Carotenoid pigments in Baltic Sea sediments*

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Abstract

Carotenoid pigments were examined in vertical profiles of Baltic sediments in early summer (June 1994). High-performance liquid chromatograms (HPLC) displayed high concentrations of carotenoids in an anoxic environment in cores from the Gotland Basin, Bornholm Deep and Gdańsk Deep. Cyanobacterial pigments were found to be present in the surface sediments of the Gdańsk and Bornholm Deeps. Dinoflagellate and diatom carotenoids were the major contributors of pigments in the Gotland Basin. Carotenoids were used as biogenic indicators of the diagenesis of organic matter and the condition of Baltic Sea sediments.

1. Introduction

The analysis of carotenoids in marine sediments has been used as a tool in the identification of the auto- and heterotrophic organisms contributing to sedimentary matter. In recent years much research has been done on pigments as chemotaxonomic indicators in biogeochemical studies (Liaaen-Jensen, 1978; Rowan, 1989; Everitt *et al.*, 1990). Major classes of the marine phytoplankton, as well as a large number of bacteria, zooplankton and benthic organisms synthesise characteristic pigment structures which can serve as natural biomarkers (Rowan, 1989; Everitt *et al.*, 1990;

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Bianchi *et al.*, 1996). High-performance liquid chromatography (HPLC) has been used extensively for separating and quantifying individual carotenoids and their breakdown products. Wright and Shearer (1984) made the first attempt at using this technique in a study of a benthic community and sediments. However, it should be noted that the complex mixture of pigments typical of most sediments and the lability of these compounds pose considerable problems for the analyst. Hence, the transformation products resulting from chemical or metabolic processes remain unidentified. For example, while a partial analysis of carotenoids in surface sediments from the Peru upwelling area revealed 200 pigments, only 37 of them were actually identified (Repeta and Gagosian, 1987).

The studies of carotenoids from Baltic sediments have concentrated on pigment stability under anoxic and oxic conditions (Abele, 1988; Abele-Oeschger, 1991). Even so, very little is known about these components in the Baltic environment. The carotenoid content in the deep, muddy, anoxic sediments, as well as the vertical biomarker pigment profiles in sediment cores from the Baltic Sea are presented in this paper.

2. Material and methods

Material

The sediment samples were collected with a Niemistö core sampler at 3 different stations in the Baltic Sea (Gotland Basin, Gdańsk Deep and Bornholm Deep, Fig. 1) during a cruise of r/v 'Oceania' in June 1994.

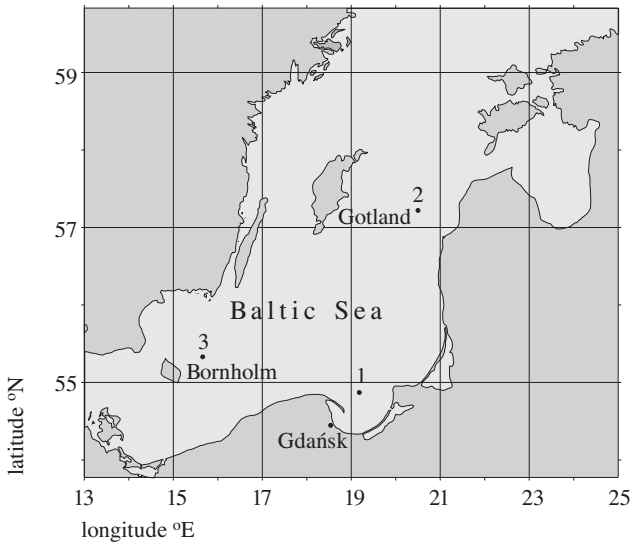


Fig. 1. Location of sampling stations (Baltic Sea, 1994)

The stations were situated in anoxic areas or within the strong oxygen minimum, where sediments were rich in organic compounds. More detailed information on the sampling areas is given by Ehlin (1981), and Gudelis and Jemielianow (1982). After sampling, a square portion of the cores was cut into 10 mm sections and frozen at –20°C until analysis. Pigment analysis was performed within 4 weeks of sampling. The characteristic environmental conditions, *i.e.* temperature, salinity and ignition loss, of the sampling areas are given in Tab. 1.

Table 1. The characteristic environmental conditions at the sampling stations

Station	Position		Depth	Temperature at bottom	Salinity at bottom	Ignition loss*
	ϕ	λ	[m]	[°C]	[PSU]	[mg g^{-1} d.w.]
Gdańsk Deep (1)	54°52′	19°11′	110	4.2	13.2	250
Gotland Basin (2)	57°13′	20°30′	90	4.8	9.5	109
Bornholm Deep (3)	55°20′	15°40′	95	3.1	13.7	97

* in the surface layer of the sediment (0–1 cm).

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

Pigment extraction

A frozen sediment sample was allowed to thaw and water was removed by centrifugation (15 min at 2500 rpm, GS–6R Beckman Centrifuge). The material (2.5–15 g) was extracted by ultra-sonication in 90% acetone (*ca* 2 ml g^{-1} sediment), (Cole Parmer Ultrasonic Homogenizer 4710 Series, 15 min at 20 kHz), then incubated for 2 hours in the dark at 5°C. After centrifugation (as above) the supernatant was decanted and the extraction procedure repeated twice. The extracts were analysed by UV–VIS spectrophotometry (spectra 350–750 nm, Beckman DU–68 Spectrophotometer).

Pigment separation with HPLC

The carotenoids were analysed in a chromatographic system consisting of a Hewlett–Packard Series 1050 chromatographic pump, a UV–Visible detector (Variable Wavelength Detector HP1050 Series) set at 440 nm and 450 nm, a fluorescence detector (HP1046) with excitation set at $\lambda_{Ex} = 431\,nm$ and emission at $\lambda_{Em} = 660\,nm$, two integrators (HP3396 Series II, HP3394A) and a Model 7125 Rheodyne injector with a 20-μl loop. The chromatographic system used an RP–18 ODS-type pre-column and a $125\,mm \times 4\,mm$ analytical column packed with Spherisorb ODS–2 (5 μm particle size).

Separation of pigments was achieved in a gradient mixture of methanol, acetone and water. At injection the solvent composition was methanol--acetone-water (80:5:15 v/v/v) and the flow-rate 0.8 ml min^{-1}. After 10 min the water content was linearly reduced and that of acetone increased in order to bring the proportion of acetone up to 20% in the 50th minute after injection. The final composition of the gradient mixture was methanol-acetone (80:20 v/v).

Calibration and standards

Calibration was carried out using the following standards: β,β-carotene, β,ε-carotene, lutein, canthaxanthin (Hoffmann-La Roche and Sigma Chemical Company). The xanthophylls (diadinoxanthin, peridinin, diatoxanthin, zeaxanthin, astaxanthin) were obtained from 3 species of phytoplankton: *Anabaena variabilis* (Cyanophyceae), *Heterocapsa triquetra* and *Peridiniella catenata* (Dinophyceae) and 1 species of zooplankton *Daphnia pulex* (Cladocera). These organisms have a well-documented carotenoid composition (Matsuno and Hirao, 1989; Liaaen-Jensen, 1978; Davies, 1976).

The purity of the pigments was tested with a HPLC Waters 991 System equipped with a UV–VIS diode array detector (conditions as above). The concentrations were determined from the data in Mantoura and Llewellyn (1983). The chloropigments were recorded with fluorescence and UV–VIS detectors but were not analysed in detail. For lutein and zeaxanthin separation the method described by Wright *et al.* (1991) was used.

3. Results

The phytoplankton composition in the sampling area (below the halocline) is shown in Tab. 2. At stations 1 and 3 Cyanophyceae (Cyanobacteria, Cyanoprokaryotes) mainly from the genera *Aphanizomenon*, *Nodularia* and *Microcystis* were dominant. At station 2 Bacillariophyceae and Dinophyceae were present in large numbers. The results of microscopic analyses of phytoplankton samples were generally in agreement with the surface sediment

Table 2. Dominant of phytoplankton species in samples collected from the Baltic Sea in June 1994

Station	Layer [m]	Number of cells [indiv. dm^{-3}] > 4 μm	Dominant species
Gdańsk Deep (1)	60–110	0.2×10^6	*Aphanizomenon flos-aquae* (L.) Ralfs ex Bornet et Flah. *Nodularia spumigena* Mert. in Jürgens ex Bornet et Flah. *Peridiniella catenata* (Levander) Balech
Gotland Basin (2)	60–90	1.2×10^6	*Paralia sulcata* (Ehrenberg) Cleve *Peridiniella catenata* (Levander) Balech *Prorocentrum* sp. *Gyrodinium* sp.
Bornholm Deep (3)	60–95	0.4×10^5	*Aphanizomenon flos-aquae* (L.) Ralfs ex Bornet et Flah. *Microcystis* sp. *Ebria tripartita* (Schumann) Lemm.

pigment content determined by HPLC. High concentrations of zeaxanthin (Cyanophyceae marker) were found in sediments from the Gdańsk Deep and Bornholm Deep. Fucoxanthin and peridinin (Bacillariophyceae and Dinophyceae markers) predominated in the Gotland Basin (Tab. 3).

Table 3. Diagnostic pigments for the characterising different algal groups in the Baltic Sea and their concentration in the surface sediments

Phytoplankton group	Pigment biomarkers	Carotenoid content [μg g^{-1} d.w.]		
		(1)	(2)	(3)
Cyanophyceae	zeaxanthin	48.7	18.3	22.4
Bacillariophyceae	fucoxanthin	13.2	62.5	10.7
Dinophyceae	peridinin	8.4	20.7	4.5
Cryptophyceae	alloxanthin	4.0	2.3	–
Chlorophyceae	lutein	3.8	4.1	2.0

The total carotenoid contents in the surface layer of sediments, ranging from 140 μg g^{-1} d.w. in the Bornholm Deep to 560 μg g^{-1} d.w. in the Gdańsk Deep, are presented in Fig. 2.

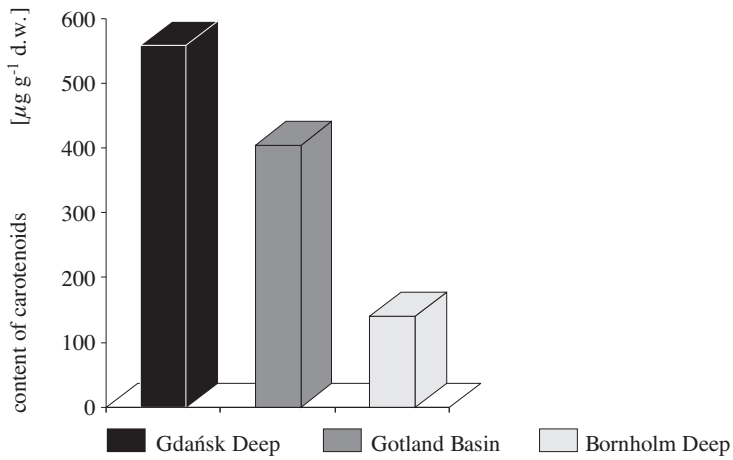


Fig. 2. Carotenoid content in surface sediments from the Baltic Sea

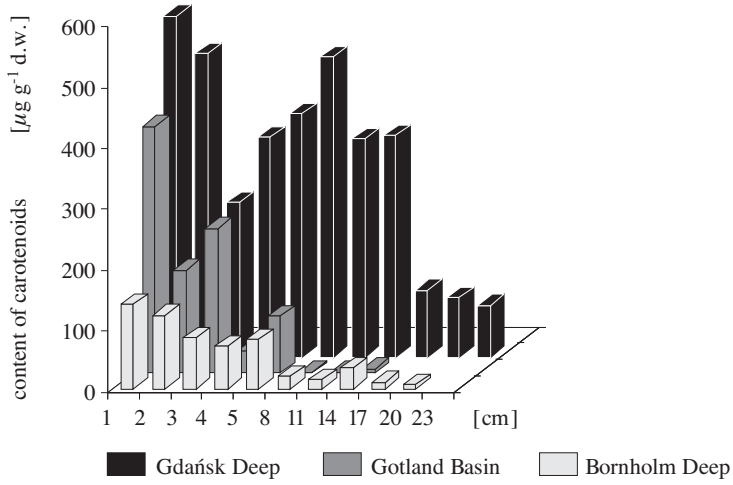
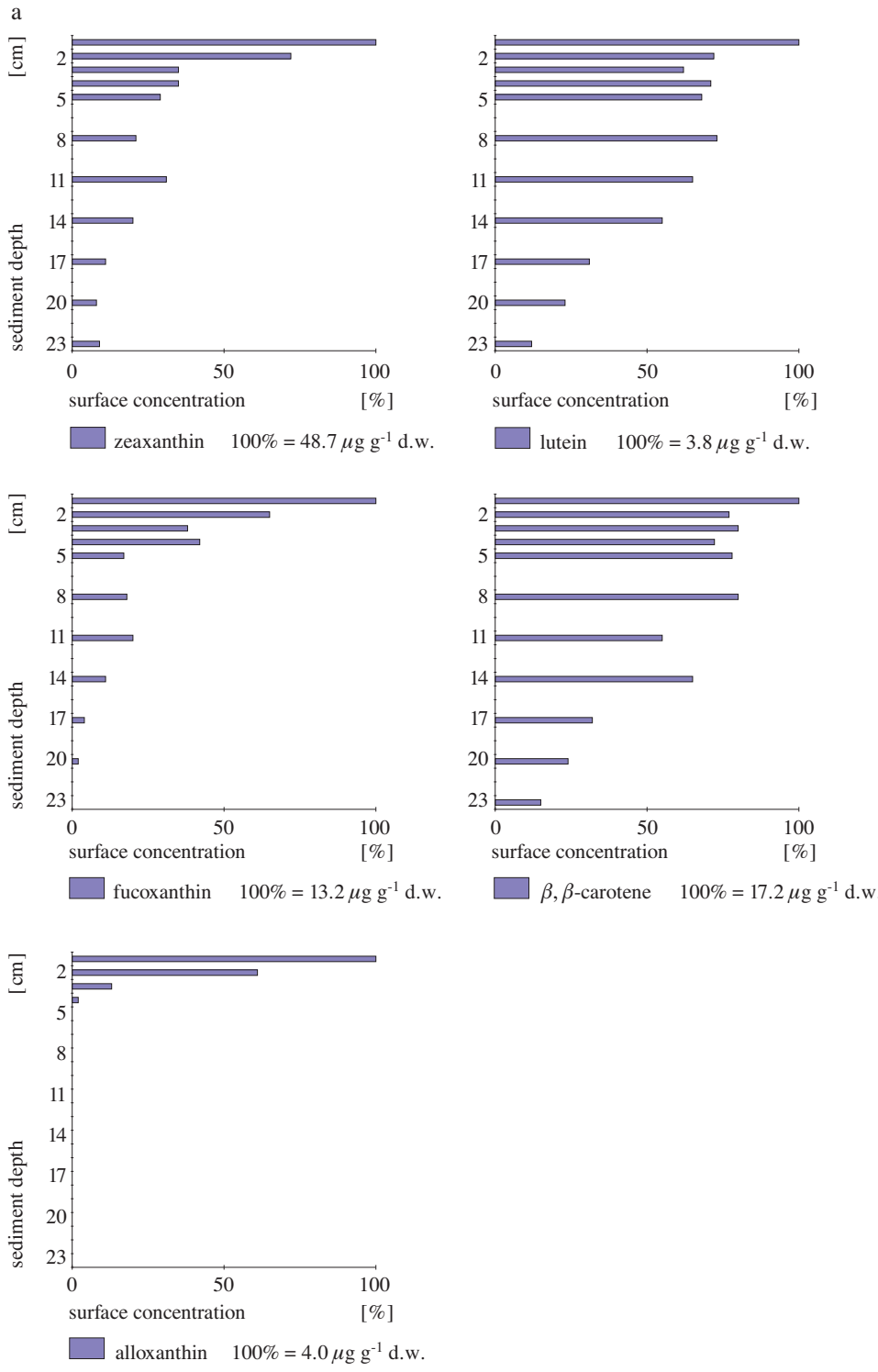
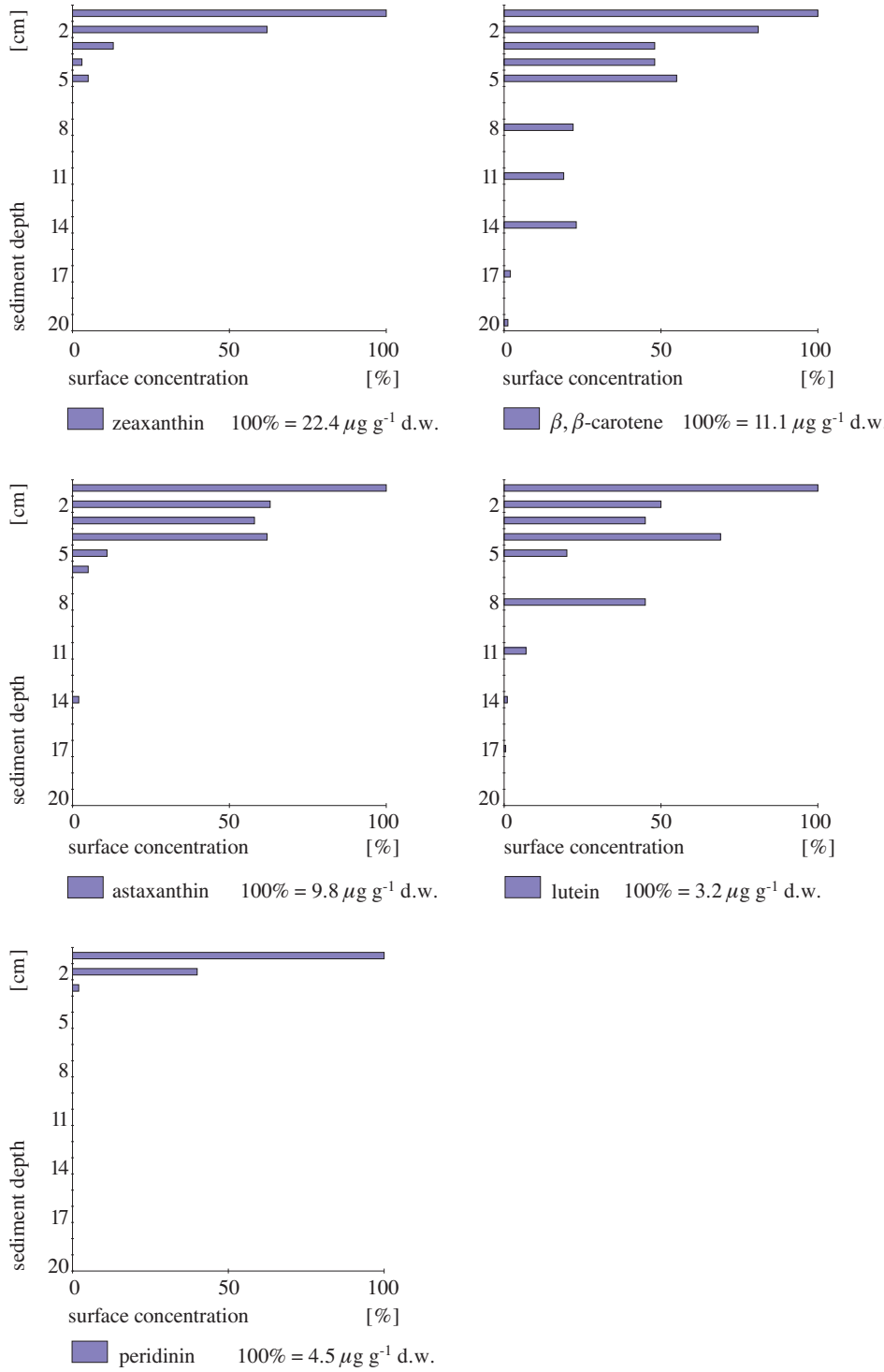


Fig. 3. Vertical distribution of carotenoids in sediment cores from the Baltic Sea

Vertical pigment profiles were measured in the sediment cores from all stations (Fig. 3) and display pronounced differences in pigment stability (Fig. 4).


a
sediment depth
[cm]
2
5
8
11
14
17
20
23
0
50
100
surface concentration
[%]
zeaxanthin 100% = 48.7 μg g-1 d.w.
lutein 100% = 3.8 μg g-1 d.w.
fucoxanthin 100% = 13.2 μg g-1 d.w.
β,β-carotene 100% = 17.2 μg g-1 d.w.
alloxanthin 100% = 4.0 μg g-1 d.w.

b


[cm]
sediment depth
2
5
8
11
14
17
20
0
50
100
surface concentration
[%]
zeaxanthin 100% = 22.4 μg g-1 d.w.
β, β-carotene 100% = 11.1 μg g-1 d.w.
astaxanthin 100% = 9.8 μg g-1 d.w.
lutein 100% = 3.2 μg g-1 d.w.
peridinin 100% = 4.5 μg g-1 d.w.

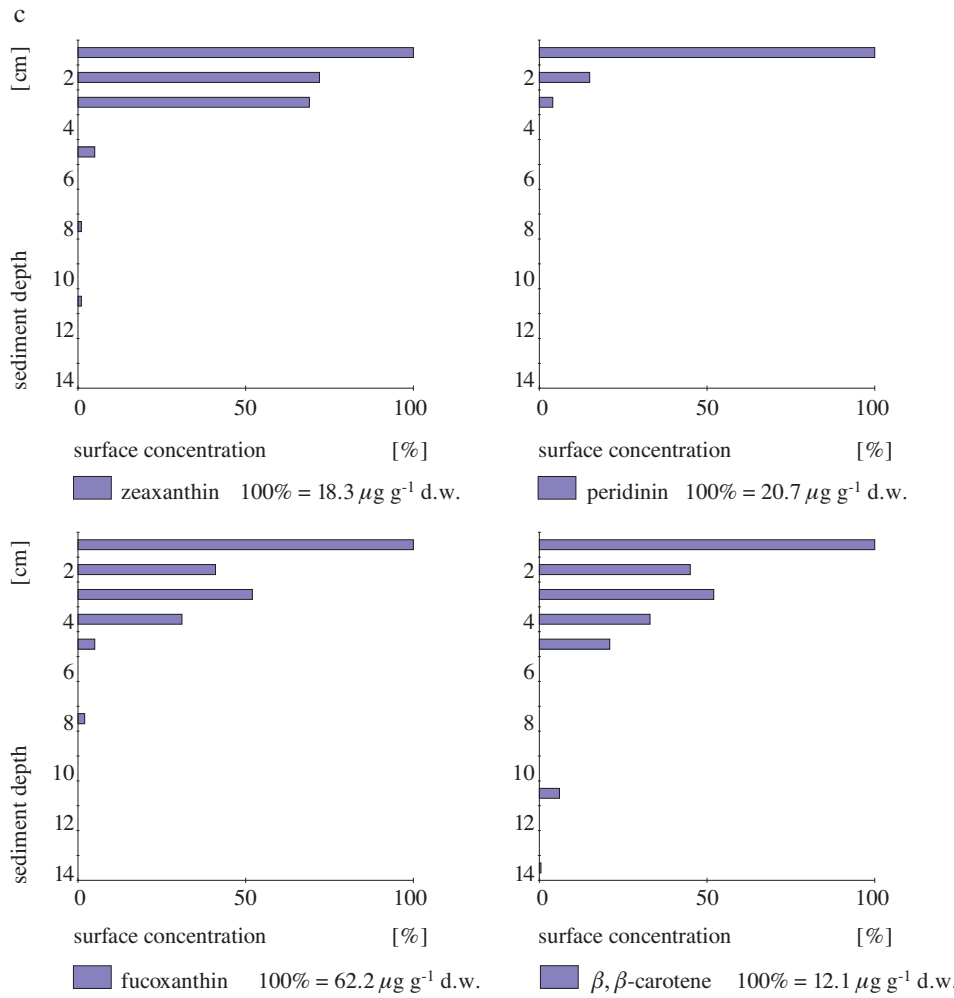


Fig. 4. Depth profiles of diagnostic pigments in the Gdańsk Deep (a), Bornholm Deep (b) and Gotland Basin (c) sediments

While peridinin, fucoxanthin, zeaxanthin and alloxanthin were subject to rapid degradation in the 0–3 cm or 0–5 cm layers of the sediment cores, the concentrations of lutein and β,β-carotene did not change to such an extent within the upper 14 cm in the Gdańsk Deep or in the 0–5 cm layer in the Gotland Basin and Bornholm Deep (Fig. 4). The concentrations of diagnostic pigments in surface sediments were the highest for fucoxanthin and zeaxanthin (62.5 μg g^{-1} d.w. and 48.7 μg g^{-1} d.w.) and $< 20.7\,\mu$g g^{-1} d.w. for the other pigments. Furthermore, astaxanthin, the biogenic marker of Crustacea and Bacteria (Matsuno and Hirao, 1989), was separated from the Bornholm Basin sediment (Fig. 4).

Being the principal pigment in various species of macrophytes, phytoplankton, bacteria and several animals, β,β-carotene was measured at all stations (Britton *et al.*, 1995).

4. Discussion and conclusions

The carotenoid concentrations in Baltic Sea deep sediments in June 1994 ranged from 140 to 560 μg g^{-1} d.w. The highest pigment concentration reported in the literature was 2–3 mg g^{-1} d.w. in the sediment from the Peru continental shelf (Repeta and Gagosian, 1987). Quantitatively, this sediment sample contained as many total pigments on a dry weight basis as phytoplankton do in culture. By contrast, Bianchi *et al.* (1996) reported 0.04–0.74 μg g^{-1} d.w. of these pigments in sediments from the eastern Mediterranean. In addition, carotenoids are labile organic compounds that are subject to degradation at the onset of diagenesis (Klein and Riaux-Gobin, 1991). The degradation rates can vary significantly, depending to a considerable extent on the light, oxygen and temperature conditions (Leavitt, 1988; Abele-Oeschger, 1991). It is well known that carotenes are rather stable in diagenesis (Watts and Maxwell, 1977) and that non-polar xanthophylls are more stable than polar xanthophylls (Abele, 1988). Differences in pigment stability were found in the sediments examined in this study, *i.e.* the rapid degradation of fucoxanthin, peridinin, alloxanthin and the slow breakdown of β,β-carotene and lutein in recent Baltic surface sediments was noted. In general, the concentration of most of the pigments was found to decrease with increasing depth profile of the sediments in question. These findings parallel the literature data (Abele-Oeschger, 1991; Klein and Riaux-Gobin, 1991).

From the chemotaxonomic point of view, as outlined by Liaaen-Jensen (1979), the thirty-seven pigments characterised in marine environmental analysis can be divided into four classes: phytoplankton, zooplankton, bacterial pigments and transformation products. Quantitatively, phytoplankton pigments are the most important in oceanographic studies. Five of them – zeaxanthin, fucoxanthin, peridinin, alloxanthin and lutein – characteristic of the marine phytoplankton, were present in the material examined. Changes in plant pigment concentrations in the surface sediments depended on the temporal and spatial dynamics of blooms in the Baltic Sea. The cyanobacterial bloom (*Aphanizomenon flos-aquae*, *Nodularia spumigena*, *Microcystis* sp.) at stations 1 and 3 was most probably responsible for the high zeaxanthin concentration (22.4–48.7 μg g^{-1} d.w.). The spring blooms of diatoms and dinoflagellates (*Paralia sulcata*, *Skeletonema costatum* and *Peridiniella catenata*, *Prorocentrum* sp.) at station 2 were reflected in the high levels of fucoxanthin (62.2 μg g^{-1} d.w.) and peridinin (20.7 μg g^{-1} d.w).

Alloxanthin is the principal carotenoid of cryptomonads (Pennington *et al.*, 1985) and is a minor component of a number of marine organisms (Liaaen-Jensen, 1979). On the basis of microscopic analyses, it can be concluded that cryptomonads were present in the Gotland and Gdańsk Deep. Since the samples were collected in the oxygen minimum zone, the contributions from the benthic macrofauna were probably not significant (Henrichs and Farrington, 1984). Indeed, no evidence of benthic macrofauna was found during the microscopic examination of the sieved subsamples. Yokoyama *et al.* (1996) reported astaxanthin-synthesising bacteria in marine sediments. In the light of these results, the benthic micro-organisms must be considered as potential sources of astaxanthin in the Bornholm Deep sediment too.

Finally, the analysis demonstrated that Baltic sediments contain a complex mixture of carotenoids with contributions from cyanobacteria, diatoms, dinoflagellates, cryptomonads, green algae, and very probably from bacteria. In many anoxic sediments they exist in sufficiently high concentrations to carry out quantitative analysis. Therefore, carotenoids are good chemotaxonomic biomarkers in environmental studies.

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