm 1.99$ $\mu$g dm$^{-3}$ in the gulfs and $1.12 \pm 1.58$ $\mu$g dm$^{-3}$ in open waters), decreasing in other seasons to reach its lowest values in winter ($0.18 \pm 0.06$ $\mu$g dm$^{-3}$ in the gulfs and

$0.12 \pm 0.13$ $\mu$g dm$^{-3}$ in open waters). The main seasonal differences in the chlorophyll group concerned divinyl chlorophyll *a* and chlorophyll *c*3

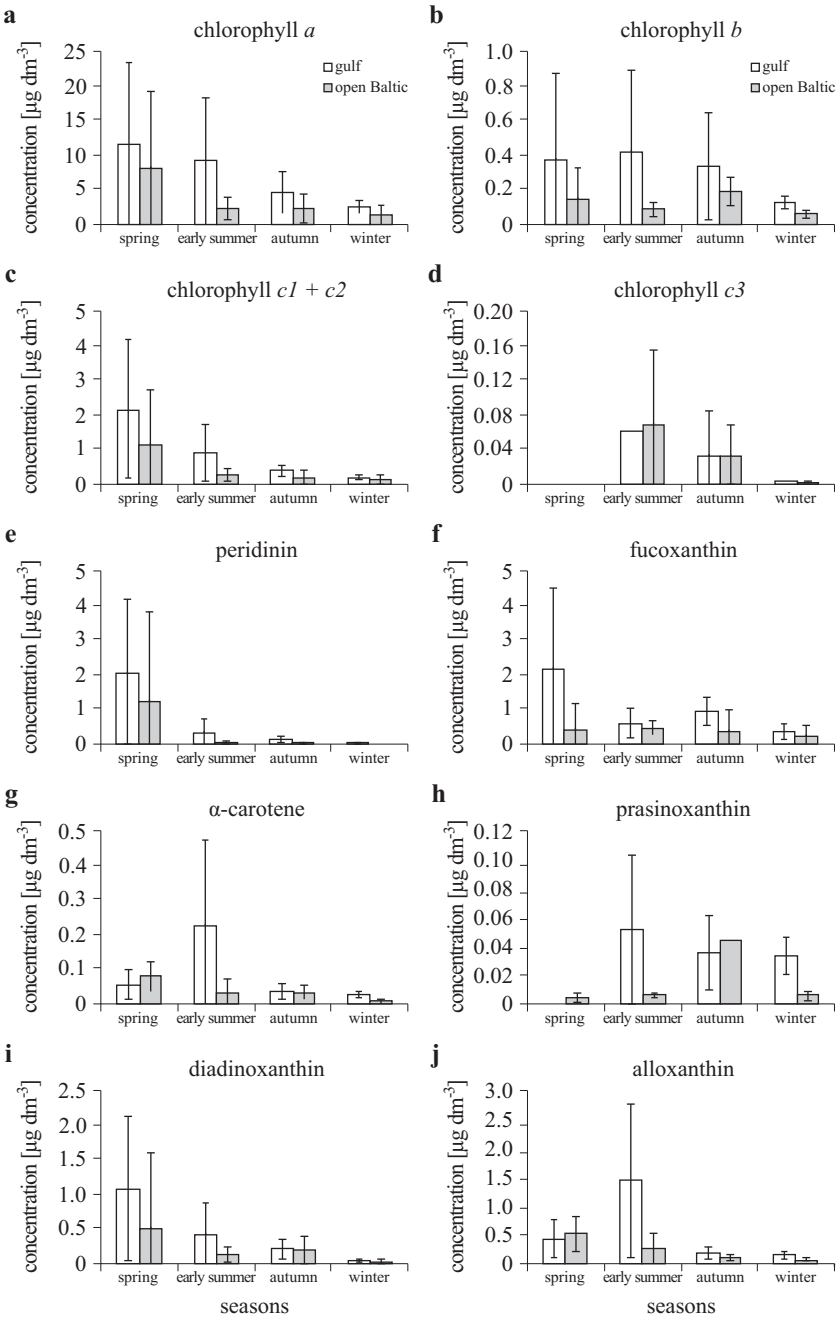


**Figure 4.** (*continued next page*)

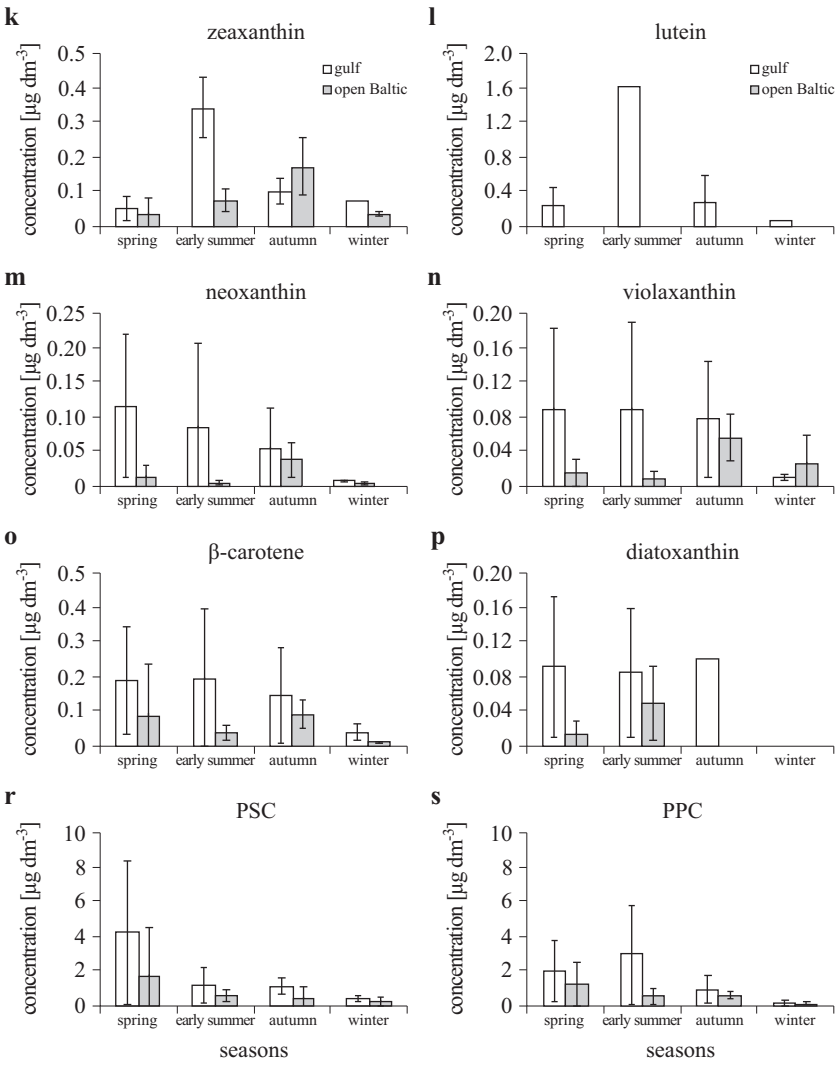


**Figure 4.** Concentrations of the main pigments (mean and standard deviation) identified in the gulfs and open waters of the southern Baltic in different seasons

(Figure 4d). Divinyl chlorophyll *a* was sporadically identified in spring samples in the gulfs (mean $0.015 \pm 0.007$ $\mu$g dm$^{-3}$) and in winter samples from the open Baltic (0.030 $\mu$g dm$^{-3}$). Chlorophyll *c*3, the pigment noted in cells of *Prymnesiophyceae* and *Chrysophyceae*, failed to be identified only in spring samples. Concentrations did not exceed 8% of the total chlorophylls *c* concentration in the samples.

There were seasonal and spatial differences in the qualitative distribution of photosynthetic carotenoids. Peridinin, fucoxanthin and $\alpha$-carotene were

identified in every season in dominant quantities. The seasonal distribution of peridinin (Figure 4e) shows decreasing concentrations during the year (highest mean values in spring, $2.05 \pm 2.11$ $\mu$g dm$^{-3}$ in the gulfs and $1.24 \pm 2.60$ $\mu$g dm$^{-3}$ in open waters). Changes in fucoxanthin abundance (Figure 4f) in the measuring seasons shows two periods of high concentration, conspicuous mainly in the gulf region. This is due to the spring and autumn phytoplankton blooms in this region of the Baltic Sea. The concentration of fucoxanthin ranged from 0.40 to 7.60 $\mu$g dm$^{-3}$ (mean $2.15 \pm 3.32$ $\mu$g dm$^{-3}$) in spring and from 0.63 to 1.78 $\mu$g dm$^{-3}$ (mean $0.95 \pm 0.40$ $\mu$g dm$^{-3}$) in autumn. The distribution of $\alpha$-carotene (Figure 4g) shows the highest concentration of this pigment in gulfs (in early summer mean $0.23 \pm 0.24$ $\mu$g dm$^{-3}$) and in open waters in spring (mean $0.08 \pm 0.04$ $\mu$g dm$^{-3}$). 19'hex-fucoxanthin was occasionally found only in early summer samples from open waters. Prasinoxanthin (Figure 4h) was found mainly in gulf waters, except in spring. Other PSC pigments – canthaxanthin, echinenone and 19'but-fucoxanthin – were noted sporadically and did not make any significant contribution to the total PSC concentration in the various seasons (maximum – 5.5% of the total PSC content). The distribution of the total PSC content in samples (Figure 4r) decreased during the year, with a maximum in both regions in spring.

Photoprotecting carotenoids, such as diadinoxanthin, alloxanthin, zeaxanthin, neoxanthin, violaxanthin and $\beta$-carotene, were identified in samples from each season and region. Diadinoxanthin and alloxanthin are considered to be the two dominant pigments in the total PPC content in both regions (Figures 4i,j). The concentration of diadinoxanthin was highest in the gulfs in spring (mean $1.09 \pm 1.04$ $\mu$g dm$^{-3}$), while that of alloxanthin was highest in early summer (mean $1.45 \pm 1.32$ $\mu$g dm$^{-3}$). The spring alloxanthin concentration was higher in open waters than in the gulfs. The zeaxanthin concentration was highest in early summer (like the alloxanthin distribution), but in open waters it reached its highest values in autumn (Figure 4k). Levels of neoxanthin (Figure 4m) and violaxanthin (Figure 4n) were the highest in open waters in autumn; in the gulfs, however, mean violaxanthin concentrations were similar, but neoxanthin levels were the highest in spring (mean $0.12 \pm 0.10$ $\mu$g dm$^{-3}$). The other pigments – lutein, diatoxanthin, myxoxanthophyll and antheraxanthin – were qualitatively diverse. For example, lutein was identified only in the gulfs (Figure 4l), myxoxanthophyll only in the gulfs in autumn, and diatoxanthin and antheraxanthin were not found in winter samples. The total PPC distribution was determined by the diadinoxanthin and alloxanthin contents, affecting the seasonal and regional differences (Figure 4s), the highest levels were noted in the gulfs in early

summer (mean $2.98 \pm 2.88$ $\mu$g dm$^{-3}$) and in open waters in spring (mean $1.20 \pm 1.28$ $\mu$g dm$^{-3}$).

The spatial and seasonal differences in the phytoplankton pigment distribution (see Figure 4) are the effect of interactions of many environmental factors that combine to produce a given set of conditions for algal cell development and phytoplankton growth. The higher concentrations of pigments in the gulfs than in open waters are a reflection of the greater phytoplankton biomass resulting from the large quantities of nutrients carried to the sea by river waters (Wasmund & Uhlig 2003).

## 4. Discussion

The qualitative composition and quantitative distribution of pigments in different ecosystems depends mainly on the occurrence of specific algal classes and cyanobacteria in a given water body, and on the season and physiological condition of the cells present there. It is common knowledge that the pigment composition of cells is a characteristic feature of individual algal classes (Wright et al. 1991, Jeffery & Vesk 1997), and also that the occurrence of pigment derivatives reflects, among other things, the physiological state of phytoplankton cells, e.g. when phytoplankton blooms are coming to an end (Klein & Sournia 1987).

Chlorophyll *a* is present in all phytoplankton, but the presence of other accessory chlorophylls and carotenoids in the relevant proportions may suggest the presence of specific phytoplankton classes and cyanobacteria. For example, chlorophyll *b* was identified in cells of chlorophytes, prasinophytes and euglenophytes, alloxanthin in cryptophyte cells, and fucoxanthin in diatoms, prymnesiophytes and chrysophytes. The divinyl chlorophyll *a* identified in Baltic phytoplankton samples is evidence for the occurrence of prochlorophytes, because divinyl chlorophyll *a* is the main photosynthetic pigment in their cells (Goericke & Repeta 1992). Although prochlorophytes have been found in large numbers in the euphotic zones of the Atlantic and Pacific Oceans, they have also been identified in Baltic estuarine regions and, for example, in inland waters (Geiß et al. 2003). Identification of this pigment in natural samples may be also due to the disintegration of the chlorophyll *a* molecule under conditions of stress (Rüdiger 1997).

The seasonal changes in the pigments identified depends on the succession of phytoplankton classes, i.e. the seasonal changes in biomass and species composition, which is governed by a number of physical, biological and chemical processes. The development of phytoplankton during the annual cycle in the Baltic Sea shows three characteristic maxima, which coincide with changes in the distribution of chlorophyll *a* concentrations (Wasmund et al. 1996, Thamm et al. 2004). In the material analysed for the

purposes of this paper, there was an increase in biomass growth in spring, resulting from the rising surface water temperatures, the large amounts of nutrients accumulated during winter, and brought to the surface by mixing processes, not to mention the increased inflow of solar energy to the water. The upshot is first a diatom bloom (mainly *Chaetoceros* spp., *Skeletonema marinoi*, *Thalassiosira* spp.), then a dinophyte bloom (*Peridiniella catenata*, *Protoperidinium* spp., *Heterocapsa triquetra*). These bloom-forming species were recorded in the earlier years of the investigations mentioned in the literature (Wasmund et al. 1998, Gasiūnaitė et al. 2005). The duration of the spring bloom depends on geographical position and the thermal characteristics in a given year; blooms were noted from early March till May (Wasmund et al. 1996, Wasmund & Uhlig 2003, Thamm et al. 2004). The next increase in Baltic phytoplankton biomass is due to the summer (July/August) cyanobacteria bloom, produced mainly by *Aphanizomenon* sp. and *Nodularia spumigena* (Finni et al. 2001). No such bloom was recorded in the results presented in this paper, however. Finally, the autumnal increase in phytoplankton biomass (due mainly to the diatoms *Coscinodiscus granii*, *Thalassiosira baltica*, Nakonieczny et al. 1991, Wasmund et al. 1996), took place from September to October depending on the measurement region, and resulted from the inflow of nutrients from deeper layers in conjunction with the diminishing amounts of solar energy available (Wasmund et al. 2001, Thamm et al. 2004).

The seasonal increase in phytoplankton biomass in the Baltic, due to the blooms of particular species, means that the composition and quantities of pigments are highest in spring and autumn samples. For example, the highest chlorophyll *a* concentrations were noted in spring (max. 35.3 $\mu$g dm$^{-3}$ in the gulfs). Also, the high fucoxanthin concentrations in the gulfs (max. 7.60 $\mu$g dm$^{-3}$) are suggestive of a spring diatom bloom, additionally confirmed by a microscopic cell count (max. *Bacillariophyceae* biomass = 62.6 $\mu$gC dm$^{-3}$). The high peridinin concentrations also noted in spring (max. 6.13 $\mu$g dm$^{-3}$) suggest an acceleration in *Dinophyceae* development at that time (max. *Dinophyceae* biomass = 325.1 $\mu$gC dm$^{-3}$). In comparison, the mean content of these pigments in periods without visible phytoplankton biomass increase was 2–6 times lower with respect to changes in fucoxanthin content and $>6$ times lower with respect to peridinin. The composition and mutual proportions of pigments found in high concentrations in early summer samples, i.e. chlorophyll *c*3 (0.10–0.55 $\mu$g dm$^{-3}$), 19'but-fucoxanthin (0.01 $\mu$g dm$^{-3}$) and 19'hex-fucoxanthin (0.13–0.19 $\mu$g dm$^{-3}$), may provide evidence for the presence of communities consisting of *Chrysophyceae* and *Prymnesiophyceae*.

The spatial distribution of different taxonomic groups is reflected by the quantitative and qualitative composition of the pigments identified. The heterogeneous vertical and horizontal distributions of phytoplankton classes (and hence, pigment distributions) result from the interaction of many factors, both hydro-meteorological (e.g. air and water temperature, insolation, water currents, salinity gradients, density stratification) and physiological (e.g. cell tolerance of external pressure, optimum light conditions, biological adaptation of individual groups to migration), which create optimal conditions for the growth and development of algal and cyanobacteria cells. Higher algal biomasses were noted in river mouths, which are subject to seasonal fluctuations caused by large inflows of nutrients with river water masses (Wasmund et al. 1999, Wasmund & Uhlig 2003). This is reflected by the fact that pigment concentrations were about 2–3 times higher in the gulfs than in open waters. In spring, the diatom content was responsible for ca 50% of the total phytoplankton biomass at the river mouth (for comparison, ca 56% in an earlier analysis, Wasmund et al. 1999), and the phytoplankton community structure changed with distance from the river mouth, with a significant proportion of dinophytes being present (from 10% near the river mouth to 40% in more distant regions of the gulf). Balanced multispecific phytocenoses inhabited both regions in other seasons: for example, the biomass of cyanobacteria, as in the earlier analysis (Wasmund et al. 1999), did not exceed 30% of the total phytoplankton content in open waters, and diatoms and dinophytes were no more than ca 17%.

The pigment concentration in a natural sample is always the resultant of the phytoplankton composition at any time or place. Chlorophyll *a* is present in the cells of all algal classes and cyanobacteria; its concentration is therefore considered to be a universal indicator of biomass of photosynthesising organisms (Hunter & Laws 1981, Nakonieczny et al. 1991, Wasmund et al. 2001). In the experiments and analysis presented in this paper, the statistical relationships between the total phytoplankton biomass (represented by the carbon content [$\mu$gC dm$^{-3}$]) and chlorophyll *a* concentration [$\mu$g dm$^{-3}$] in the southern Baltic was assumed to be (Figure 5):

$$biomass_{phytoplankton} = 10^{0.9424 \log(C_{chl\,a}) + 0.8959}. \quad (1)$$

The empirical data used to determine this relationship include all the results (N = 53) from different seasons in both the gulfs and the open waters of the southern Baltic. Despite the large scatter of the data points (see Figure 5), the determination coefficient of the above relationship is $R^2 = 0.75$; Table 2 gives the relative statistical errors according to arithmetic and logarithmic

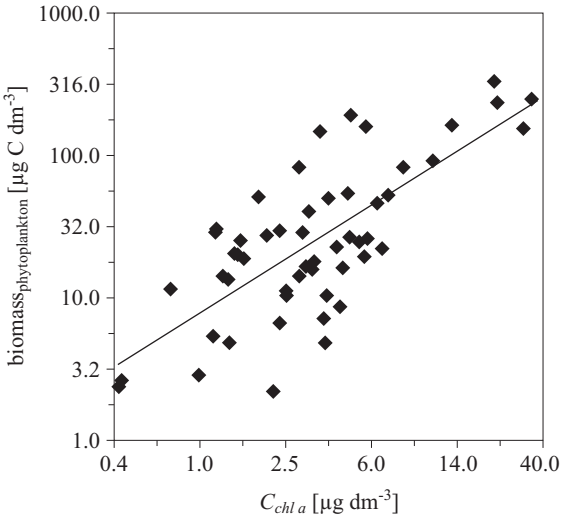


**Figure 5.** Relationship between total phytoplankton biomass, represented by the organic carbon content, and chlorophyll *a* concentration ($C_a$) in all the samples from the gulfs and open waters of the Baltic Sea (points – empirical data, line – approximation using equation (1))

statistics of this approximation. The scatter of the results may be evidence for the differentiation of the phytoplanktonic organisms with respect to their cell structures and morphological characteristics. It is well known that pigments may constitute 2–10% of the organic matter in a chloroplast (Parsons et al. 1977), and their amounts in cells may vary under stress. For example, excessive light may reduce the chlorophyll *a* content in cells by as much as five times (Goericke & Montoya 1998).

Carotenoids are regarded as markers of algal classes and cyanobacteria, so it is possible to estimate the biomass of a particular class from the concentrations of these pigments. It is standard practice to determine biomass and class composition on the basis of known identified marker pigment combinations (Latelier et al. 1993, Andersen et al. 1996, Peeken 1997), and the CHEMTAX program has found wide application in this field (Mackey et al. 1996, Henriksen et al. 2002, Eker-Develi et al. 2008).

A series of statistical relationships for approximating the biomass (represented by organic carbon [$\mu$gC dm$^{-3}$]) of individual phytoplankton classes in the southern Baltic ($biomass_{phytoplankton\,class}$) using known marker pigment concentrations ($C_i$, $i$ – pigment, [$\mu$gC dm$^{-3}$]) was obtained. They are expressed mathematically as follows:

- for the diatom biomass ($biomass_{Bacil.}$) based on the fucoxanthin concentration ($C_{fuco}$):

$$biomass_{Bacil.} = 10^{1.0829\log(C_{fuco})+0.6958}, \tag{2}$$

- for the cyanobacteria biomass ($biomass_{cyano.}$) based on the zeaxanthin concentration ($C_{zeax}$):

$$biomass_{cyano.} = 10^{0.7579\log(C_{zeax})+0.6332}, \tag{3}$$

- for the dinophyte biomass ($biomass_{Dinoph.}$) based on the peridinin concentration ($C_{peri}$):

$$biomass_{Dinoph.} = 10^{0.7002\log(C_{peri})+1.6827}, \tag{4}$$

- for the cryptophyte biomass ($biomass_{Crypto.}$) based on the alloxanthin concentration ($C_{allox}$):

$$biomass_{Crypto.} = 10^{0.0098\log(C_{allox})-0.0660}, \tag{5}$$

- for the chlorophyte biomass ($biomass_{Chloro.}$) based on the lutein concentration ($C_{lut}$):

$$biomass_{Chloro.} = 10^{0.9164\log(C_{lut})+1.7080}, \tag{6}$$

- for the total biomass of chlorophytes, prasinophytes and euglenophytes ($biomass_{Chloro.+Pras.+Eugl.}$) based on the sum of chlorophyll $b$ and neoxanthin concentrations ($C_{chl\,b+neox}$):

$$biomass_{Chloro.+Pras.+Eugl.} = 10^{1.5437\log(C_{chl\,b+neox})+1.2208}, \tag{7}$$

- for the total biomass of dinophytes, diatoms, chrysophytes and euglenophytes ($biomass_{Dinoph.+Bacil.+Chryso.+Eugl.}$) based on the diadinoxanthin concentration ($C_{diad}$):

$$biomass_{Dinoph.+Bacil.+Chryso.+Eugl.} = 10^{0.8898\log(C_{diad})+1.8697}. \tag{8}$$

Figure 6 presents these approximations, and Table 2 lists the relative statistical errors according to arithmetic and logarithmic statistics of the estimated biomasses of individual phytoplankton classes.

The best approximations were obtained for the dependence of *Bacillariophyceae* on the concentration of fucoxanthin (eq. (2)), the diagnostic xanthophyll of this class (coefficient of determination $R^2 = 0.69$, $N = 51$, the statistical error according to logarithmic statistics $\sigma_- = 61\%$), for the biomass of *Chlorophyceae* based on the lutein concentration (eq. (6), $R^2 = 0.72$, $N = 9$, $\sigma_- = 48\%$), and for the total biomass of *Dinophyceae*, *Bacillariophyceae* and *Euglenophyceae* based on the concentration of diadinoxanthin, the main carotenoid pigment in the cells of species from

**Table 2.** Relative errors in the estimation using equations (1)–(8) of phytoplankton biomass ($biomass_{phytoplankton}$) and the biomass of individual phytoplankton classes ($biomass_{phytoplankton\ class}$) represented by their organic carbon content [$\mu$gC dm$^{-3}$]

| Equation | N | Arithmetic statistics | | Logarithmic statistics | | | |
|---|---|---|---|---|---|---|---|
| | | systematic error $<\varepsilon>$ [%] | statistical error $\sigma_\varepsilon$ [%] | systematic error $<\varepsilon>_g$ [%] | standard error factor $x$ | statistical error $\sigma_-$ [%] | statistical error $\sigma_+$ [%] |
| (1) | 53 | 41.0 | 136.1 | −6.17E − 09 | 2.31 | −56.6 | 130.5 |
| (2) | 51 | 69.1 | 254.9 | 3.76E − 08 | 2.54 | −60.7 | 154.2 |
| (3) | 32 | 105.2 | 227.0 | 5.73E − 09 | 3.75 | −73.3 | 274.9 |
| (4) | 41 | 114.3 | 397.6 | 9.39E − 08 | 3.25 | −69.2 | 224.5 |
| (5) | 48 | 77.4 | 228.8 | −9.1E − 11 | 2.93 | −65.9 | 193.1 |
| (6) | 9 | 21.4 | 80.3 | −5.5E − 08 | 1.94 | −48.4 | 93.8 |
| (7) | 37 | 183.3 | 520.6 | −6.9E − 08 | 4.38 | −77.6 | 337.7 |
| (8) | 51 | 60.3 | 240.5 | 8.01E − 08 | 2.50 | −60.0 | 150.1 |

where:
N – number of data,
$\varepsilon = (biomass_{phytoplankton\ class,\ C} - biomass_{phytoplankton\ class,\ M})/biomass_{phytoplankton\ class,\ M}$ – relative error,
$biomass_{phytoplankton\ class,\ C}$, $biomass_{phytoplankton\ class,\ M}$ – phytoplankton biomass (represented by the organic carbon content), determined using equations (2)–(8) – index $C$, and measured – index $M$,
$<\varepsilon>$ – arithmetic mean of errors,
$<\varepsilon>_g = 10^{[<\log(biomass_{phytoplankton\ class,\ C}/biomass_{phytoplankton\ class,\ M})>]} - 1$ – logarithmic mean of errors,
$<\log(biomass_{phytoplankton\ class,\ C}/biomass_{phytoplankton\ class,\ M})>$ – mean of $\log(biomass_{phytoplankton\ class,\ C}/biomass_{phytoplankton\ class,\ M})$,
$\sigma_\varepsilon$ – standard deviation of errors (statistical error),
$\sigma_{\log}$ – standard deviation of $\log(biomass_{phytoplankton\ class,\ C}/biomass_{phytoplankton\ class,\ M})$,
$x = 10^{\sigma_{\log}}$ – standard error factor,
$\sigma_+ = x - 1$ and $\sigma_- = \frac{1}{x} - 1$.

these classes (eq. (8), $R^2 = 0.63$, N = 51, $\sigma_- = 60\%$). Fucoxanthin and diadinoxanthin are present not only in the cells of these algal classes: besides being a marker pigment of diatoms, fucoxanthin is regarded as the main xanthophyll in *Prymnesiophyceae* and *Chrysophyceae* (Jeffrey & Vesk 1997), which have also been recorded in Baltic phytoplankton communities. This may be why the arithmetic and logarithmic errors have the particular values listed in Table 2. The organic carbon of cyanobacteria (eq. (3)) can be estimated from the zeaxanthin content in samples with a relative statistical error $\sigma_- = 73\%$. Zeaxanthin is a carotenoid unique to the cells

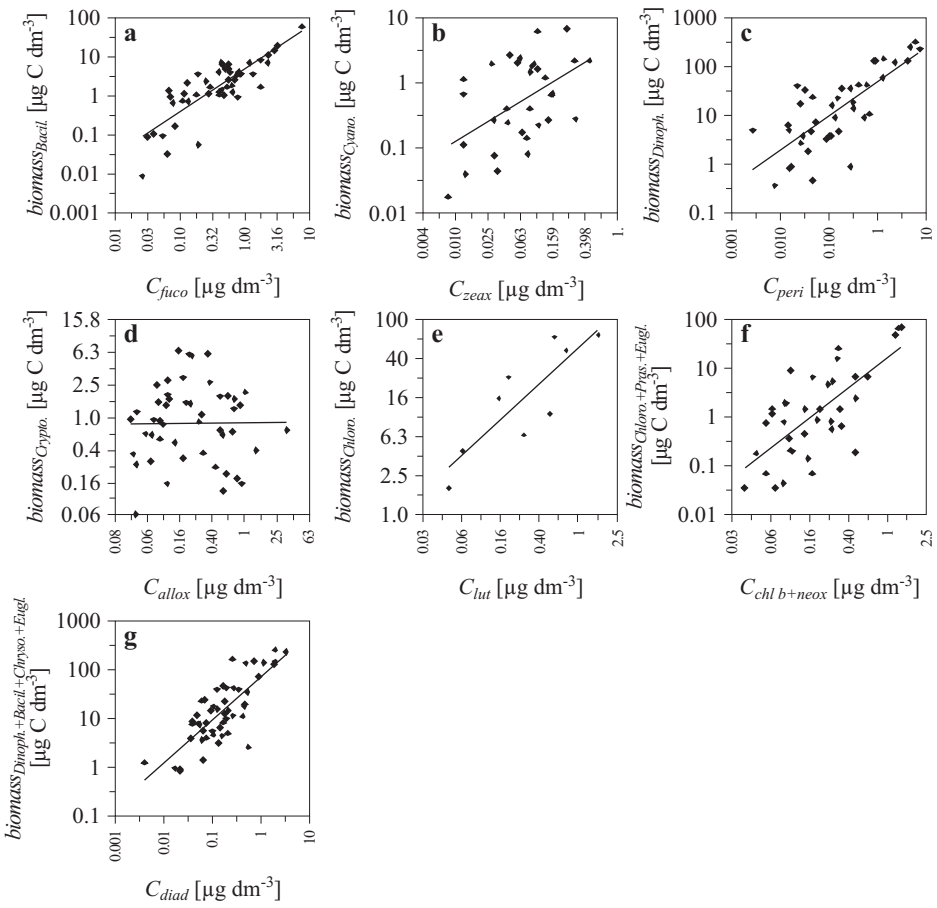


**Figure 6.** Relationships between the biomass of phytoplankton classes and cyanobacteria (represented by the organic carbon content) and the concentration of suitable marker pigments (points – empirical data, line – approximation using the relevant formula): a) *Bacillariophyceae* – fucoxanthin (eq. (2)); b) cyanobacteria – zeaxanthin (eq. (3)); c) *Dinophyceae* – peridinin (eq. (4)); d) *Cryptophyceae* – alloxanthin (eq. (5)); e) *Chlorophyceae* – lutein (eq. (6)), f) the total biomass of *Chlorophyceae*, *Prasinophyceae* and *Euglenophyceae* – sum of chlorophyll *b* and neoxanthin (eq. (7)), g) the total biomass of *Dinophyceae*, *Bacillariophyceae*, *Chrysophyceae* and *Euglenophyceae* – diadinoxanthin (eq. (8))

of these organisms, while the phycobilins (allophycocyanin, phycoerythrin and phycocyanin), which were not taken into consideration here, are used as pigment markers of cyanobacteria. The high errors of this estimate can be put down to the photoprotecting role of zeaxanthin in algal cells, where the concentration of this pigment is governed by changing light conditions and light intensity (known as the 'xanthophyll cycle': Demmig-Adams & Adams

1996, Larkum 2003). The sequence of two reversible enzymatic reactions – epoxidation and de-epoxidation – leads to the intercellular conversion of zeaxanthin – antheraxanthin – violaxanthin, changing their relative concentrations by means of the non-photochemical quenching of the excess energy absorbed by the cells.

Errors were least for the estimate of *Chlorophyceae* biomass as a function of lutein concentration, the standard error factor of this relationship being 1.94 (eq. (6)). The largest error of estimation was in the approximation of the total biomass of *Chlorophyceae*, *Prasinophyceae* and *Euglenophyceae* based on the sum of the chlorophyll *b* and neoxanthin concentrations (eq. (7)); the relative statistical error according to logarithmic statistics here was $\sigma_{-} = 77\%$.

Additionally, the large errors of these estimates (eqs. (4), (2), (7)) may be due to the various proportions and relative concentrations of these pigment markers in different algal species, the wide spectrum of cell sizes in the different taxonomic groups (from 0.1 $\mu$m to a few hundred $\mu$m), the effects elicited in phytoplankton cells by stress factors such as excessive light and nutrient limitation, and also errors inherent in the measuring techniques (Britton et al. 2003).

## 5. Final remarks

This analysis yielded a series of seasonal and regional regularities in the occurrence of phytoplankton pigments in different water bodies in the southern Baltic.

The chlorophylls identified do not qualitatively differentiate the investigated regions; it is the carotenoids that are the main markers of biological differentiation. The pigment composition is directly related to taxon, although the individual pigment content may change as a result an organism's adaptation to ambient physical and chemical conditions.

A series of mathematical expressions describing the distributions and concentrations of individual phytoplankton pigments with respect to biotic factors were found. Relationships were established between the total phytoplankton biomass in the Baltic Sea and the chlorophyll *a* concentration, and also between the biomass of the main algal classes and the concentrations of suitable marker carotenoids. Given the precision of the approximations obtained, the total phytoplankton biomass (eq. (1)) could be estimated with relative statistical errors according to logarithmic statistics $\sigma_{-} = 56\%$. The best approximations were obtained for the dependences of the *Bacillariophyceae* biomass on the fucoxanthin concentration (eq. (2), $\sigma_{-} = 60\%$), the *Chlorophyceae* biomass on the lutein concentration (eq. (6), $\sigma_{-} = 48\%$), and the total biomass of *Dinophyceae*, *Bacillariophyceae*,

*Chrysophyceae* and *Euglenophyceae* on the concentration of diadinoxanthin, the main carotenoid pigment present in the cells of the species from these latter four classes (eq. (8), $\sigma_- = 60\%$).

The results complement and broaden existing knowledge of biologically active compounds isolated from different compartments of the Baltic Sea environment and are applicable to the validation of the bio-optical models used in the remote-sensing research of marine ecosystems (Ostrowska et al. 2007, Majchrowski et al. 2007, Woźniak et al. 2008, Darecki et al. 2008).

The problems concerning the influence of environmental factors on composition and pigment resources in phytoplankton samples in different ecosystems are extremely complex. The results presented in this paper by no means exhaust this topic and will constitute the basis for further investigation and analysis.

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