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A century-scale record of the preservation of chlorophyll and its transformation products in anoxic sediments

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Abstract—We have determined the chlorophyll pigment composition by liquid chromatography (LC) and LC/MS/MS in a 1.45-m long freeze core, representing 157 years of annually varved sedimentation, from Saanich Inlet, B.C., Canada. We investigated the very early diagenetic processes of chlorophyll *a* alteration in these anoxic sediments and the possible implications for palaeoproductivity studies. Excellent preservation of pigments is indicated by high total pigment concentrations, and the presence of labile compounds such as chlorophyllide *a*. The lack of systematic down core changes in both the total pigment concentration and the chlorin composition indicates that no detectable alteration of the pigment composition has occurred during the past 157 years. The sedimentary pigment composition is the result of processes occurring in the water column, or within few months after deposition. Chlorophyll derivatives corresponding to different diagenetic processes have distinct down core profiles. Profiles of compounds related to grazing activity steryl pyropheophorbide esters (SPE) and pyropheophytin *a*, are very similar. In contrast, dephytylated compounds (chlorophyllide *a* and phaeophorbide *a*), which are related to chlorophyllase activity during the degradation of ungrazed diatom cells, show an independent pattern. Quantifying pigment composition in Saanich Inlet sediments can help constrain processes regarding the transport of algal pigments to the sediments. Copyright © 2000 Elsevier Science Ltd

1. INTRODUCTION

Research into climatically driven changes in organic carbon cycling and burial in the ocean has led to the search for sedimentary tracers of marine production. The chlorophyll *a* concentration in seawater is commonly used as a measure of the standing stock of primary producers, and the accumulation of chlorins, transformation products of chlorophyll, in ocean sediments has been proposed as an indicator of changes in total primary production over glacial–interglacial time scales (Summerhayes et al., 1995; Harris et al., 1996). Although a correlation between the concentration of sedimentary chlorins and overlying productivity has been described (Sun et al., 1994), diagenetic factors can distort severely the productivity signal accumulated in the sedimentary record.

Laboratory studies have shown slower degradation rates and better preservation of chlorophylls in anoxic conditions compared to oxic ones (Sun et al., 1993a,b). These observations are consistent with higher pigment concentrations typically found in anoxic environments (Kowalewska, 1994; Sun et al., 1994; King, 1995). Chlorophyll diagenesis in oxic sediments usually results in a dramatic decrease in the concentration of acetone-extractable pigments in the top 0.2–8 cm of the sedimentary column (Furlong and Carpenter, 1988; Sun et al., 1994; Stephens et al., 1997). Modeling experiments have suggested that the half-life of chlorins in oxic environments ranges from 40 to 200 days (Furlong and Carpenter, 1988; Sun et al., 1994;

Stephens et al., 1997). In sediments underlying high productivity areas, the initial rapid degradation of chlorins is not complete (Sun et al., 1994) and residual compounds degrade according a much slower process with a half-life of about 450 years (Stephens et al., 1997). The occurrence of this pool of refractory chlorins in oxygenated sediments has been attributed to protective effects associated with the organic matrix.

Sampling of surface sediments in anoxic environments is often complicated due to their unconsolidated nature (Crusius and Anderson, 1991) and detailed studies of the alteration of chlorophyll compounds in the upper few centimeters of anoxic sediments are scarce. Down core profiles of carotenoids in anoxic sediments from the Black Sea do not show any decreasing trend, suggesting no apparent degradation within the sediments (Repeta, 1993). These results contrast markedly with the finding of a decrease of most lipid components (fatty acids, sterols, alkanes, alcohols) in the first 2 cm of the sediment column (Sun and Wakeham, 1994). In contrast, a moderate decrease of chlorin concentrations has been described in the top 10 cm of anoxic sediments of the Baltic Sea (Kowalewska, 1994).

During the past two decades, several chlorophyll *a* degradation compounds have been described in the marine systems that can be useful tracers of specific diagenetic processes. Phaeophorbides have been used to trace grazing processes in the water column (Jeffrey, 1974). However, the use of phaeophorbides as tracers for grazed materials in the sedimentary environment is complicated by other possible processes such as bacterial attack of algal cells (Spooner et al., 1994a), the release of endogenous enzymes during algal senescence (Daley, 1973; Daley and Brown, 1973; Spooner et al., 1994a), and the activity of chlorophyllase (Villanueva et al., 1994a). More recently, a series of steryl pyropheophorbide *a* and carotenol pyropheo-

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phorbide *a* compounds have been described in marine sediments and in the water column (King and Repeta, 1991; Eckardt et al., 1992; Kowalewska, 1994; Pearce et al., 1998; Goericke et al., 1999) that are specific markers of grazing processes. Also, chlorophyllide *a* is related to the activity of chlorophyllase, an endogenous enzyme abundant in many diatoms (Jeffrey and Hallegraeff, 1987; Louda et al., 1998). The usefulness of these degradation products to trace processes occurring in the water column has been demonstrated and studies performed in the particulate matter and in sediment traps have often used these compounds to trace grazing processes in the water column. However, the lack of understanding of the postdepositional diagenetic processes has complicated the use of those derivatives in the sedimentary environment. A significant effort has recently been devoted in determining new chlorophyll derivatives and new degradation pathways (Eckardt et al., 1992; King and Repeta, 1994; Harris et al., 1995b; Louda et al., 1998; Goericke et al., 1999), but there is a lack of quantitative studies of chlorophyll derivatives in sediments and its potential to assess biogeochemical processes in the past.

In this article we present the first detailed study of the chlorin composition in annually varved marine sediments during the past 157 years. Using a freeze corer to sample these unconsolidated sediments ensures the recovery of the sediment–water interface, and reliably preserves each lamination. Down core chlorin determinations indicate that no detectable alteration of chlorophylls in the sediment occurred between 1997 and 1840. The pigment composition in these marine sediments varies from year to year. We argue that these changes are related to processes that take place in the water column or at the sediment–water interface. This study demonstrates the potential of some sedimentary chlorophyll derivatives to trace specific biogeochemical processes in the past, such as phytoplankton decay and grazing.

2. SITE DESCRIPTION

Saanich Inlet (Vancouver Island, British Columbia, Canada) is a highly productive fjord with a maximum depth of 236 m and a 75 m sill at the entrance (Fig. 1). Restricted circulation in the basin limits the renewal of bottom water, and the high production of the surface waters coupled with the isolation of the deep waters results in anoxic conditions below 150 m (Richards, 1965). Renewal of deep water occurs each year during late summer or early fall when dense, oxygen-rich water flows over the sill and into the basin, partially replacing the anoxic deep water (Anderson and Devol, 1973).

Production is dominated by intense episodic diatom blooms. A major bloom is triggered by the seasonal increase in irradiance in April and May (Takahashi et al., 1977; Hobson, 1983). This is followed by a series of shorter and less intense blooms during the summer months (Takahashi et al., 1977). These events are related to the influx of nutrient-rich waters from Haro Strait through Satellite Channel (Mackas and Harrison, 1997) and the tidal mixing of surficial and intermediate waters (Takahashi et al., 1977; Parsons et al., 1983; Stucchi and Whitney, 1997). Another major diatom bloom generally occurs in late fall (Takahashi et al., 1977). During winter, productivity is light-limited and phytoplankton are relatively scarce and subject to extensive grazing (Takahashi et al., 1978; Takahashi

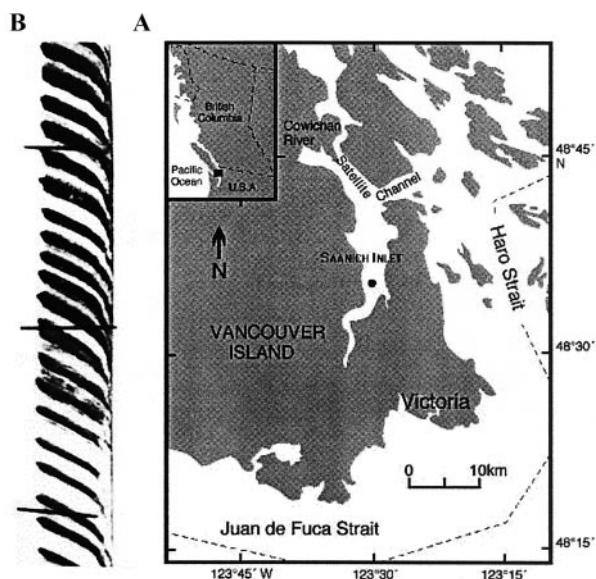


Fig. 1. (A) Location of the study site, Saanich Inlet, British Columbia. Dot indicates sampling site at 48°35.1'N, 123°29.5'W. The dashed line is the Canada/USA border. (B) X-radiography of a slice of the freeze core. The age model was obtained by visually counting successive dark–light couplets.

and Hoskins, 1978). The settling particulate material during the summer is dominated by diatom flocs, often with intact chloroplasts (Sancetta and Calvert, 1988). Fecal pellets are a minor fraction of the sinking particles in the summer. During the winter, sinking materials are composed primarily of detrital particles (Sancetta and Calvert, 1988) originating from the Cowichan and the Fraser Rivers outside the fjord (Herlinveaux, 1962).

Sediments of the central basin are finely laminated and have a very high porosity (0.93–0.95). The laminae consist of alternating detrital and siliceous layers that represent annual varves (Gucluer and Gross, 1964; Fig. 1). Dark layers have a high content of detrital materials supplied during the rainy winter period. Light bands have high contents of diatom silica and organic matter and represents the spring and summer deposition (Takahashi et al., 1977; Sancetta and Calvert, 1988; McQuoid and Hobson, 1997).

3. MATERIALS AND METHODS

3.1. Sample Collection

Collection of undisturbed cores of the surficial sediment layers from Saanich Inlet is not possible using conventional coring techniques because of their highly unconsolidated nature. For this study we used a freeze-core technique (Shapiro, 1958) modified by Crusius and Anderson (1991). A rectangular aluminum tube filled with a mixture of dry ice and ethanol was inserted into the sediment for 20 min. The sediment freezes onto the outer surface of one side of the corer and preserves the laminations in situ.

We collected a 20 cm wide, 2 cm thick, 145 cm long sediment core at 220 m of water depth (48°35.1'N, 123°29.5'W) in July 1997. The surface of the sediments was overlain with clear frozen seawater, indicating that the sediment–water interface was obtained. X-radiographs show alternating dark and light layers (Fig. 1) that correspond to interbedded laminae with high and low detrital content, respectively.

3.2. Chronology and Sediment Sampling

The core chronology was established by counting the varves on the X-radiographs (Fig. 1). Each light–dark couplet was counted as 1 yr, assuming that the uppermost dark layer corresponds to the winter of 1996–1997. The 145 cm core contains 157 varves, extending from 1997 to 1840. Uncertainty in varve counting was estimated to be ± 3 yr at 100 yr BP. The average varve thickness was 0.85 ± 0.4 cm (1s, $n = 157$). Because of sediment compaction, the upper 15 varves showed a uniform decrease in thickness with depth, from 3 to 0.9 cm. Varves corresponding to 1996, 1973, and 1920 were unusually thick (6.1, 2.8, and 5.8 cm, respectively) corresponding to massive layers deposited by subaqueous debris flows (Blais-Stevens et al., 1997). To study annual changes in the accumulation of chlorophyll pigments, we sampled every varve.

3.3. Extraction

Wet sediment samples (3 mL) were weighed into a glass test tube, centrifuged, and the overlying water was discarded. Pigments were extracted three times with HPLC grade acetone with sonication in a water bath at 4°C. The combined solvent extracts were evaporated under a N₂ stream to a final volume of 4 mL. The sediments were dried at 60°C overnight to obtain the dry weight and percent water. To purify the extract, 2 mL of 10% aqueous NaCl were added and the resulting mixture was extracted with HPLC grade ethyl acetate until complete decoloration. Extracts were dried with anhydrous Na₂SO₄, the solvent evaporated under a N₂ stream, and redissolved in 4 mL of acetone for instrumental analysis.

3.4. Visible Spectra and Liquid Chromatography

Visible spectra of total extracts were obtained with a Cary 3 spectrophotometer (model 3000) scanning from 350 to 700 nm at 100 nm/min with 1 nm resolution. Total chlorins were quantified by UV absorbance at 665 nm after background correction and are expressed in terms of equivalents of phaeophorbide *a* (nmol/g dry sediment, nm/gdw). The background level was estimated by averaging the absorbance measured at 585 and 750 nm.

$$A = A_{665} - A_{corr}$$

$$= A_{665} - \left(\left(\frac{750 - 665}{750 - 585} \right) \times (A_{585} - A_{750}) + A_{750} \right)$$

A Waters 510 liquid chromatograph equipped with an automatic injector (Waters 717+) and a photo-diode array detector (190–700 nm, Waters 996) was used to separate and identify individual compounds. The detector was operated at 665 and 410 nm for selective monitoring of chlorophyll derivatives. Separation was performed on a C₁₈ column (Econosil, 5 μm particle diameter, 250 × 4.6 mm, Alltech Associates Inc.) using a modification of the method described by Zapata et al. (1987). Aliquots of 150 μL were mixed with 50 μL of 0.5 mol/L ammonium acetate in water before analysis. The elution gradient was programmed from 100% solvent A (80% MeOH and 20% of 0.5 mol/L ammonium acetate in water) to 100% solvent B (50% MeOH and 50% acetone) for 20 min followed by an isocratic elution for 30 min. The solvent flow was 1 mL/min for the first 29 min and 2 mL/min for the remaining time.

3.5. Liquid Chromatography–Mass Spectrometry (LC/MS and LC/MS/MS)

A Hewlett-Packard HP-1090 liquid chromatograph delivery instrument coupled to a VG Quattro mass spectrometer through an electrospray interface was used for compound specific analysis. Separation was performed on a C₁₈ column as described above. The analysis of fractions three and four (see Identification) was performed at 1 mL/min using a binary solvent gradient program from 60% to 30% solvent A for 10 min, holding this final mixture for isocratic elution for 10 min. Fraction 2 was analyzed at 2 mL/min using isocratic elution with 10% A and 90% B. The composition of solvents A and B were 60/40 acetonitrile/0.001 mol/L ammonium acetate in water, and 100% acetone, respectively. A postcolumn splitter reduced the flow reaching the

MS to 100 μL/min. The temperature of the source and the cone voltage were set at 100°C and 40 V, respectively. Mass spectral information was obtained in the positive mode scanning from 300 to 1000 dalton in 3 s.

The mass spectra obtained under these conditions were largely dominated by the protonated molecule. No fragmentation of the chlorins was apparent. To obtain more structural information, MS/MS experiments were performed. For each compound, the daughter spectrum of the protonated molecule was obtained using argon (3×10^{-4} mbar) with a collision energy of 40 eV.

3.6. Identification

The analysis of crude pigment extracts by LC-MS has the inconvenience of being time consuming and expensive chromatographic procedures (60–90 min per sample). The drastic solvent gradients needed to elute the chlorophyll derivatives complicates the optimization of the ionizing conditions in the MS, including source temperature and cone voltage. Moreover, we found that the presence of many interfering compounds in the crude extracts complicated the acquisition of MS spectra of many chlorins. Accordingly, a purification step is required to obtain reliable mass spectral information. To solve these inconveniences, we developed a simple and rapid method to purify the pigment extracts by column chromatography.

A pigment extract corresponding to a typical sample was separated into four fractions by column chromatography and each fraction was analyzed separately by LC and LC/MS. The chromatographic columns (30 × 1.1 cm inner diameter) were packed with 6 g of silica gel 40 (70–230 mesh, Merck) previously suspended in a mixture of 50/50 dichloromethane/*n*-hexane (DCM/Hex).

The **first fraction** was collected after the addition of 35 mL of 50/50 DCM/Hex. The eluate had an intense yellow color due to the presence of carotenoids. The presence of carotenes and the absence of chlorophyll derivatives and xanthophylls was confirmed by LC.

The **second fraction** was collected after the addition of 30 mL of 95/5 DCM/acetone and included a distinct and intense brown band. Analysis by LC indicated the presence of compounds 6 to 12 (Table 1) as well as carotenoids. Compounds 6 and 7 had the same retention time, and visible and mass spectra as chlorophyll *a* purchased from Fluka Co. and its epimer, respectively. Compounds 8 to 11 coeluted and had visible and mass spectra similar to synthetic pyrochlorophyll *a*, phaeophytin *a*, phaeophytin *a'*, and pyropheophytin *a*, respectively.

Synthetic phaeophytin *a* and its epimer were obtained using the method described by Brown (1968), by placing a solution of chlorophyll *a* in acetone in contact with an atmosphere with hydrochloric acid for 5 min. Absorbance measurements at 665 nm showed that the recovery was close to 100% and LC and LC/MS analyses showed the absence of by products. Synthetic pyrochlorophyll *a* and pyropheophytin *a* were obtained from chlorophyll *a* and phaeophytin *a*, respectively. The original compound was dissolved in pyridine previously distilled over KOH, and heated at 90°C for 90 min in a low oxygen atmosphere. The recovery by this procedure was better than 90% and no other chlorophyll derivatives were formed during the reaction. Mass spectra obtained by LC/MS/MS were identical to those obtained by LC/MS using atmospheric pressure chemical ionization (Harris et al., 1995a).

A series of compounds that eluted between 35 and 42 min (compound 12; Table 1 and Fig. 2) were identified as a series of steryl pyropheophytin ester (SPE) compounds. Mass spectra of these compounds are similar to those previously published (King and Repeta, 1991; Prowse and Maxwell, 1991; Eckardt et al., 1992), with an intense protonated molecule ($[M + H]^+ = 887\text{--}945$ Da/e) and the loss of the steryl moiety ($[M + H\text{-stere}]^+ = 535$ Da/e). To confirm the presence of steryl moieties, fraction 2 was repeatedly injected into an LC system and the purified compounds were collected. The combined extracts were evaporated to dryness, redissolved with 5% H₂SO₄/methanol, flushed with N₂ and sealed to exclude oxygen, and left overnight at room temperature. The alcohols were recovered with hexane and were derivatized with a silylating agent before the analysis by gas chromatography (GC) and GC/MS. The alcohol fraction consisted of a complex mixture of sterols, confirming compound 12 as a mixture of steryl pyropheophorbides *a*.

The **third fraction**, collected after elution with 30 mL of 90/10

Table 1. LC/visible and LC/MS/MS structural information used for the identification of chlorophylla and its derivatives in Saanich Inlet sediments.

Compound	Retention Time (min)	Absorbance Maxima (nm)	MS Diagnostic Ions ^a	Extinction Coefficient ^b	Source ^c
1. Chlorophyllide <i>a</i>	13.8	432/665	629	100.7×10^3	1
2. Phaeophorbide <i>a</i>	18.9	410/665	607/547	66.8×10^3	1
3. Phaeophorbide <i>a'</i>	19.6	410/665	—	66.8×10^3	
4. Pyropheophorbide <i>a</i>	21.5	410/665	549/521	66.8×10^3	
5a. Chlorophyllone <i>a</i>	21.1	410/665	533/515	66.8×10^3	
5b. Chlorophyllone <i>a</i>	21.6	409/668	533/515	66.8×10^3	
6. Chlorophyll <i>a</i>	26.9	433/665	893/615/583/555	100.7×10^3	2
7. Chlorophyll <i>a'</i>	27.3	433/665	—	100.7×10^3	2
8. Pyrochlorophyll <i>a'</i>	27.7	410/665	833	100.7×10^3	3
9. Phaeophytin <i>a</i>	30.1	410/665	871/593/533	66.8×10^3	3
10. Phaeophytin <i>a'</i>	30.5	410/665	871/593/533	66.8×10^3	3
11. Pyropheophytin <i>a</i>	32.3	410/665	813/535	66.8×10^3	3
12. Sterylpyropheophorbides <i>a</i>	35–41	410/665	887-945/535	66.8×10^3	

^a MS of phaeophorbide *a* and pyropheophorbide *a* correspond to the methylated compounds.

^b Molar extinction coefficient at 665 nm ($\text{cm}^2 \text{M}$) from Brown (1968). Phaeophytins and phaeophorbides were assumed to have the same molar extinction coefficient.

^c Standards have been obtained from: 1) Culture of *Phaeodactylum tricornutum*; 2) commercially available; 3) synthesised from chlorophyll *a* (see text).

DCM/acetone, consisted of two chlorins (5a,b; Table 1), which had visible spectra similar to phaeophytin *a*. These compounds partially coeluted with pyropheophorbide *a* (Fig. 2). Their LC/MS/MS spectra were identical and were dominated by the protonated molecule ($[\text{M} + \text{H}]^+ = 533 \text{ Da/e}$) and a fragment of 515 Da/e, probably related to the loss of water. The column chromatographic and LC behavior, mass and visible spectra, and the presence of two isomers are consistent with the properties of chlorophyllone found to be abundant in several marine and lacustrine sediments (Harris et al., 1995a,b).

The **fourth fraction** or “polar fraction” was obtained after elution with 30 mL of 8/2 DCM/methanol. Analysis by LC showed high abundance of carotenoids and the presence of three chlorophyll derivatives (2, 3, and 4) with phaeophorbide-type visible spectra. Compounds 2 and 3 showed the same retention time as phaeophorbide *a* and phaeophorbide *a'* obtained after acidification of a solution of chlorophyllide *a*. This fraction was methylated with diazomethane and analyzed by LC-MS and LC/MS/MS. The mass spectra of methylated 2, 3, and 4 coincided with the previously published mass spectra for methyl esters of phaeophorbide *a*, phaeophorbide *a'*, and pyropheophorbide *a*, respectively (Eckardt et al., 1991; Keely and Maxwell, 1991; Villanueva et al., 1994a).

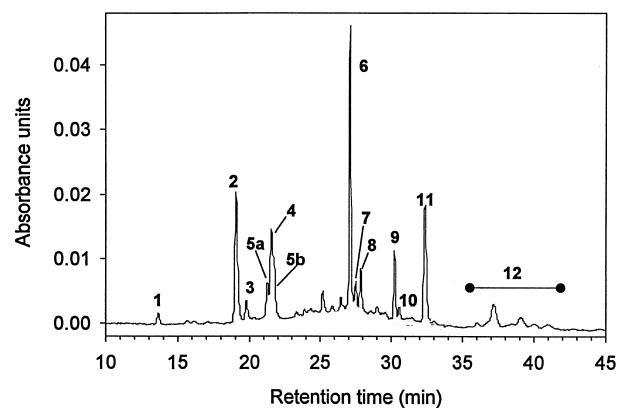


Fig. 2. Example of a LC elution profile from a Saanich Inlet extract recorded at 665 nm. See Table 1 for peak assignments.

3.7. Quantification

The molar absorbance coefficient (ϵ) used for the quantification of chlorophylls and the phaeoderivatives are listed in Table 1. A similar coefficient for all phaeoderivatives has been assumed on the basis that they possess the same ring chromophore. The coefficient for chlorophyllide *a* and pyrochlorophyll *a* was assumed to be the same as chlorophyll *a*. Because of partial coelution, pyropheophorbide *a* and chlorophyllone were quantified together. Replicates analyses performed on one sediment sample resulted in a reproducibility better than 10% for all compounds.

4. RESULTS

4.1. Visible Spectra

The absorbance spectra of the extracts (Fig. 3A) are typical of recent anoxic sediments (Baker and Louda, 1986). The two distinct maxima at 410 and 666 nm indicate the dominance of phorbins. The shoulder at 680 nm on the 666-nm peak is probably related to the occurrence of pigments associated with high molecular weight polymeric materials (Fig. 3B; see below). This shoulder is not related to the occurrence of 13(2), 17(3)-cyclophaeophorbide *a* enol, a compound recently described in other anoxic sediments (Ocampo et al., 1999) and that has not been identified in this study. The absorbance ratio 410/666 ranges between 4 and 5, a value typical of recent sediments (Baker and Louda, 1986; Summerhayes et al., 1995). This ratio is significantly higher than the characteristic values for chlorophyll and phaeopigments (1 and 2, respectively). This is related to the presence of carotenoids, which are also responsible for the shoulders at 450–500 nm, and to the presence of a high molecular weight fraction with very high $A_{410}:A_{665}$ ratios (Fig. 3B).

4.2. LC: Qualitative Analysis

The chromatographic profile obtained from a typical Saanich Inlet solvent extract shows the presence of 13 individual compounds that absorb at 665 nm (Fig. 2). The dominant peak in

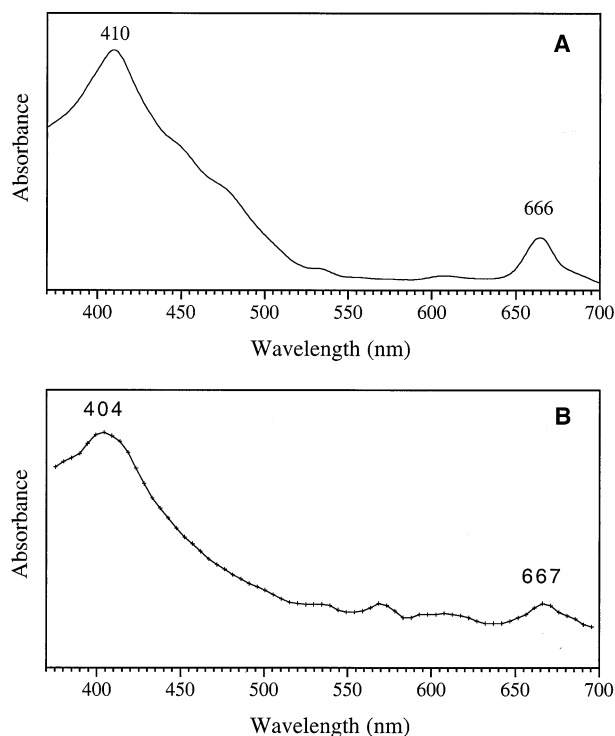


Fig. 3. (A) Absorbance spectrum of a pigment extract from Saanich Inlet. (B) Absorbance spectrum of the unresolved complex mixture (UCM).

each sample is unaltered chlorophyll *a* (6). The additional 12 compounds identified represent >95% of the sedimentary chlorins and are the result of one or several transformation processes that alter chlorophyll *a*, including (Table 1, Fig. 2):

1. loss of the central Mg ion (compounds 2, 3, 4, 5, 9, 10, 11, 12);
2. loss of the C₁₃ methoxycarbonyl moiety (formation of pyro derivatives, 4, 8, 11, 12);
3. loss of the phytol moiety (1, 2, 3, 4, 12);
4. transesterification of the phytol group by a sterol (formation of SPE, 12);
5. formation of chlorophyllone *a* (5).

The occurrence of these specific diagenetic processes has been related to enzymatic reactions during the accumulation of algal material to the sediments. With the exception of chlorophyllone (5), all these chlorophyll derivatives have been described in decaying algal cultures, senescent algal blooms, fecal pellets, sediment traps, and surficial sediments (Daley, 1973; Daley and Brown, 1973; Vernet and Lorenzen, 1987; Hurley and Armstrong, 1990; Leavitt and Carpenter, 1990; Gieskes et al., 1991; Keely and Maxwell, 1991; Sun et al., 1991; Head and Harris, 1992; Spooner et al., 1994a,b; Villanueva et al., 1994b; Harris et al., 1995a; King, 1995). Accordingly, they are the result of alteration processes during senescence of algae, grazing, and attack by bacteria. Steryl pyropheophorbide ester compounds have been described in sediment traps and sediments (King and Repeta, 1991; Eckardt et al., 1992; Kowalewska, 1994; Pearce et al., 1998), fecal pellets (King and

Wakeham, 1996), and grazing experiments (Harradine et al., 1996) and they have been proposed as a reliable marker for grazing processes. Chlorophyllone *a* has been described in sediments (Harris et al., 1995b) and some diatoms (Sakata et al., 1990). However, its origin in the sedimentary environment is not well constrained at present (Harris et al., 1995b). The absence of significant amounts of other chlorophyll derivatives, such as allomers, meso derivatives, and porphyrin compounds (excepting chlorophylls *c*₁ and *c*₂) confirms that the pigments in Saanich Inlet sediments are in a very early diagenetic stage and have not been subjected to extensive structural alteration (Baker and Louda, 1986).

A broad hump found in all chromatographic profiles between 22 and 33 min (Fig. 2) suggests the presence of an unresolved complex mixture (UCM) of compounds. The visible spectrum of this UCM is dominated by two absorbance maxima centered at 404 and 667 nm (Fig. 3B), suggesting that they are polymeric materials of diagenetic origin that have incorporated chlorophyll derivatives in their structure. A similar UCM in sediment extracts from surface sediments of the Black Sea has previously been described as high molecular weight (HMW) material (King and Repeta, 1994).

4.3. LC: Quantitative Analysis

The most remarkable feature observed in the down core profiles of the eight major chloropigments (Fig. 4) is the lack of monotonic down core changes. Although concentrations vary considerably between annual laminations, the average concentration of each compound does not increase or decrease over the century and half-time span of the core. Other important features include the presence of distinct down core patterns for different compounds and a higher degree of interannual variability for chlorophyllide *a* and phaeophorbide *a* than for the phaeophytins and chlorophyll *a*.

The mean composition of chlorophyll derivatives for the entire core (Fig. 5) provides information on the relative concentration of chlorophyll *a* and its degradation products. Chlorophyll *a* represents an average of 17% of the individual compounds detected in Saanich Inlet sediments. The relative contribution of each derivative is quite similar (10–23%), with the exception of chlorophyllide *a* (1%) and pyrochlorophyll *a* (3%), which are the only chlorophyll derivatives that have not undergone demetallation (loss of Mg). Overall, the composition of the chlorophyll derivatives in Saanich sediments is similar to other anoxic recent anoxic sediments, such as the Black Sea (King, 1995) and the Baltic Sea (Kowalewska, 1994). All three studies show that chlorophyll *a* represents about 15–20% of the solvent-extractable compounds. In contrast, the hydrolyzed compounds in Saanich sediments represent a higher proportion (46%) of the extractable chlorins, compared to the Black Sea (15%) or the Baltic Sea (13%).

4.4. Down Core Diagenetic Trends

4.4.1. Degradation

As shown in Figures 4 and 6, no systematic decreases in the down core concentration of total and individual pigments can be detected. This feature indicates that diagenetic processes involving breakdown of the porphyrin macrocycle are not signif-

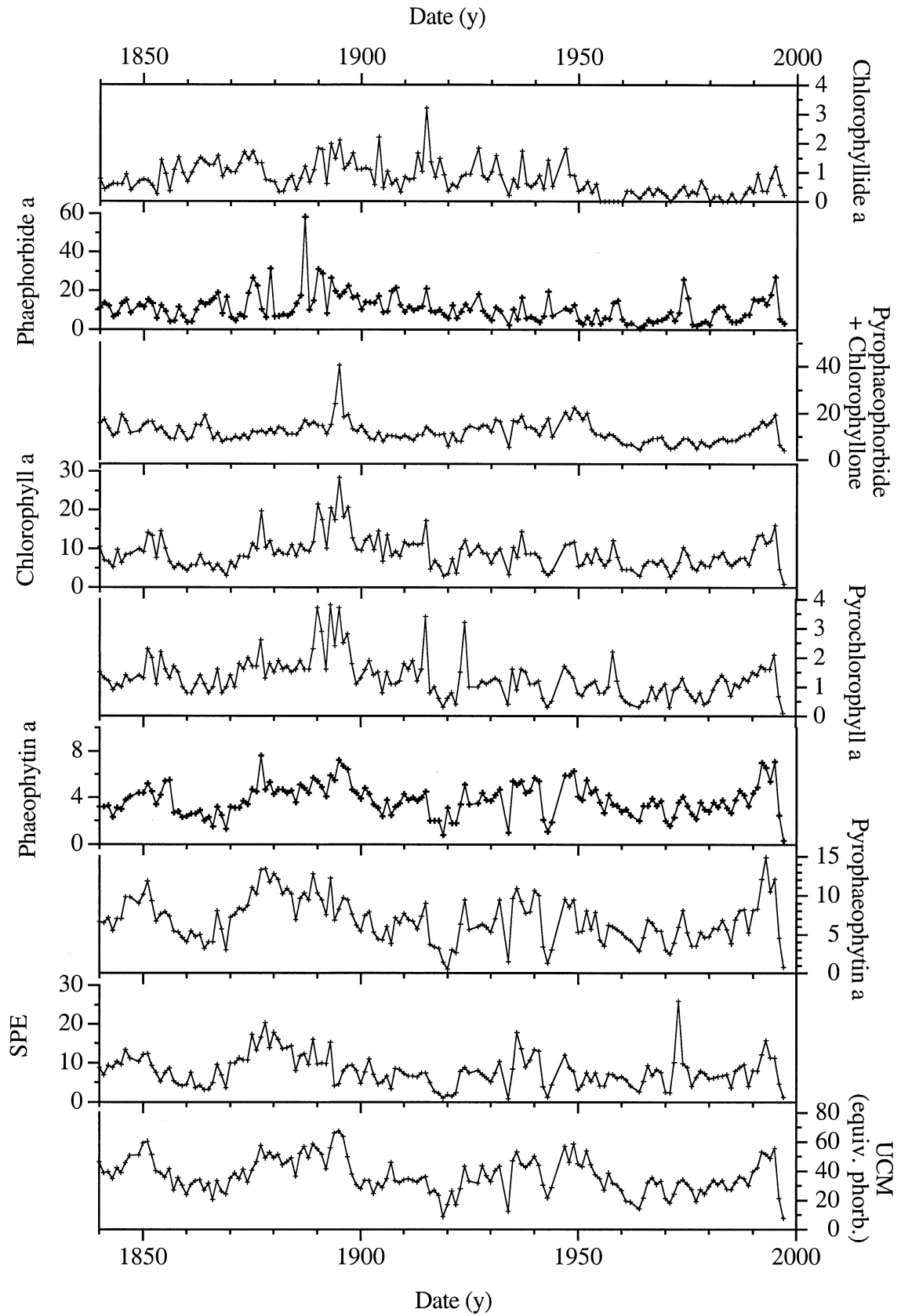


Fig. 4. Concentration profiles of chlorophyll *a* derivatives (in nmol/gdw) from annually laminated sediments of Saanich Inlet.

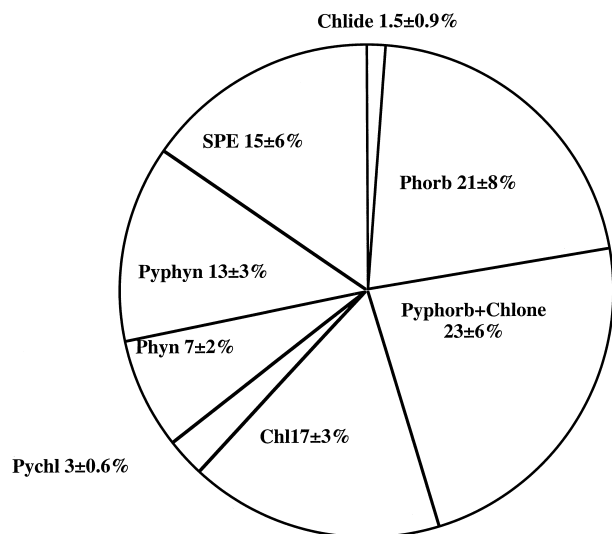


Fig. 5. Averaged composition (on a molar basis) of chlorophyll *a* pigments in the top 1.45 m of Saanich Inlet sediments.

icant in these anoxic sediments over a 150-yr period. In contrast to typical oxic sediments the data reveal a substantial increase in the total pigment concentration over the first five varves (ca. 14 cm). This unexpected trend is observed irrespective of whether pigment concentration is expressed in grams per volume of sediment or grams per wet weight, indicating that it is not an analytical artifact caused by uncertainties in the determination of porosity or water content.

4.4.2. Incorporation into the polymeric fraction

The summed absorbances at 665 nm of all the individual chlorophyll derivatives identified by LC (A_{LC} , expressed in equivalents of phaeophorbide *a* per gram of sediment) is substantially lower than the measured absorbance of the total extract (A_{UV} ; Fig. 6A). The discrepancy between these estimates is eliminated when the absorbance associated with the UCM in the LC trace is taken into account (Fig. 6B). Because the UCM is related to porphyrins and chlorins associated with extractable HMW compounds (King and Repeta, 1994), we used A_{HMW} to quantify the extractable HMW chlorins, expressed as equivalents of phaeophorbide *a* (A_{HMW} ; Fig. 6C). According to our estimates $39 \pm 5\%$ ($1s$, $n = 157$) of the absorbance at 665 nm of extracts from Saanich is related to the HMW fraction. This value is close to that previously published for Black Sea sediments of approximately 50% (King and Repeta, 1994).

The down core profiles of the individual compounds (A_{LC}) and the high molecular fraction (A_{HMW}) are similar, and the relative contribution of these two components to the total absorbance does not show any significant variability (Fig. 6C). This down core uniformity suggests that there is no evidence for incorporation of chlorophyll pigments into the polymeric fraction during the 157 yr time period represented by the core.

4.4.3. Alteration of individual chlorophyll compounds

Chlorophyll *a* undergoes three primary early diagenetic reactions: demetallation, loss of the C_{13} -COOMe moiety, and

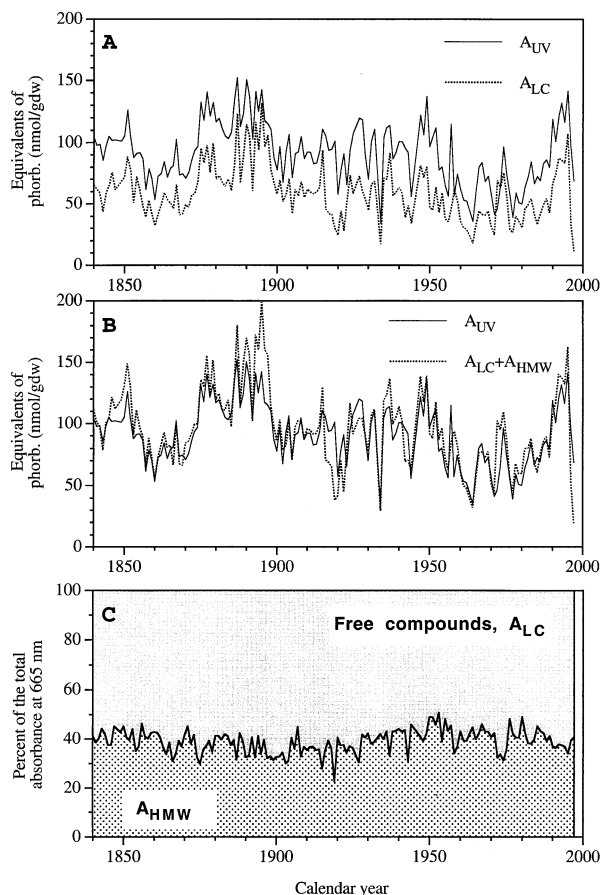


Fig. 6. (A) Comparison of chlorin concentrations measured by UV visible absorbance (solid line) and individual compounds measured by HPLC (dotted line). (B) Comparison of chlorin concentrations measured by UV visible absorbance (solid line) and individual compounds measured by HPLC summed with UCM (dotted line). (C) Percentage of the total absorbance at 665 nm related to the individual compounds and to the HMW fraction.

hydrolysis of the phytol chain. To estimate the relative extent to which each of these degradative reactions has occurred, we calculated the percent of total free compounds that have resulted from each degradation process (Fig. 7). Demetallation is the preferred process, involving an average of $79 \pm 4\%$ ($1s$, $n = 157$) of the total compounds. The remaining 21% include intact chlorophyll *a* as well as minor amounts of chlorophyllide *a* and pyrochlorophyll *a*. Formation of pyroderivatives is the second preferred mechanism that involved an average of $55 \pm 8\%$ of the individual compounds. The hydrolysis of phytol occurs less extensively and involves an average of only $44 \pm 10\%$ of the total compounds. The most remarkable feature of all three profiles is the absence of an increasing trend of alteration, demonstrating that none of the three degradation reactions is observed in these sediments.

5. DISCUSSION

Our data demonstrate that there is no detectable diagenetic alteration of the chlorin composition within the top 1.5 m of the sedimentary column of Saanich Inlet. This reveals the critical

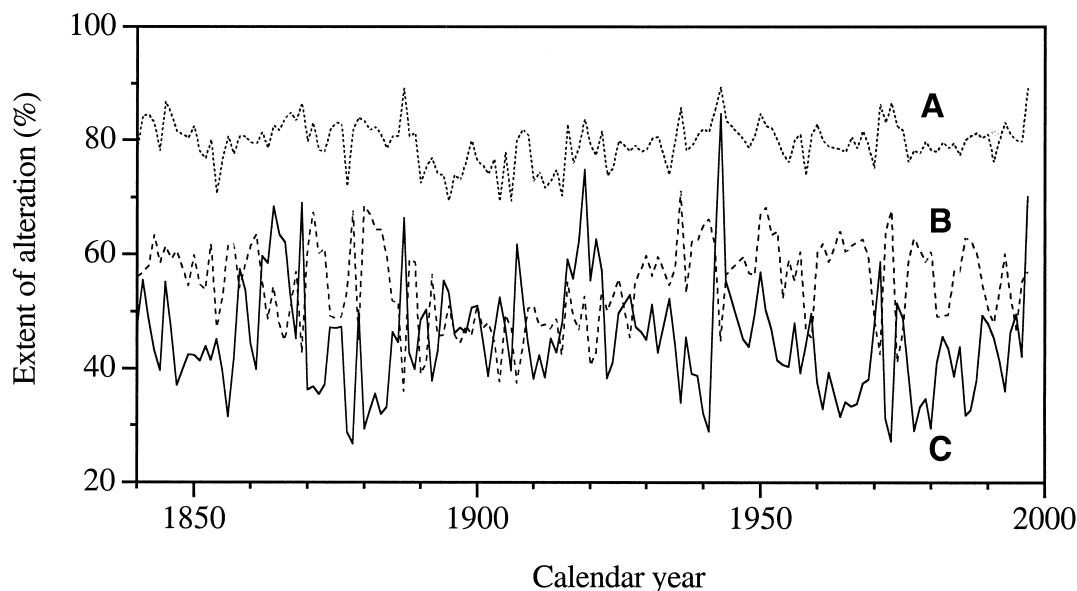


Fig. 7. Down core record of the percentage of chloropigments that have lost the central Mg atom (solid line), the methoxycarbonyl moiety (dashed line) and the phytol group (dotted line).

importance of oxygen in the preservation and degradation of chlorophylls derivatives. In similar coastal environments where oxygen is present in overlying bottom water and in surficial sediments, chlorins are readily degraded.

The presence of substantial amounts of chlorophyll degradation products in these sediments is related to processes occurring in the oxic photic zone, at the anoxic sediment–water interface, or shortly (hours to months) after deposition. With the exception of chlorophyllone, the chlorophyll derivatives detected in Saanich Inlet sediments are commonly found in the water column. This suggests that the pigment composition of Saanich sediments may be the result of biological and geochemical processes occurring before sedimentation. The pigment signature resulting from diagenetic processes that occur within months is preserved for more than a century. Thus, the pigment composition of different varves offers reliable information about past processes occurring in the water column or at the sediment–water interface. The geochemical information

contained in the pigment composition has not been altered by postdepositional alteration.

Although the average annual production in Saanich Inlet is highly variable (Takahashi et al., 1977, 1978; Hobson, 1983), and is expected to be reflected in the total sedimentary chlorin content, nonetheless we would expect the relative chloropigment composition to remain fairly constant. This is not the case. The relative amount of different chlorophyll derivatives contributing to the total chlorin content changes substantially as reflected by differing ranges of compound: precursor ratios, which are also depicted in the regression coefficients between different pigment derivatives (Table 2; $0.01 < r^2 < 0.81$). These data demonstrate that the relative pigment composition accumulated in the sediments changes from year to year. Determining which compounds are correlated in our sedimentary record reveals important information related to the mechanisms and controls on pigment diagenesis in the water column and shortly after sedimentation.

Table 2. Correlation coefficients (r^2) between the different chlorophyll derivatives.

r^2	Clide <i>a</i>	Phor <i>a+a'</i>	Pyphorb+ Chlone	Chl <i>a+a'</i>	PyChl <i>a</i>	Phyn <i>a+a'</i>	PyPhyn	SPE
Clide <i>a</i>		0.25	0.21	0.27	0.31	0.05	0.05	0.02
Phor <i>a+a'</i>			0.15	0.30	0.31	0.11	0.14	0.07
Pyphorb+Chlone				0.36	0.26	0.32	0.16	0.03
Chl <i>a+a'</i>					0.77	0.60	0.41	0.17
PyChl <i>a</i>						0.45	0.42	0.19
Phyn <i>a+a'</i>							0.72	0.42
PyPhyn								0.81
SPE								

Abbreviations: Clide *a*, Chlorophyllide *a*; Phor *a+a'*, phaeophorbide *a* and its epimer; Pyphorb+Chlone, the sum pyropheophorbide *a* and chlorophyllone *a*; Chl *a+a'*, chlorophyll *a* and its epimer; PyChl *a*, pyrochlorophyll *a*; Phyn *a+a'*, paeophytin *a* and its epimer; PyPhyn, pyropheophytin *a*; SPE, steryl pyropheophorbides *a*.

R^2 values above 0.6 are in bold.

Using the regression coefficients between different chlorophylls, we can describe the existence of covarying compounds and derivatives with an independent behaviour. Compounds with related chemical structures show similar behaviour:

1. Chlorophyll *a*, pyrochlorophyll *a*, phaeophytin *a*, pyropheophytin *a* are linearly correlated, as indicated by high regression coefficients ($0.41 < r^2 < 0.77$), and are correlated with the total pigment concentration ($0.63 < r^2 < 0.77$, Vand DH unpublished data), but have poor or no linear correlation with the dephytylated compounds ($0.05 < r^2 < 0.36$).
2. SPE compounds show the highest regression coefficient with pyropheophytin *a* ($r^2 = 0.81$) and total pigments ($r^2 = 0.77$), but low values with the other compounds ($0.02 < r^2 < 0.42$).
3. The dephytylated compounds (phaeophorbide *a*, chlorophyllide *a*, and the sum of pyropheophorbide *a* and chlorophyllone *a*) have much lower degrees of correlation with other compounds ($r^2 < 0.31$, 0.31 , and 0.36 , respectively) or with the total pigment concentration.

The four compounds in the first group have similar down core profiles, and the best linear relationships correspond to chlorophyll *a* and its pyro derivative ($r^2 = 0.77$) and demethylated ($r^2 = 0.60$) analogs. We also find good relationships between phaeophytin *a* and its pyro derivative ($r^2 = 0.72$). The existence of linear relationships implies that a variation in the input of chlorophyll *a* or phaeophytin *a* is linked to a proportional change in the input of its pyro and phaeo derivative. A fairly constant precursor-to-derivative ratio for pyro and phaeo derivatives is observed down core: chlorophyll-to-phaeophytin = 2.3 ± 0.6 and chlorophyll-to-pyrochlorophyll = 7.1 ± 1.8 . Changes in the production and degradation of chlorophylls in the water column do not substantially alter the degree of formation of pyro and phaeo derivatives.

Down core changes in the concentration of SPE compounds in Saanich Inlet closely parallel those of pyropheophytin *a* (Fig. 8C; $r^2 = 0.81$) and are poorly correlated with other chlorophyll derivatives such as phaeophorbide (Fig. 8B). Remarkably, SPE and pyropheophytin *a* are the main chlorophyll derivatives formed during grazing activity (Harris et al., 1995b). This suggests that these compounds could be of use to trace past changes in grazing activity.

The compounds that have undergone hydrolysis of the phytyl chain show a different down core profile compared to the phytyl-containing molecules. Thus, an increase in the input of the phytyl-containing derivatives is not accompanied by a proportional increase in the hydrolyzed compounds. The different behaviour of phaeophorbides has already been described in a collection of surface sediments from the Baltic Sea (Kowalewska, 1994), demonstrating that this feature is not restricted to our site. In Saanich sediments, chlorophyllide *a* and phaeophorbide *a* (Fig. 8A) show a higher degree of interannual variability than the phaeophytins and chlorophyll *a*. Indeed, concentration maxima of chlorophyllide *a* usually coincide with maxima of phaeophorbide *a*. Although differences in the magnitude of these spikes cause low regression coefficients ($r^2 = 0.25$), their temporal coincidence suggests a direct mechanistic link between both derivatives.

The occurrence of phaeophorbides in sediments can be related to grazing processes, to bacterial attack of algal cells (Spooner et al., 1994a), to the release of endogenous enzymes during algal senescence (Daley, 1973; Daley and Brown, 1973; Spooner et al., 1994a), and to the activity of chlorophyllase (Villanueva et al., 1994a). In contrast, chlorophyllide *a* is only related to the activity of the enzyme chlorophyllase and is one of the main derivatives formed during disruption and bacterial attack of diatom cells (Spooner et al., 1994a). This pigment does not survive well in the acidic environment of the zooplankton guts and is seldom encountered in fecal pellets (Gieskes et al., 1991; Head and Harris, 1992). The high degree of correlation between phaeophorbide *a* and chlorophyllide *a* (Fig. 8A) contrasts with the distinctive patterns with grazing tracers such as SPE ($r^2 = 0.07$; Fig. 8B). These correlations suggest that chlorophyllide *a* and phaeophorbide *a* are related to inputs of ungrazed diatoms to the sediments. Sancetta and Calvert (1988) showed that a significant fraction of the diatom frustures that reach the sediments are related to flocculated material, and the pellets can account for only 25% of the mass flux during summer and spring, confirming that a major source for phaeophorbides in this environment is the activity of chlorophyllase in senescent diatoms cells.

The temporal variability of chlorophyllide and phaeophorbide can be used to constrain possible changes in biological and geochemical processes in Saanich Inlet. Two explanations could account for the different behaviour of phytyl-containing and hydrolyzed compounds. The first involves the assumption that the variability is related to annual changes in the preservation of the pigment composition formed in the water column. Chlorophyllide *a* is relatively unstable and is rarely reported in sediments. Environmental changes in the water column or at the sediment surface, such as the depth of the redox boundary or the intensity of the annual flush of oxygenated bottom waters, may play an important role in modulating the sedimentary chlorophyllide *a* content. Accordingly, sedimentary chlorophyllide might offer useful information about environmental conditions affecting the preservation and degradation of pigments or other organic compounds in Saanich sediments. The second possible interpretation is that dephytylated compounds trace the input of ungrazed diatoms accumulated in the sediments. In this case, these compounds could be used to trace the relative amount of chlorins transported by fecal pellets and by ungrazed diatom cells. This involves the assumption that preservational effects do not play a major control in the down core patterns of chlorophyllide and phaeophorbide. The excellent pigment preservation observed in Saanich sediments favors the second hypothesis over the first, but the implications and limitations related to each hypothesis need to be further investigated.

5.1. Total Chlorins as a Production Signal

Because diagenetic processes do not affect the pigment composition within the top 1.45 m of Saanich Inlet sediments, total chlorins can also be used to trace variations in the delivery of chlorophyll to the sediment, which are, in principle, related to changes in algal production. Partial flushing of the anoxic bottom waters with dense, oxygenated water from outside the basin occurs some years in late summer (Anderson and Devol,

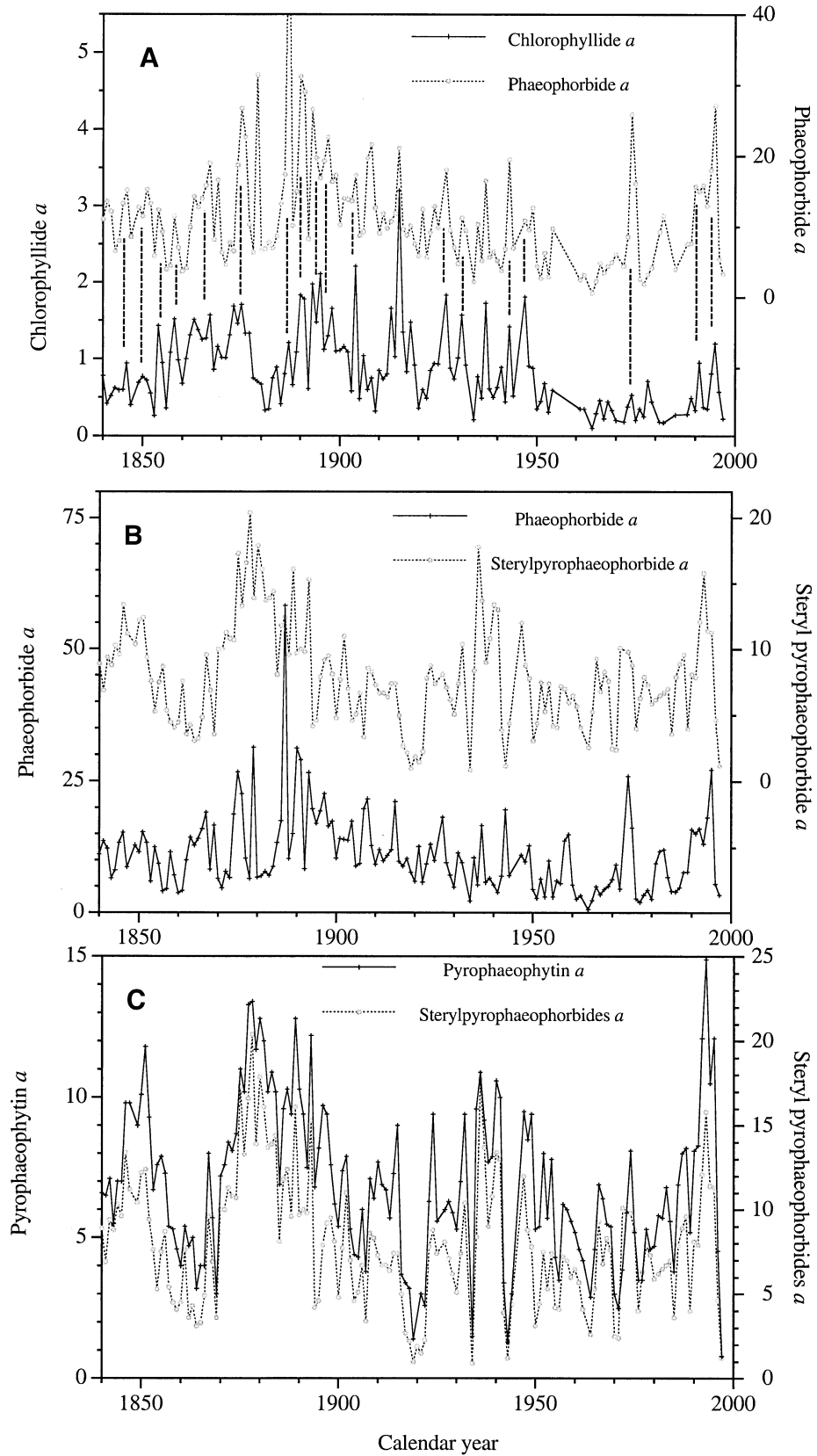


Fig. 8. Comparison of the concentration (in nmol/g) profiles obtained for: (A) chlorophyllide *a* and phaeophorbide *a* ($r^2 = 0.25$); (B) phaeophorbide *a* and SPE ($r^2 = 0.14$); and (C) pyropheophytin *a* and SPE ($r^2 = 0.81$).

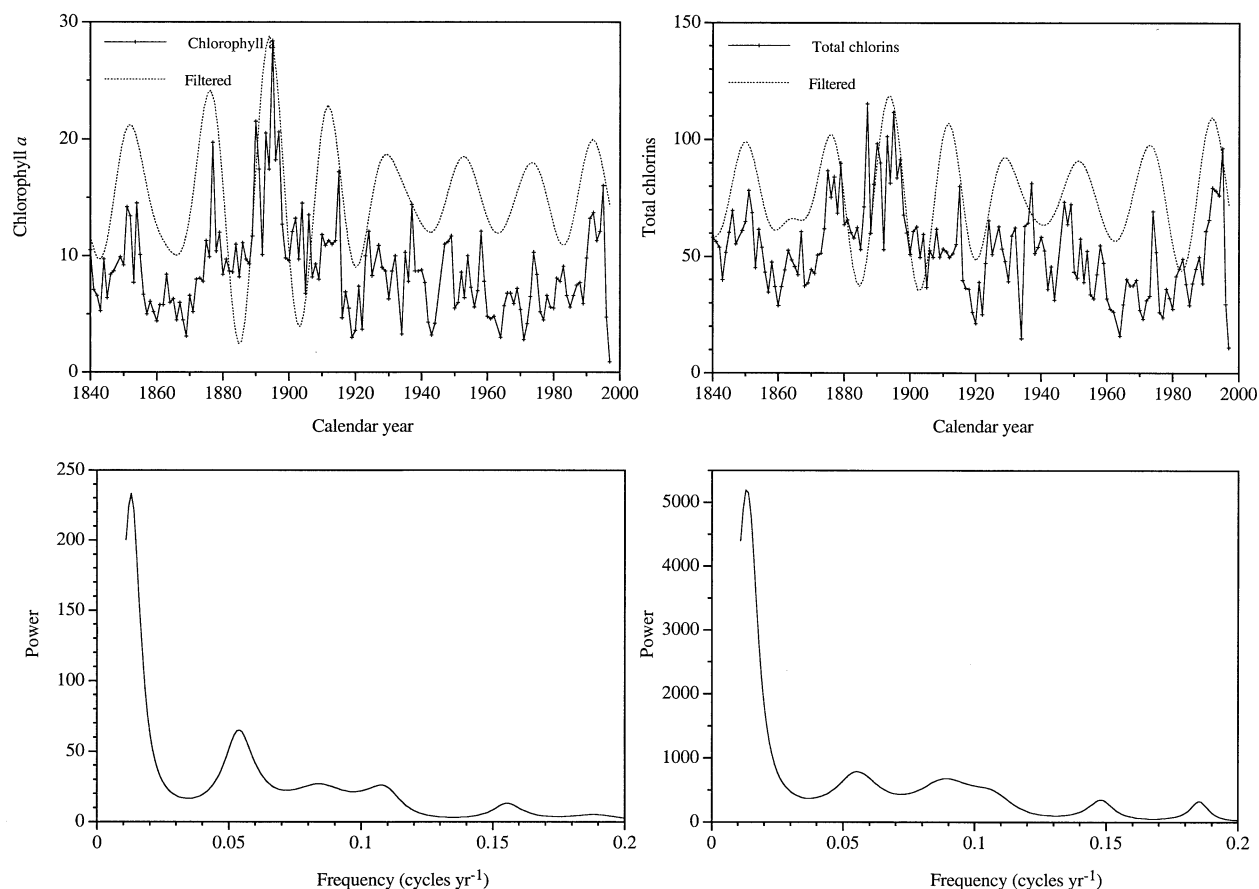


Fig. 9. (Top) Chlorophyll *a* and total chlorins concentration profiles (nmol/g, solid lines) and the filtered signal using a Gaussian filter (centered at $1/18.5 = 0.054 \text{ yr}^{-1}$). (Bottom) Maximum entropy frequency spectra of the same profiles (Software Analyseries, Paillard et al., 1996).

1973). Because chlorophyll *a* is especially sensitive to degradation by dissolved oxygen (Hurley and Armstrong, 1990; Leavitt and Carpenter, 1990; Sun et al., 1993a,b), annual variability in chlorophyll concentrations could be due to the extent of bottom water renewal and bottom water oxygen concentration. We attempted to determine if degradation by O_2 at the sediment–water interface was a primary control of chlorophyll concentrations by comparing our annual record of sedimentary chlorins with a compilation of over 275 deep water ($>170 \text{ m}$) oxygen profiles dating back to 1953 (D. Stucchi, Institute of Ocean Sciences, Sidney, B. C., unpublished data). No correlation was found, which supports the argument that changes in overlying production is a primary control.

Possible changes in the transport efficiency of pigments through the water column or at the sediment–water interface can affect the fraction of total chlorins produced in the photic zone that ultimately reach the sediments, and therefore, could substantially alter the production signal. No relationship was found between the sedimentary chlorophyll *a* concentration and the relative abundance of its derivatives. For example, we have shown in this article that the extent to which specific degradation reactions occur, such as loss of Mg, formation of pyro derivatives, or incorporation into the polymeric fraction, remains constant in spite of fivefold changes in the total pigment

concentration. Also, the year-to-year variability in the extent of phytol-hydrolysis is not correlated with the total chlorins. Although the lack of correlation with bottom water oxygen and the fairly constant pigment composition argues against degradation as a major control of the accumulation of chlorins in the sediment, the possible influence of oxic degradation in the productivity signal before incorporation into sediments cannot be completely ruled out. Thus, interpretation of production changes from the pigment data should be done with caution.

Spectral analyses based on the maximum entropy method show similar features for both chlorophyll *a* and total chlorins; there are prominent peaks at 0.009 and 0.053 cycles/yr, or 110 and 18.9 yr, respectively (Fig. 9). Because the pigment record covers a time span of only 157 yr, the 110-yr cycle is not statistically significant. Six cycles with a periodicity of 18.9 yr are visible in both profiles (Fig. 9), implying a long-term process that modulates the pigment accumulation in Saanich sediments with a periodicity of 18–19 yr. A variety of meteorologic phenomena, such as droughts (Currie, 1984; Currie and Fairbridge, 1984) and ocean/atmospheric temperatures (Loder and Garret, 1978), have been found to fluctuate at this periodicity and have been suggested to have an undetermined causal relationship with the lunar nodal tidal cycle of 18.6 yr.

In the case of Saanich Inlet, short-term (e.g., daily and

weekly) changes in primary production have been attributed to the variability in the tidal mixing outside the mouth of the Inlet (Haro Strait) that modulates the input of nutrients to the surface waters of the inlet, and the stratification of surface waters that favors the development of an algal bloom. Both physical processes are controlled by the spring-neap tidal cycle, giving rise to a biweekly production pattern (Parsons et al., 1983; Stucchi and Whitney, 1997). Mackas and Harrison (1997) have shown that nitrate concentrations are perennially above 10 mmol/L in the surface waters of the eastern part of Juan de Fuca Strait and in Haro Strait due to the subsurface input of nutrient-rich waters from offshore and the vigorous mixing in the island archipelagoes of the southern Strait of Georgia. Accordingly, changes in the tidal mixing intensity have a profound effect on production inside the inlet. The 18.9 yr periodicity in the pigment record could be the result of changes in the basin production as modulated by the tidal constituent with a periodicity of 18.6 yr. Other factors controlling interannual production changes, and hence the sedimentary pigment content of the corresponding varve, also include freshwater discharge from the Fraser River or the density of bottom waters.

6. CONCLUSIONS

Saanich Inlet is a unique depositional environment in which changes in overlying production and the diagenesis of chloropigments can be studied in detail as the laminated sediments allow accurate annual sampling, and because bottom water anoxia retards chlorin degradation. We have been able to show that the total pigment content (total absorbance at 665 nm) of a 1.45 m long freeze core, representing 157 yr of sedimentation, compares very well (<10% difference) with the determination of individual compounds by LC when combined with the UCM, which represents the extractable HMW fraction. Average pigment composition indicates that demetallation is the most extensive diagenetic reaction, as 79% of the individual compounds have lost the central Mg ion, followed by formation of pyroderivatives (55%), and the hydrolysis of phytol (44%).

No consistent down core variation was observed in the concentration of any of the compounds, the HMW fraction, total chlorins, or the relative fraction of any one compound or class of compounds. This indicates that no detectable degradation of chloropigments occurs over the 157 yr time period represented by the core, and suggests that the transformation products are formed in the water column, or at the sediment-water interface. Postdepositional reworking does not significantly affect the pigment signature formed in the water column, and the geochemical information is preserved in the sediments. Therefore, our record reflects the annual changes in the production of pigments, and allows a study of the changes in pigment composition accumulated in these sediments.

On the basis of their temporal variability, chlorophyll *a* derivatives can be classified into three groups:

1. Chlorophyll *a*, phaeophorbide *a*, and their pyro derivatives, which have a low degree of interannual variability in the extent of demetallation and demethoxycarbonyl degradation reactions.
2. Compounds related to grazing activity (SPE and pyropheophytin *a*), which show very similar down core profiles.

3. Dephytylated compounds (chlorophyllide *a* and phaeophorbide *a*) are related to chlorophyllase activity during the degradation of ungrazed diatom cells.

Chlorophyll derivatives corresponding to different transformation pathways have distinct down core profiles. We speculate that phaeophorbides and SPE compounds could be used to estimate the relative weight of flocculated and grazed materials to the sediments.

The profiles of total chlorins and chlorophyll *a* show a significant variability in the 18.9 yr, which is probably the result of changes in the basin production as modulated by the tidal constituent with a periodicity of 18.6 yr.

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