Chlorins in sediments of the Gotland Deep (Baltic Sea)*

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Abstract

The distributions of chlorin components as well as chlorophylls *c* and β-carotene in postglacial sediments (down to *ca* 5 m depth), collected from the Gotland Deep in July 1997, have been determined. The major pigments were similar to those identified in recent (0–10 cm) sediment extracts of the Gdańsk Deep. High

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amounts of well preserved chlorins, including intact chlorophyll *a*, provide evidence for high primary production in the past, anoxic sedimentation and favourable postdepositional conditions. In addition, the results reveal several extremely rapid changes in the Baltic environment over the last 8000 years caused by different physico-chemical conditions and subsequent changes in phytoplankton occurrence, biomass and algal species.

1. Introduction

Chlorophyll diagenesis is greatly influenced by the environmental conditions prevailing both at the time of deposition and afterwards. The occurrence of highly transformed chlorophyll derivatives (alkyl porphyrins) in sediments has been recognised for over sixty years (Treibs 1936). However, the early diagenetic chlorophyll transformation products (chlorins), which are less stable than porphyrins, were recognised much later. Recently developed HPLC techniques have made the analysis of such compounds possible (*e.g.* Jeffrey *et al.* 1997), although their geochemistry is still not fully understood.

The aim of this study was to investigate the distributions of chlorin components in postglacial sediments from the Gotland Deep as parameters reflecting environmental conditions.

2. Experimental

Sediments

The Gotland Basin, together with the Bornholm Basin and the North Central Basin, is one of three representative areas chosen for a detailed study of the paleoecology of the Baltic Sea as part of the EU-funded BASYS Project (Baltic Sea System Study). The areas were chosen on the basis of the following criteria:

- the basin should be large in size and situated along the axis of inflow of highly saline North Sea waters through the Danish Straits,
- sedimentation should have been more or less continuous for the last 8000 years,
- anoxic conditions should preferentially prevail, to ensure minimum bioturbation and the formation of undisturbed laminated sediments.

The Gotland Basin, the largest and the deepest (*ca* 250 m) of the three basins, is one of the most intensively studied areas in the Baltic Sea. The BASYS sediment samples were cored in the southeastern part of the Basin, the area with the thickest recent and sub-recent sediments. The site was, however, sufficiently far from the eastern slope to minimise the effect of coastal erosion and river input via direct down-slope processes, and it can be assumed that possible saline inflows would have a direct impact on deposition. The sampling site, in an area of the Gotland Deep which is supposed to be minimally influenced by material sliding down from the slopes of the basin or other sediment transport along the seafloor, was chosen to ensure that the bulk of the material was deposited directly from suspended material in the water column. A Side Scan Sonar survey was conducted around the coring site to ascertain the intactness of the seabed. No features which could be related to slumping or major gravity flows were observed in the detailed acoustic profiling conducted prior to sampling. The sediments in the area proved to be extremely soft and of low reflectivity. No indication of possible gas or water seeps was apparent.

The topography of the Gotland Basin is intermediate between the basin fill type of the Bornholm Basin and the typical conformable deposition pattern of the North Central Baltic Basin (Winterhalter 1992). The *ca* 4.5 m thick marine mud sequence is clearly divided into darker laminated sequences and frequent lighter, more homogenous sequences. To a first approximation the laminated units are found in the topmost 10 cm and in the intervals 45–110 cm, 235–355 cm and 370 cm to the base of the marine sequence at 444 cm. The laminated units probably represent long periods corresponding to anoxic conditions in the bottom water.

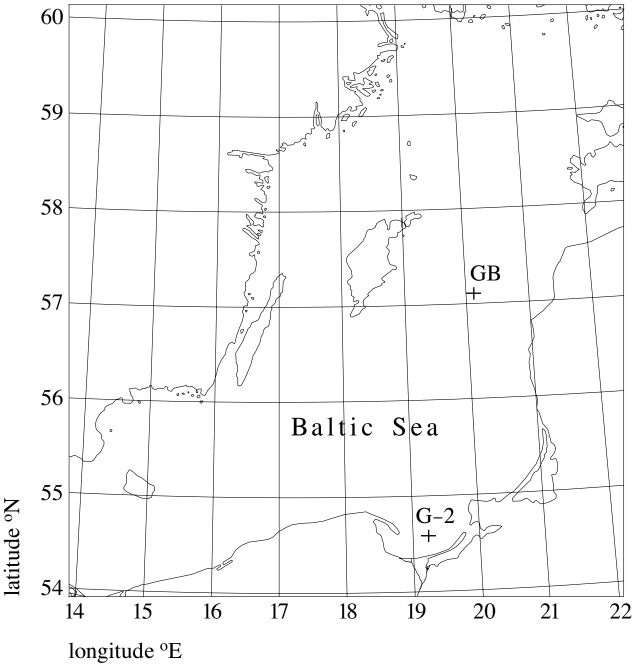

Fig. 1. Location of the sampling sites

2.1. Sampling

Sediments were collected during the r/v 'Petr Kottsov' cruise (20 July–2 August 1997) from the Gotland Deep (57°17.028′N, 20°07.138′E) using a Kastenlot box corer (30 cm × 30 cm, 10 m length); water depth was 241 m (GB, Fig. 1). The core was sub-sampled in 4 cm thick sections from 8 to 512 cm depth and kept frozen until analysis. For comparison, the topmost 10 cm of sediment was collected in March 1997 from the Gulf of Gdańsk, the most chlorin-rich area of the southern Baltic (G–2, Fig. 1). The Gdańsk Deep (anoxic, *ca* 100 m water depth) is a sink for particulate matter transported by the River Vistula.

Pigment extraction

The extraction procedure has been described previously (Kowalewska *et al.* 1996, Kowalewska 1997) and was in part similar to that used routinely in other studies of chlorins (Eckardt *et al.* 1991, 1992). Typically, an aliquot of the frozen sample (*ca* 1–10 g) was left to thaw and excess water was removed by centrifugation. The samples were extracted ultrasonically with acetone and chlorins from the acetone extract were re-extracted into benzene. The benzene extract was evaporated to dryness under a stream of argon and the residue was dissolved in a small volume of acetone (200–1500 μl) and analysed by HPLC.

HPLC

Extracts were analysed by HPLC (Knauer) using two detectors, a diode-array (Barspec Chrom-a-Scope) and a fluorescence detector (Shimadzu RF–551), the procedure having been described previously (Kowalewska 1997). An aliquot (20 μl) of the extract was injected on a Merck Lichrospher 100 RP 18e column (250 × 4 mm, 5 μm) with the same precolumn (4 × 4 mm, 5 μm). Separations were typically carried out in an acetone-water gradient system as follows: 0 min 80:20, 10 min 85:15, 20 min 95:15, 40–55 min 100% acetone, 65 min 80:20, at a flow rate of 1.0 ml min^{-1} and for determination of chlorophylls *c* at 0.5 ml min^{-1}. Fluorescence analyses were carried out for the determination of chlorophylls *c* (Ex/Em 440/630 nm) and very low concentrations of chlorins; in other cases the diode-array detector was used. Specific pigments were identified on the basis of comparison of their retention times and electronic spectra with those of standards and with literature data. Standards of chlorophyll *a*, phaeophytin *a*, phaeophorbides and β-carotene were prepared from higher plant leaves and those of chlorophylls *c* from cultures of the diatom *Cyclotella meneghiniana*, using TLC (CAMAG–Linomat IV). Components were quantified from their diode-array chromatograms and UV–VIS absorption spectra and for fluorescence measurements from calibration against diode-array chromatograms determined for the same extracts (Kowalewska 1997).

HPLC–MS

Two of the extracts were also analysed using HPLC (Waters MS600 silk quaternary) with mass spectrometric detection. Reversed phase HPLC with a Phenominex ODS3 column (150 mm × 4.6 mm i.d., 1 ml min^{-1}) with an ODS3 guard column (30 mm × 4.6 mm i.d.) was used. An on-line Waters 991 PDA detector provided electronic spectra of individual components from 350 to 700 nm. Gradient elution was as follows: with 80% B, 20% C isocratic (5 min), then to 30% A, 60% B, 10% C after 15 min and isocratic to 20 min, then to 60% A, 30% B, 10% C after 30 min, then to 80% A, 10% B, 10%C after 45 min, then to 90% A, 5% B, 5% C after 55 min and isocratic thereafter to 120 min, where A = acetone, B = methanol and C = water. The system was linked to a Finnigan MAT TSQ 700 quadrupole mass spectrometer via a Finnigan MAT atmospheric pressure interface (APCI; Harris *et al.* 1995a). Interface conditions were: vapouriser 450°C; capillary 300°C; corona 7 μA ($\cong$ 5 kV), sheath gas pressure 20 psi with the auxiliary gas turned off. Positive ion spectra were recorded by scanning from m/z 400–1200 in 2 s. Data acquisition was performed using an ICIS data system. Components were assigned by comparison with literature data.

Organic carbon analyses

Organic carbon content was determined by the acid wet titration method (Goudette *et al.* 1974).

3. Results and discussion

The sediment extracts to a depth of *ca* 450 cm revealed the presence of similar complex mixtures of chlorins (*e.g.* Fig. 2), only with the components in different proportions; similar mixtures have been observed previously in extracts from North Central Basin sediments and the upper layers of the Bornholm Basin sediments (Kowalewska *et al.* 1998). The major chlorins were assigned as: phaeophorbide *a*-like components, chlorophyll *a*, chlorophyll a', phaeophytin *b*, phaeophytin *a*, pyrophaeophytin *a*, and a range of steryl chlorin esters (SCEs; *e.g.* Eckardt *et al.* 1991); the latter comprised not only derivatives of chlorophyll *a* (*i.e.* sterol esters of pyrophaeophorbide *a*) but also of chlorophyll *b* (sterol esters of pyrophaeophorbide *b*; *cf* Cariou-Le Gall *et al.* 1998). The chromatograms of the deep layer extracts down to a depth of ~450 cm, were

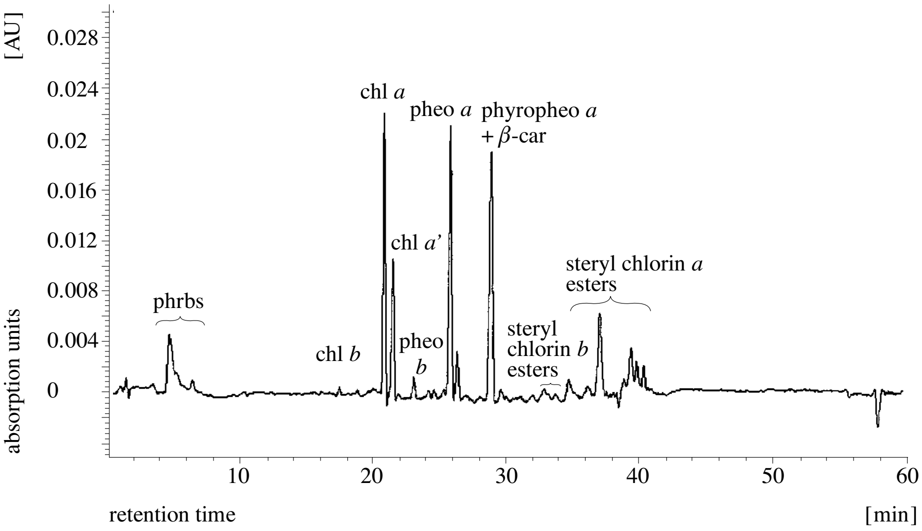

Fig. 2. Diode-array HPLC chromatogram (660 nm) of a Gotland Deep sediment extract (layer 68–72 cm)

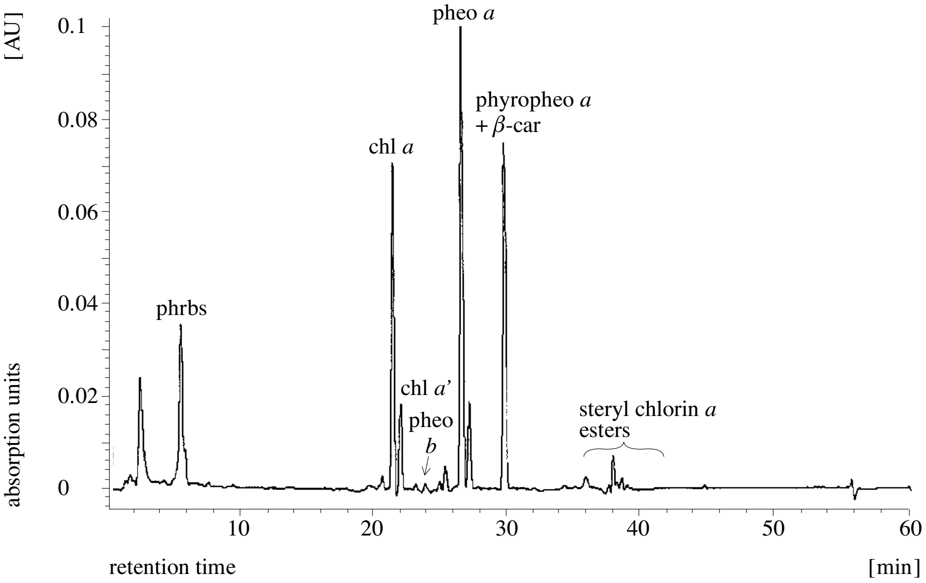

Fig. 3. Diode-array HPLC chromatogram (660 nm) of a recent (0–1 cm) sediment extract from the Gdańsk Deep

comparable to that of the surface sediment (0–1 cm) from the Gdańsk Deep (Fig. 3). HPLC–MS analysis of two samples from 68–72 cm and 418–422 cm

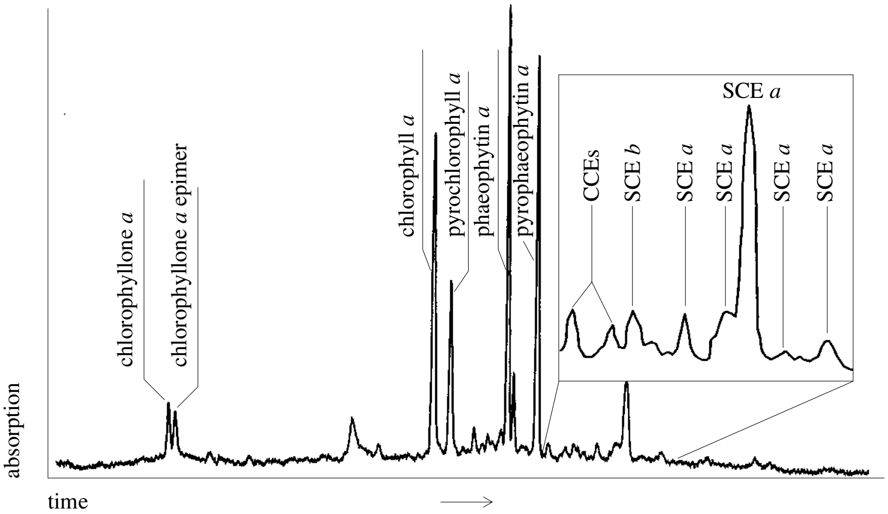

Fig. 4. HPLC–MS diode-array chromatogram (660 nm) of a Gotland Deep sediment extract (layer 68–72 cm)

identified (Fig. 4) the component denoted as chlorophyll a' in Fig. 2 as pyrochlorophyll *a* (both having the same electronic spectrum as chlorophyll *a*), chlorophyllone *a* and its epimer denoted as the phaeophorbide *a*-like components in Fig. 2. (Harris *et al.* 1995b). A range of carotenoid chlorin esters was also detected (CCEs – carotenoid alcohols esterified to pyrophaeophorbide *a* (Goericke *et al.* 1998). The mass spectra confirmed

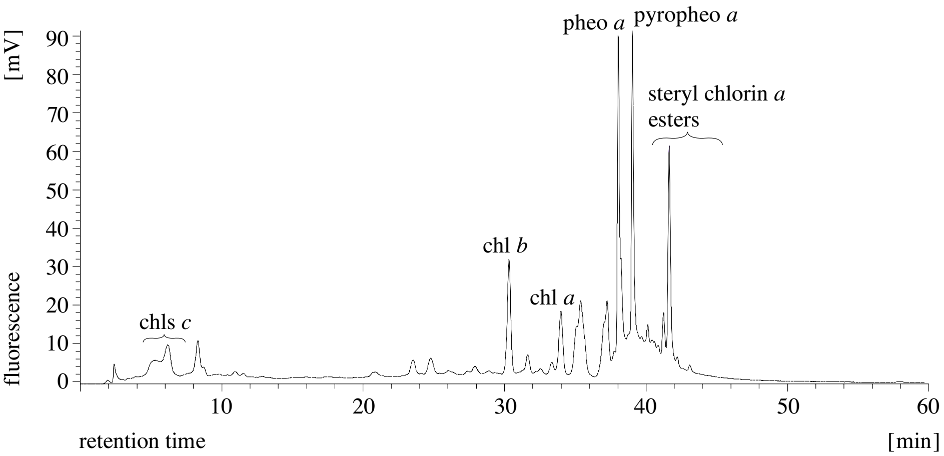

Fig. 5. Fluorescence HPLC chromatogram (Ex/Em – 440/630 nm) of a Gotland Deep sediment extract (layer 78–82 cm)

the assignment of the other components in Fig. 2. Fluorescence detection was used to detect and quantify the chlorophylls *c* (Fig. 5).

Fig. 6 shows the concentration depth profiles of chlorophyll *a* and the major chlorophyll *a*-derived chlorins and Fig. 7 the profiles of the chlorophylls *b* and *c* and the carotenoid pigment β-carotene. In the extracts from below 450 cm only the more stable derivatives of chlorophyll *a*, *i.e.* SCEs and pyrophaeophytin *a*, were present in very low abundance, along with even lower concentrations of phaeophytin *a*; chlorophyll *a*, chlorophyll a' (pyrochlorophyll *a*) and phaeophorbide *a*-like chlorins (chlorophyllones) were absent. This indicates that oxic conditions were present at the time of deposition of these sediments.

In general, the depth profile characteristics of the major pigments are similar, with three regions of high concentrations containing maxima around 70, 300 and 420 cm. These regions of high concentration coincide with the regions containing the laminated units which are thought from the lithological description to represent periods of bottom water anoxia (see above). The summed concentrations of the major chlorins *a* show the greatest and sharpest maximum at *ca* 70 cm and are comparable to those found in the surface sediment (0–10 cm) of the Gdańsk Deep, collected in 1997 (Fig. 8). In addition, the high amounts of intact chlorophyll *a* provide further evidence for anoxic conditions in the bottom waters. For comparison, the concentrations of chlorophyll *a* in recent sediments from the Gdańsk Deep, determined over the last few years are shown in Fig. 9, where a rapid decomposition of chlorophyll *a* within the top 10 cm is apparent. Hence, given the age of the deeper sediments from the Gotland Deep, it appears that conditions were more favourable than at present for chlorophyll preservation. On the other hand, the proportion of chlorophyll *a* to the summed chlorins *a* is similar right through the core except for the two samples at 110 and 190 cm (Fig. 10). This leads us to conclude that the conditions within the sediments were more or less similar throughout but that there were rather different and rapidly changing conditions in the euphotic zone of the water column, leading to algal blooms, this higher productivity being reflected in the chlorins *a* (Figs. 6 and 7) and β-carotene maxima (Fig. 7).

The correlation coefficient with organic carbon is high for the sum of the major chlorins *a* (Fig. 11), $r > 0.9$ – exponential correlation; the correlation is poorer, however, for the steryl chlorins *a* and *b* and the chlorophylls *c* (Fig. 12). The good correlation with the chlorins *a* shows that the prevailing part of the organic matter in the sediments is of phytoplanktonic origin

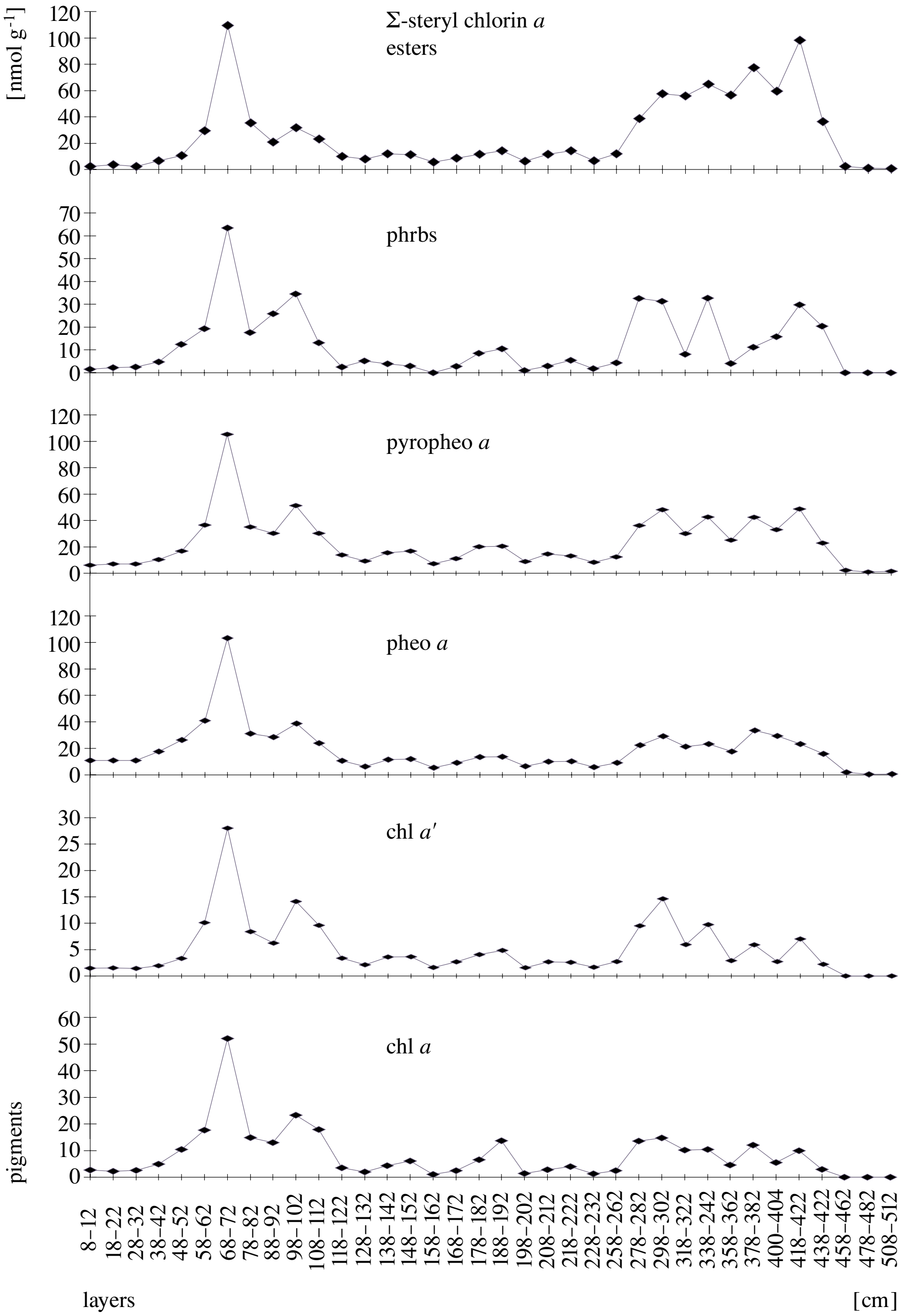

Fig. 6. Concentration profiles of chlorins *a* in the Gotland Deep sediment core: chl *a* – chlorophyll *a*, chl *a*′ – chlorophyll *a*′ (pyrochlorophyll *a*), pheo *a* – phaeophytin *a*, pyropheo *a* – pyrophaeophytin *a*, phrbs (chlone) – phaeophorbides *a*-like (chlorophyllone epimers), steryl chlorin *a* esters – SCEs

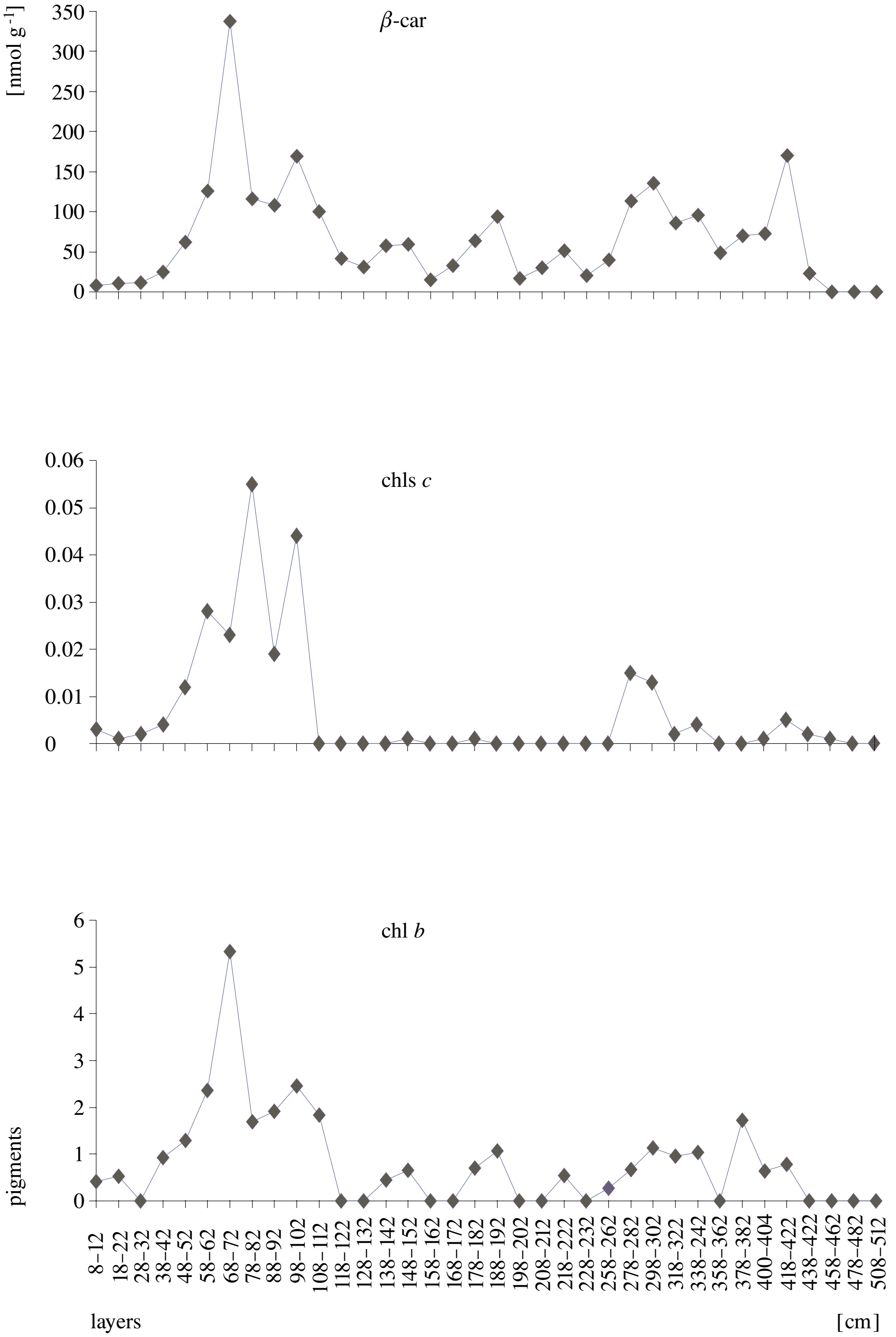

Fig. 7. Concentration profiles of chl *b* – chlorophyll *b*, chls *c* – chlorophylls *c*, *β*-car – *β*-carotene in the Gotland Deep sediment core

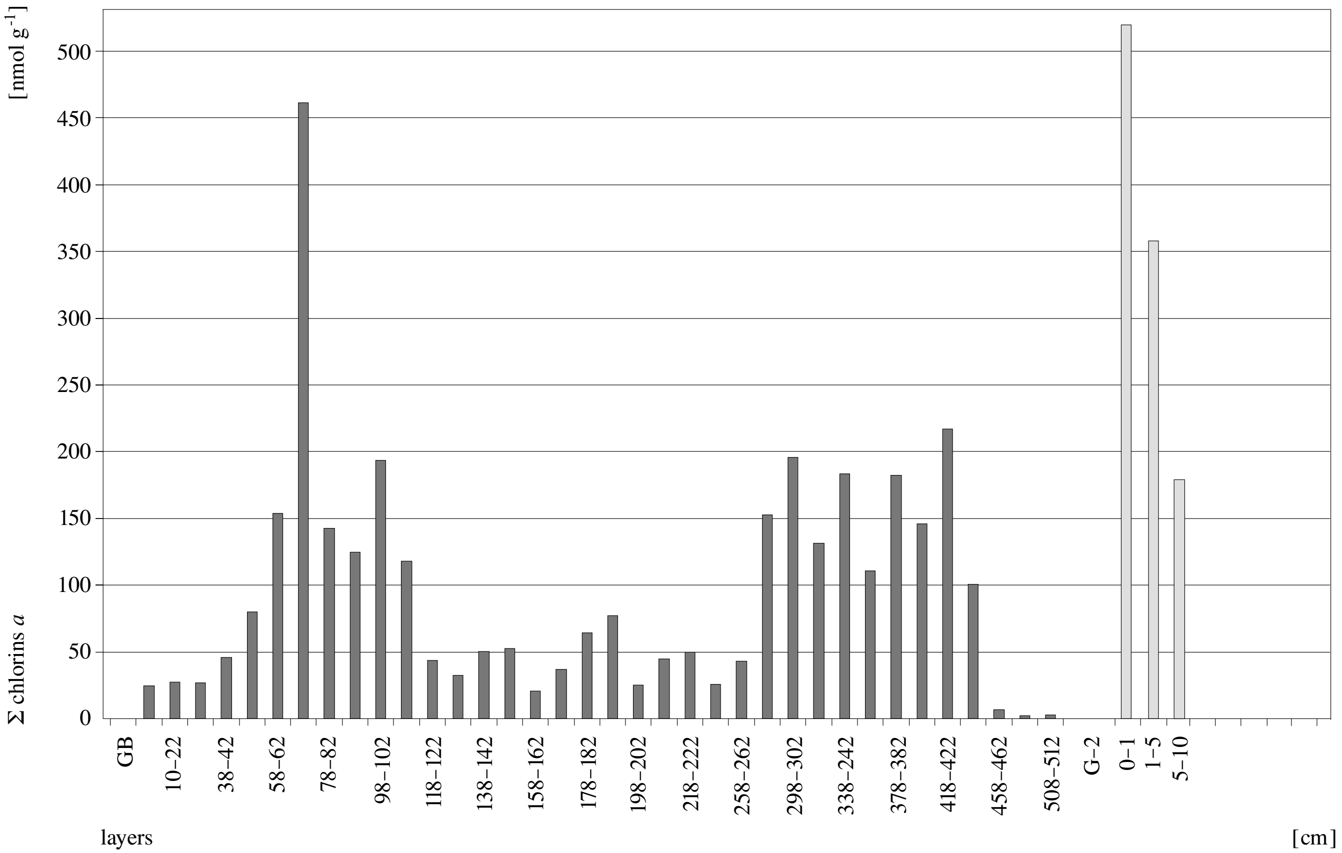

Fig. 8. Summed concentrations of chlorins *a* in the Gotland Deep (GB) sediments and recent (0–10 cm) sediments from the Gdańsk Deep (G–2) collected in March 1997

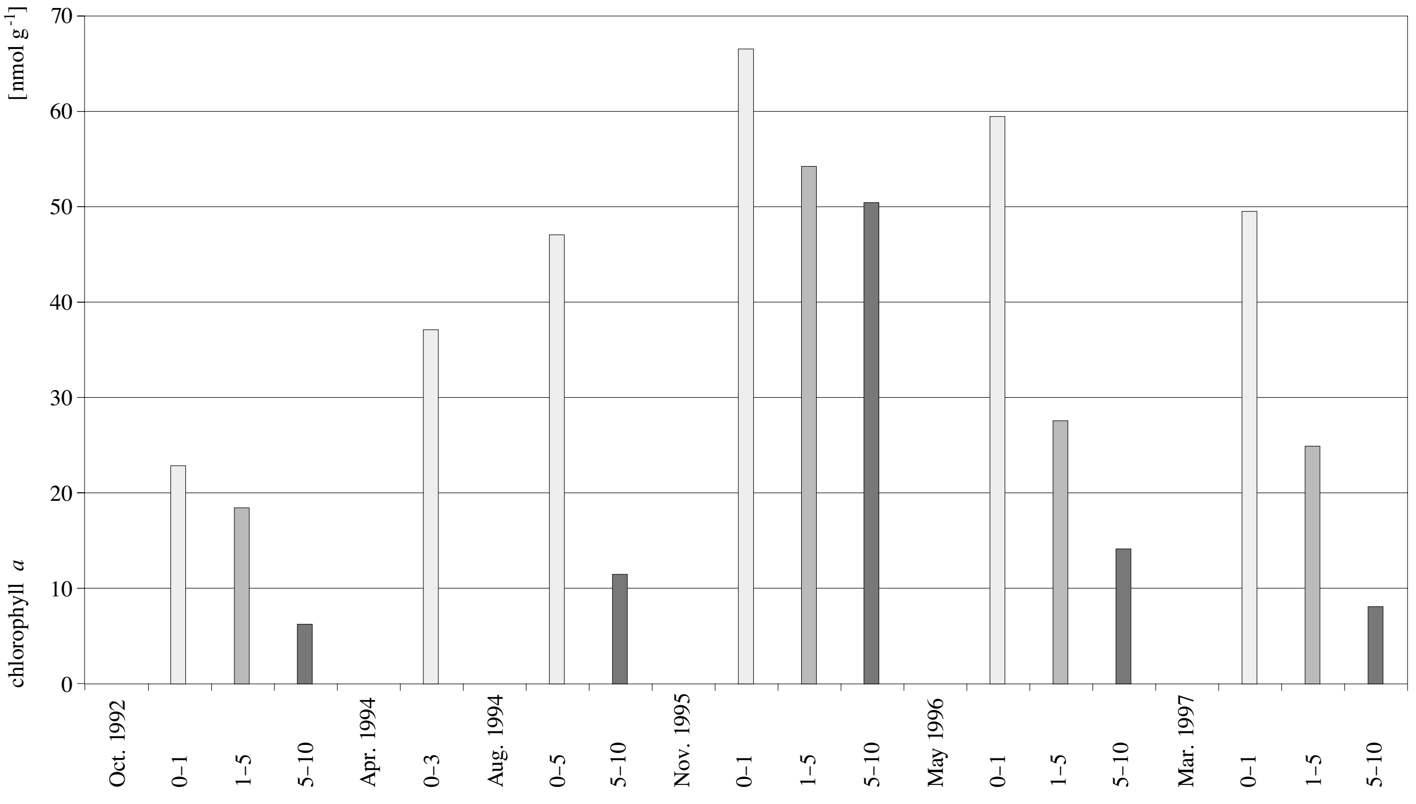

Fig. 9. Chlorophyll *a* concentrations in recent sediments from the Gdańsk Deep, collected in 1992–1997

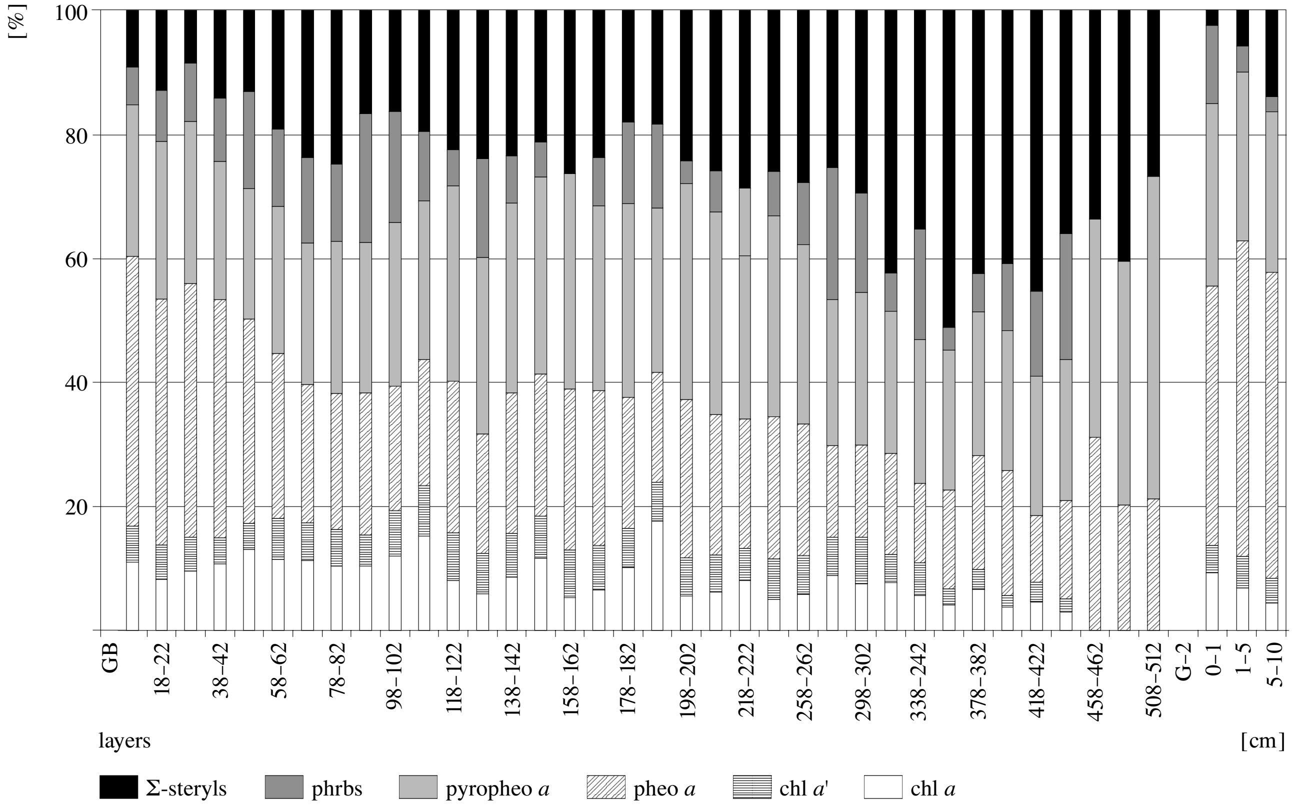

Fig. 10. Proportion [%] of major chlorins *a* [nmol g^{-1}] relative to the sum of chlorins *a* in the Gotland Deep (GB) and Gdańsk Deep (G–2) sediments (the short forms used in above legend are the same as those in the caption to Fig. 6)

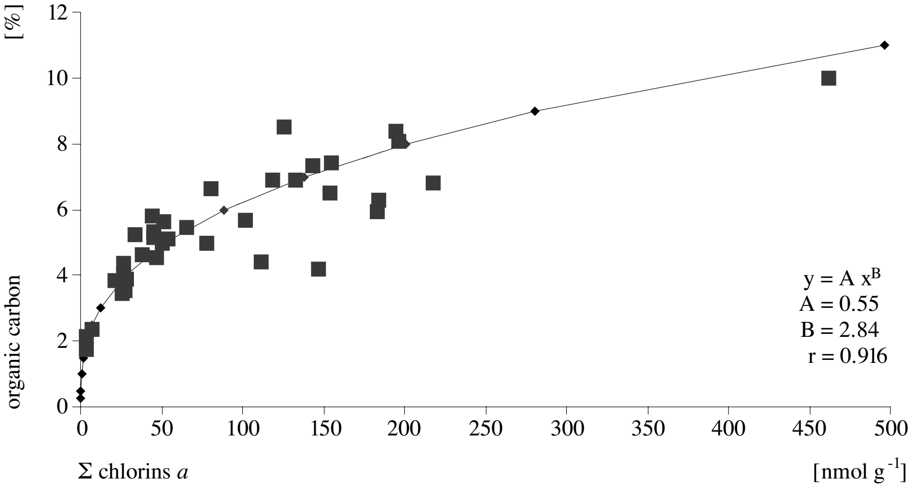

Fig. 11. Plot of the sum of major chlorins *a* [nmol g^{-1}] vs organic carbon [%]

and is not highly degraded. The contributions to this organic matter might include blue-green algae and species containing chlorophyll *b*, such as green algae (higher plants not totally excluded), with a smaller input of chlorophyll *c*-containing species such as diatoms and/or dinoflagellates.

The sediments around 70 cm are exceptional and correspond to an enormous and rapid increase in the primary production of algae containing chlorophylls *a* and *b* or, less possible, an input from the higher plants (Figs 6 and 7), which was followed (78–82 cm) by a marked increase in the proportion of species containing chlorophylls *c* (Fig. 7) as a result of a rapid change in the conditions in the water column. Further evidence comes from the low correlation coefficient between the chlorophylls *c* and organic carbon. This is also reflected in the low correlation coefficient for the SCEs, which may be products of zooplankton grazing (Harradine *et al.* 1996) and are not formed equally effectively from all algal species during grazing (Talbot *et al.* unpublished results).

Overall, the high amounts of well preserved chlorins provide evidence for high primary production and conditions favourable for the preservation of such compounds, *i.e.* low temperature, lack of water mixing and of bioturbation and anoxic conditions in sediments. In addition, the results indicate several extremely rapid changes in the Baltic environment, over the last 8000 years especially in the water masses, caused by different conditions of temperature, salinity *etc.* and leading to productivity changes and the phytoplankton succession.

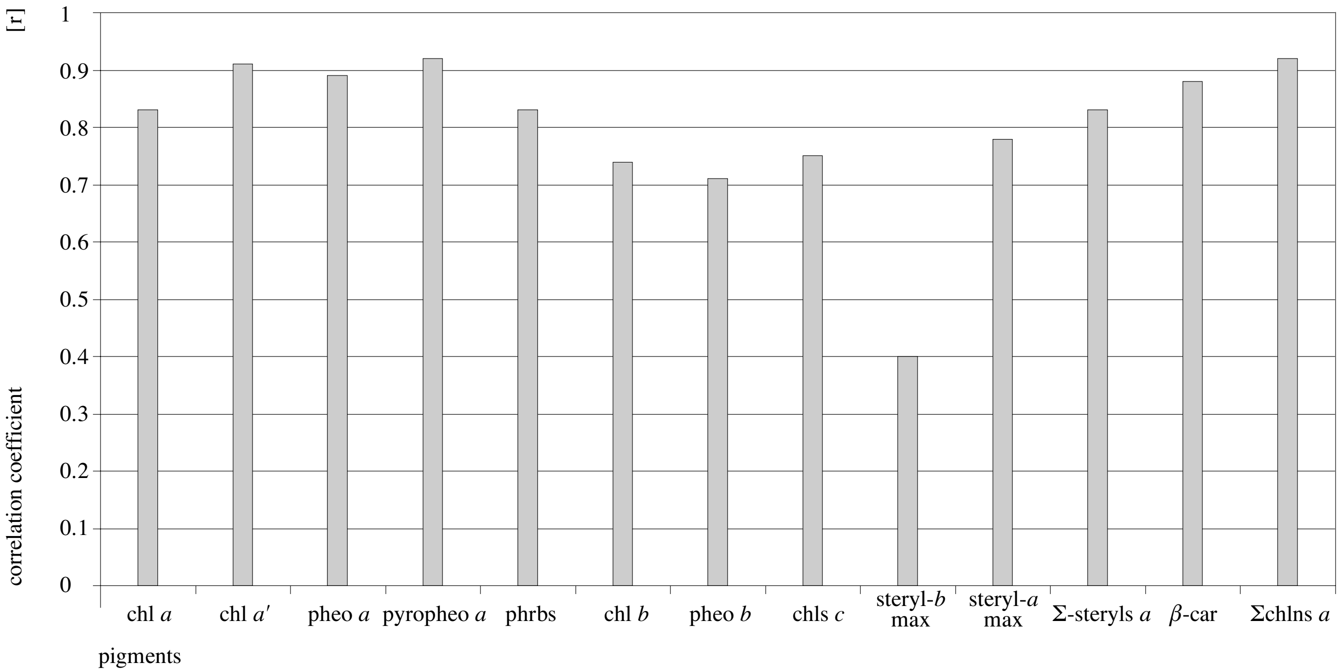

Fig. 12. Plot of exponential correlation coefficients of the determined pigments with organic carbon (the short forms used above are the same as those in the captions to Figs. 6 and 7)

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