Early diagenetic alteration of chlorophyll-a and bacteriochlorophyll-a in a contemporaneous marl ecosystem; Florida Bay

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Abstract

This report covers the analyses of tetrapyrrole pigments in sediments and numerous source biota from northern central Florida Bay. Sediment cores were all carbonate marls and ‘bedrock’ (Pleistocene limestones) was typically at 0.6–1.2 m bsf (below sea floor). Extraction of the sediments was performed using tetrahydrofuran, shown to be 3–7 times as effective as more common solvents. Surficial sediments were found to contain a pigment distribution indicating a diatomaceous-cyanobacterial biofilm/mat underlain with purple sulfur bacteria as the microphytobenthos. Downhole trends in pigment diagenesis revealed differences due to paleoenvironment. That is, when bacteriochlorophyll-a (BCHL-a) plus derivatives were elevated, indicating strong syn-depositional anoxia, the pheophytins-a (PTINsa) were the major chlorophyll-a (CHL-a) derivatives. Conversely, when BCHL-a plus derivatives were low, indicating oxic to dys-oxic syn-depositional conditions, then pyropheophytin-a (pPTINa) and 132,172-cyclopheophorbide-a (CYCLO) were strongly dominant. The generation of cyclopheophorbide in carbonate marls with weakly anoxic to dysoxic syn-depositional conditions may yield an oil-source paleoenvironmental marker for bitumen containing compounds such as bicycloalkanoporphyrins (BiCAPs). Downhole conversions were observed for the following diagenetic steps; CHL-a to pheophytin-a (PTINa), PTIN-a to pyropheophytin-a (pPTINa), pPBIDa to CYCLO, BCHL-a to bacterioopheophytin-a (BPTINa), and BPTINa to bacteriopyropheophytin-a (BpPTINa). © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Diagenesis; Pigments; Chlorophyll-a; Bacteriochlorophyll-a; Microphytobenthos; Florida Bay; Pheophytins; Carbonates; Chemotaxonomy; Cyclopheophorbide-a-enol; Bicycloalkanoporphyrins; BiCAP

1. Introduction

The link between precursor biologic pigments, such as the chlorophyll and heme families, and their geologic products, namely the true deoxophylloerythroetioporphyrin (DPEP) series and certain etioporphyrin (ETIO) structures respectively, is accepted as having initiated organic geochemical thought and practice (Baker and Palmer, 1978; Baker and Louda, 1986). The theoretical conversion of biotic precursor to geologic product, based upon a series of increasingly severe reactions, is also well known and is referred to the Treibs’ Scheme (Baker and Palmer, 1978; Baker and Louda, 1986; Keely and Maxwell, 1990, Callot, 1991). In essence, considering the chlorophyll/DPEP scenario: Chlorophyll looses Mg, phytol, and the carbomethoxy moiety; undergoes keto reduction/dehydration/reduction (’O’ loss); and the resulting deoxomesopyropheophorbide (DOMPP) is then dehydrogenated and decarboxylated to yield the specific phylloporphyrins known as deoxophylloerythrin (DPE) and DPEP, respectively (cf. Treibs, 1936; Baker and Palmer, 1978; Baker and Louda, 1986; Keely and Maxwell, 1990). Variations upon this theme and a myriad of possible ‘side reactions’ are indeed possible and can lead to the highly complex arrays of geoporphyrins found in moderately organically mature samples (cf. Baker and Louda, 1986; Callot, 1991).
The conversion of chlorophyll to DPEP and other geoporphyrins encompasses both biologic (viz. enzymatic, senescent/death: Louda and Baker, 1986; Louda et al., 1998a) and geologic (Baker and Louda, 1986) reactions. The present study covers the combined effect of these phenomena, senescence/death and diagenesis, upon the early sedimentary history of chlorophyll-a and bacteriochlorophyll-a in a contemporaneous marine carbonate marl ecosystem.

The system studied, Florida Bay, is an area of considerable current ecological concern and provides the authors the ability to study both chemotaxonomic ecological evaluation (Louda et al., 1998b, 1999) and, the topic of this report, organic diagenesis.

Florida Bay is a triangular shaped sub-tropical lagoon ('estuary') at the southern tip of mainland Florida (Fig. 1) and has experienced widespread seagrass wasting since the Summer of 1987. These dead and dying seagrasses, notably turtle grass (*Thalassia testudinum*) apparently spawned algal blooms as they release nutrients, especially phosphorous, during the decay process and generate highly anoxic sulfidic surficial sediments (Porter and Muelstein, 1989; Robblee et al., 1991; Carlson et al., 1994; Fourqurean and Frankovich, 1996; Landesberg et al., 1996).

Relevant to the present study, these recent algal blooms afford known high productivity episodes of various phytoplankton, one of the sources of the chlorophylls to the underlying sediments. Obviously, a great many phytoplankton species exist in the Florida Bay ecosystem. However, only a few species have been targeted as being major contributors to either the 'normal' (=healthy) biota or as bloom forming organisms (Phlips and Badylak, 1996; Steindinger and Phlips, 1996; Vargo et al., 1996). In particular, the cyanobacterium *Synechococcus elongatus*, the diatom *Cyclotella choctawatcheana* and "an eukaryotic picosphere" are the main three organisms implicated in certain common "blue-green" or "blue-green/diatom/picosphere" blooms within Florida Bay (Steindinger and Phlips, 1996). These authors also reported specific blooms of other diatom species and gave examples of dinoflagellate blooms. *Synechococcus* blooms elsewhere have been reported to be able to reach such proportions that OM supply to and resultant H2S production in sediments have exacerbated seagrass dieoffs and fisheries (mullet, eel) collapse (Sorokin et al., 1996). The "2-micron picosphere" has recently been shown, by pigment-based chemotaxonomy to be a prokaryote due to the presence of only chlorophyll-*a* and the cyanobacterial carotenoid array myxoxanthophyll, zeaxanthin and echinenone (Louda et al., 1998b, 1999).

Periphyton is another easily identified source of chlorophyll within this ecosystem and also responds to nutrient and other stresses. In the present case, periphyton loading of seagrasses/macroalgae generally increases from the oligotrophic east to the eutrophic west in upper Florida Bay (Frankovich and Fourqurean, 1994).

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Fig. 1. Map of Florida Bay with sampling sites indicated. TKB = Twin Key Basin, RKB = Rabbit Key Basin, SKB = Sandy Key Basin, PEKB = Pass-Eagle Key Bank, JFK = Jim Foot Key, WR Nos. 1 and 2 = Whipray Basin sites Nos. 1 and 2, respectively. Dotted line indicates 1 m isobath.
Among reported epiphytes, a wide variety of diatom species are known to exist on the leaves of turtle grass (\textit{T. testudinum}) within Florida Bay (DeFelice and Lynts, 1978). Thus, periphyton, as well as phytoplankton and macrophytes, require attention as potential chlorophyll sources to the sediments.

The microphytobenthos is emerging as a major contributor to overall biomass and productivity in many ecosystems in which the photic zone reaches the benthos (see Schreiber and Pennock, 1995; Barranquet et al., 1997; references in Schlesinger, 1997). The activity of the microphytobenthos is driven by nutrients delivered both as dissolved species and as solids, namely sedimenting phytoplankton and seston (Marcus and Boero, 1998; Sagan and Thouzeau, 1998). Considering the obvious intimate relationship between the microphytobenthos and the sediments per se, the organic matter including pigments generated in this syndepositional situation should record overall system productivity quite readily. Further, pigment preservation, barring resuspension and oxygenation, should be enhanced. As will be detailed below, the microphytobenthos of Florida Bay has been somewhat overlooked as a source of primary organic matter and, specific to our study, photoautotrophic pigments.

The present report covers our efforts to trace the early diagenetic processing of the chlorophylls-a/-c and bacteriochlorophyll-a within the highly sulfidic carbonate marls of Florida Bay. Essentially, we have studied the source biota (phytoplankton, seagrasses, drift algae, and microphytobenthos) and sediment cores.

2. Experimental

2.1. Sites and samples

Seven sampling sites are identified within Fig. 1. These are abbreviated as follows; TKB = Twin Key Basin, RKB = Rabbit Key Basin, SKB = Sandy Key Basin; PEKB = Pass-Eagle Keys Bank, JFK = Jim Foot Key and WRB-1/WRB-2 = Whirpay Basin Sites nos. 1 and 2. TKB, RKB and SKB were locations of water samples collected during a SE–NW bay transect conducted by the Florida Department of Environmental Protection, Marine Research Institute (DEP-MRI) in Marathon Florida. Sites PEKB, WRB-1/-2 and JFK were carbonate banks which were cored in liaison with the United States Geologic Survey (USGS). WRB-1/-2 and JFK are sites in the southern and northwestern portions of Whirpay Basin, respectively. The core labeled WRB2 was investigated in the most detail to date. Water depth at all coring sites was approximately 0.5 m at mid tide (± 0.2 m).

Samples of various seagrasses and macrophytes were collected by hand using snorkeling techniques. These samples were placed into polyethylene bags underwater, closed with expulsion of most but not all seawater, and returned to deck were they were placed upon ice until (2–4 h) frozen upon return to shore.

In addition to field samples, three pure cultures of phytoplankton known to be primary bloom formers (Philps and Badylak, 1996; Steindinger and Philps, 1996) were provided by Dr. C. Tomas of Florida DEP-MRI in St. Petersburg, Florida. These were a diatom (\textit{Cyclotella choctawathceana}) and 2 cyanobacteria (\textit{Synechococcus elongatus} and the so-called ‘2-micron picosphere’ cf. Steindinger and Philps, 1996).

Water samples and pure cultures were collected by filtration onto Whatman GF/F glass filters, in subdued light and the filters were immediately placed upon dry ice for transport to the FAU-OGG laboratory in Boca Raton.

Sediment cores were collected using food grade 4 inch i. d. acrylic tubes fitted with a double “O”-ring sealed piston. The tubes were driven into the carbonate marl with the weight of 2 people, the piston cable secured in place, and the core pulled either manually or using a winch mounted on a tripod over a ‘moonpool’ in the center of the USGS 25 foot catamaran sampling vessel. As the bottom of the core reached the sediment — water interface, it was immediately capped using a polyethylene ‘capplug’. Upon retrieval to deck the cores were immediately wrapped in several layers of aluminum foil for return to land. On land at the National Undersea Research Center (NURC) laboratory in Key Largo, Florida, the cores were sectioned into 2, 4 or 5 cm slices, each section quartered, and the quarter sections individually bagged, and placed onto dry ice for transport.

Samples of surficial sediment ‘flocs’ were collected while snorkeling. In these cases, we opened a 0.5 gallon polyethylene food bag (“ZipLock’T’) while moving it slowly into (<1 cm) and parallel with the flocculent sediment surface. As the bag opened material was drawn in and immediately closed for transport to the boat. Solids were recovered in the laboratory by centrifugation.

Filtered water, surficial sediment and core samples were maintained in a frozen state until thawed for sieving and analyses.

2.2. Extractions

All filters of seston or pure cultures, as well as blotted seagrasses or macroalgae, were extracted using 90% aqueous acetone in a Kontes DuAll’T all glass/Teflon/ stainless steel grinder. Grinding was performed in 3–5 s intervals, with 5–10 s periods between grinding, while the glass mortar was immersed in an ice bath. The extract and dispersed filter were transferred to a chilled centrifuge tube, centrifuged for 1 min, and the supernatant removed with a syringe and passed through a 0.2 µm filter. The filtrate was considered as the crude extract and the UV/Vis absorption spectrum was recorded at
this point. For volumetric purposes the initial, not the final, volume of extractant was recorded.

The extraction of the carbonate marls of upper/central Florida Bay proved to be difficult. That is, extraction with acetone and methanol, either pure or in aqueous mixtures, was found to lead to a bleed out of pigment rather than releasing the majority within the first 1–2 extractions. Therefore, we experimented with several extraction cocktails (Table 1) and found that 100% peroxide-free tetrahydrofuran (THF) was far and away the best agent for release of pigment, extracting from 2 to 7 times the amount of pigment within the first extraction, as compared to the total which other solvents achieved after 5+ successive treatments. Extractions were made by the simple admixture of solvent with sieved (63 μm) sediment which had been centrifuged to remove bulk water. For test purposes, the first extraction used 2 ml solvent per 1 g sediment weight determined prior to centrifugation to remove excess water. Subsequent extractions were made using 1 ml solvent per gram original sediment weight. Crude estimates of total 'pheopigments-a' and 'bacterioopheopigments-a' were made using εₘ₉₈ = 53.25 and 76.0 at 664-666 and 750-700 nm, respectively (cf. pheophorbide-a, bacteriopheophytin-a: Fuhrhop and Smith, 1975). The UV/vis spectra of samples of pure pheophorbide-a ME, bacteriopheophytin-a, and one of the extracts were successively recorded in each of the solvents, evaporating between determinations. No significant alteration of absorptivity at band-I maxima in THF could be shown when compared to spectra taken in acetone or methanol.

All extractions of Florida Bay sediments were then made using 100% peroxide-free THF. Each THF extraction was pooled, evaporated in vacuo and the final pooled extract was redissolved in 100% acetone, prior to the addition of water to achieve a 90% aqueous acetone solution. This solution was then filtered through a 0.2 μm syringe filter, in order to remove any precipitated salts, and treated in the manner described above for all crude extracts prior to injection onto the HPLC system.

2.3. Bulk properties

Here we refer to the determination of water content, organic carbon and radionuclide dating. Duplicate

Table 1
Quantitation of chlorophyll-a and bacteriochlorophyll-a and derivatives extracted from Florida Bay marls using various solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Yield (μg/g-dry weight sediment)</th>
<th>Solvent</th>
<th>Yield (μg/g-dry weight sediment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ace</td>
<td></td>
<td>Ace/MeOH/THF (1:1:)</td>
<td></td>
</tr>
<tr>
<td>×1</td>
<td>2.792</td>
<td>×1</td>
<td>2.175</td>
</tr>
<tr>
<td>×2</td>
<td>2.118</td>
<td>×2</td>
<td>1.748</td>
</tr>
<tr>
<td>×3</td>
<td>0.452</td>
<td>×3</td>
<td>1.499</td>
</tr>
<tr>
<td>×4</td>
<td>0.368</td>
<td>×4</td>
<td>0.587</td>
</tr>
<tr>
<td>×5</td>
<td>0.304</td>
<td>×5</td>
<td>0.395</td>
</tr>
<tr>
<td>Total</td>
<td>6.034</td>
<td>Total</td>
<td>6.404</td>
</tr>
<tr>
<td>Ace/MeOH (1:1:1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>×1</td>
<td>0.164</td>
<td>×1</td>
<td>0.731</td>
</tr>
<tr>
<td>×2</td>
<td>0.335</td>
<td>×2</td>
<td>1.084</td>
</tr>
<tr>
<td>×3</td>
<td>0.541</td>
<td>×3</td>
<td>1.438</td>
</tr>
<tr>
<td>×4</td>
<td>0.526</td>
<td>×4</td>
<td>0.790</td>
</tr>
<tr>
<td>×5</td>
<td>0.434</td>
<td>×5</td>
<td>0.469</td>
</tr>
<tr>
<td>Total</td>
<td>2.000</td>
<td>Total</td>
<td>4.512</td>
</tr>
<tr>
<td>THF (1st run)</td>
<td></td>
<td>THF (2nd run)</td>
<td></td>
</tr>
<tr>
<td>×1</td>
<td>11.383</td>
<td>×1</td>
<td>10.790</td>
</tr>
<tr>
<td>×2</td>
<td>1.468</td>
<td>×2</td>
<td>1.460</td>
</tr>
<tr>
<td>×3</td>
<td>0.583</td>
<td>×3</td>
<td>0.388</td>
</tr>
<tr>
<td>×4</td>
<td>0.527</td>
<td>×4</td>
<td>centrifuge tube broke</td>
</tr>
<tr>
<td>×5</td>
<td>0.493</td>
<td>×5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14.4541</td>
<td>Total (1-3)</td>
<td>12.638</td>
</tr>
</tbody>
</table>

* Sediment used was a recombined and sieved (63 μm) quarter core from Whipray Basin Site no. 1 covering the interval 0–25 cm bsf. Pigments were calculated as “PPBIDa” = pheophorbide-a (εₘ₉₈ = 53.25 at 664–666 nm) and “BPTINa” = bacteriopheophytin-a (εₘ₉₈ = 76.0 at 750 nm: Fuhrhop and Smith, 1975). Extraction details are in text.
percentage dry weight determinations of sieved sediments were performed by placing a homogenized aliquot in a pre-dried ceramic crucible and heating at 108–114°C for 18 h, repeating to constant weight. This removes wet, bound, and occluded water, leaving only true waters of hydration (Pierce et al., 1958).

Organic carbon was performed by the United States Geological Survey (USGS: Dr. W. R. Orem) in Reston, Virginia, USA by fuming HCl methodology.

Radionuclide (210Pb, 137Cs, 14C) dating was performed by the USGS in St. Petersburg, Florida, USA (Dr. C. W. Holmes).

2.4. Pigment analysis

The HPLC system utilized in these studies included; a 3.9×150 mm Waters NovaPak® 4 μm ODS column, a Rheodyne Model 7120 injector fitted with a 100 μl injection loop, a Thermo Separations Products Model 4100 quaternary pump and a Waters Model 990 photodiode array detector set to collect data between 300 and 800 nm. The column, when not in use, was stored in 85% aqueous methanol.

Ninety percent aqueous acetone 0.2 μm filtered crude extracts or standard pigments were prepared for injection by the addition of ion-pairing (aka suppressing) reagents and an internal standard. The ratio of this mixture was; extract/ion-pairing solution/internal standard = 2/1/1, v/v/v. The ion pairing solution was that reported by Mantoura and Llewellyn (1983) and consisted of 15.0 g of tetrabutylammonium acetate, 77.0 g. of ammonium acetate plus water to equal 1.0 l final volume.

The internal standard chosen was copper mesoporphyrin-IX dimethyl ester (‘Cu-Meso-IX-DME’). Reasons for choosing this compound include; structural parallels to the chlorophyll derivatives, good chemical stability, a lack of UV/vis absorption at 440 nm, minimal absorption at 410 nm, and chromatographic behavior on our RP-HPLC system which gave but partial coelution only with canthaxanthin. This partial coelution, using full spectral PDA detection, still allowed quantification of Cu-Meso-IX-DME (394 nm) and canthaxanthin (472 nm), each in the presence of the other.

The ion-paired reverse-phase HPLC solvent protocol followed a modification of that given by Kraay et al. (1992), which in turn is a modification of Mantoura and Llewellyn (1983: cf. Winfree et al., 1997; Steinman et al., 1998). The solvent profile used is given as Table 2. To date, 78 known pigments, of which but 6 are tentative, have been employed to standardize the RP-HPLC system used in these studies. This data bank includes 44 tetrapyrroles, chlorophylls and derivatives, and 34 carotenoids.

Table 3 contains the retention times and UV/vis spectra, as generated by the PDA detector in the eluting solvent, for the chlorophylls and their derivatives. The carotenoids and bacteriochlorophylls other than the “a” series are omitted for clarity. Within Table 3, the following applies; (1) “-ap” and “-agg” refer to phytol and geranylgeraniol as the esterifying isoprenoid alcohols in each bacteriochlorophyll/bacteriopheophytin-a series, and (2) the retention times for pigments such as the phorbin steryl esters (PSEs) are given as a range since their exact retention times will depend upon the identity of the esterifying alcohol. PSEs are geochemical isolates and are tentatively identified based upon UV/Vis spectra and chromatographic positioning in relation to literature reports (cf. King and Repeta, 1991; Prowse and Maxwell, 1991; Harradine et al., 1996; King and Wakeham, 1996).

2.5. Abbreviations

The following abbreviations are used in text: CHL = chlorophyll; CHLide = chlorophyllide; PTIN = pheophytin; PBID = pheophorbide; p = pyro (lacks 132-carbomethoxy group); B = bacterio; PSE = phorbin steryl esters; CYCLO = 132, 171-cyclohephorbide-a-enol; “prime”, as in a’, refers to the epimer form of the pigment given.

3. Results and discussion

3.1. Source biota

As covered in the Introduction, a wide variety of photoautotrophic organisms can be implicated as the source biota for chlorophyll nuclei entering the sediments of Florida Bay. Therefore, it was decided to examine as many of these potential sources as possible in order to characterize their pigment complements and to assess early alterations which may be due to senescence/death (cf. Louda et al., 1998a) and/or heterotrophic processing (‘grazing’: cf. Head and Harris, 1992, 1996;
Bianchi et al., 1998; Harradine and Maxwell, 1998). The latter to be concluded from the examination of surficial sediments and resuspended ‘flocs’. We then undertook to examine the pigment complements of unialgal phytoplankton cultures, field samples of natural phytoplankton populations, the major seagrass, and the microphytobenthos.

### 3.1.1. Phytoplankton

The pigment distributions found for the 3 unialgal cultures of the major bloom forming phytoplankton yielded the distributions expected, except for the ‘2 micron picosphere’. Fig. 2a–c contains the HPLC/PDA 440 nm chromatograms for the cyanobacterium *Synechococcus elongatus*, the ‘2 micron picosphere’, and the diatom *Cyclotella choctawatcheena*. The extremely simple pigment distribution of *S. elongatus* (Fig. 2a) reveals chlorophyll-a, β-carotene, and considerable zeaxanthin. The diatom (Fig. 2c) contained the typical chrysophyte pigments CHLa, CHLs-c1/-c2, fucoxanthin, diadinoxanthin, β-carotene, plus a carotenol and carotenone which, though unidentified to date, may be typical of this species (Louda et al., 1998b and unpublished). The ‘eukaryotic 2 micron picosphere’ (Steindinger and Philps, 1996) was found during this study (Fig. 2b) not to be an eukaryote but to be a prokaryote, specifically a cyanobacterium. This was concluded on the basis of myxoxanthophyll, zeaxanthin and echinenone forming the accessory carotenoid complement to CHLa and β-carotene.

#### Table 3
RP-HPLC retention times and PDA-UV/vis spectra for tetrapyrrole standards

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Time(min)</th>
<th>UV/Vis(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent Front</td>
<td>~1.0–1.2</td>
<td>N/A</td>
</tr>
<tr>
<td>Chlorophyllide-a</td>
<td>2.2</td>
<td>426, 582, 616, 660</td>
</tr>
<tr>
<td>Chlorophylls-c1/-c2</td>
<td>2.7–2.9</td>
<td>446, 582, 628</td>
</tr>
<tr>
<td>Pyro-Chlorophyll-a</td>
<td>4.3</td>
<td>426, 582, 616, 660</td>
</tr>
<tr>
<td>Pheoporphide-a</td>
<td>6.8</td>
<td>408, 506, 534, 610, 668</td>
</tr>
<tr>
<td>Pyropheophorbide-a</td>
<td>10.3</td>
<td>412, 510, 540, 608, 666</td>
</tr>
<tr>
<td>Pheoporphide-b ME</td>
<td>11.5</td>
<td>436, 526, 598, 654</td>
</tr>
<tr>
<td>Pheoporphide-b’ ME (epimer)</td>
<td>12.4</td>
<td>436, 526, 598, 654</td>
</tr>
<tr>
<td>Pyropheophorbide-b ME</td>
<td>15.1</td>
<td>436, 526, 598, 654</td>
</tr>
<tr>
<td>Pheoporphide-a ME</td>
<td>16.3</td>
<td>410, 508, 538, 608,666</td>
</tr>
<tr>
<td>Pheoporphide-a' ME (epimer)</td>
<td>17.1</td>
<td>410, 508, 538, 608, 666</td>
</tr>
<tr>
<td>Pyropheophorbide-a ME</td>
<td>19.0</td>
<td>410, 508, 538, 608, 666</td>
</tr>
<tr>
<td>Bacteriochlorophyll-a&lt;sub&gt;ag&lt;/sub&gt;</td>
<td>21.0</td>
<td>360, 580, 770</td>
</tr>
<tr>
<td>Cu Mesoporphyrin-IX DME (Int.Std. = “IS”)</td>
<td>22.4</td>
<td>394, 524, 558</td>
</tr>
<tr>
<td>Bacteriochlorophyll-a&lt;sub&gt;p&lt;/sub&gt;</td>
<td>25.6</td>
<td>358, 580, 772</td>
</tr>
<tr>
<td>Chlorophyll-b</td>
<td>26.1</td>
<td>458, 596, 646</td>
</tr>
<tr>
<td>Cyclopyropheophorbide-a-enol</td>
<td>26.2</td>
<td>360, 426, 456, 628, 686</td>
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<tr>
<td>Chlorophyll-b’ (epimer)</td>
<td>27.4</td>
<td>458, 596, 646</td>
</tr>
<tr>
<td>13&lt;sup&gt;1&lt;/sup&gt;-oxydeoxx-Chlorophyll-a</td>
<td>27.8</td>
<td>416, 514, 562, 606, 654</td>
</tr>
<tr>
<td>Chlorophyll-a-allomer (‘13&lt;sup&gt;2&lt;/sup&gt;-hydroxy-Chl-a’)</td>
<td>28.2</td>
<td>430, 582, 616, 662</td>
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<tr>
<td>Chlorophyll-a</td>
<td>29.0</td>
<td>430, 582, 616, 662</td>
</tr>
<tr>
<td>Chlorophyll-a’ (epimer)</td>
<td>29.8</td>
<td>430, 582, 616, 662</td>
</tr>
<tr>
<td>Pheophtin-b-allomer (‘13&lt;sup&gt;2&lt;/sup&gt;-hydroxy-PP-b’)</td>
<td>30.8</td>
<td>436, 528, 598, 656</td>
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<tr>
<td>Bacteriopheophytin-a&lt;sub&gt;ag&lt;/sub&gt;</td>
<td>31.2</td>
<td>358, 526, 750</td>
</tr>
<tr>
<td>Bacteriopheophytin-a&lt;sub&gt;p&lt;/sub&gt;</td>
<td>31.4</td>
<td>358, 526, 750</td>
</tr>
<tr>
<td>Pheophtin-b</td>
<td>31.8</td>
<td>436, 528, 598, 656</td>
</tr>
<tr>
<td>Bacteriopheophytin-a&lt;sub&gt;p&lt;/sub&gt;</td>
<td>32.0</td>
<td>358, 526, 750</td>
</tr>
<tr>
<td>Pheophtin-b’ (epimer)</td>
<td>32.1</td>
<td>436, 528, 598, 656</td>
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<td>Pheophtin-a-allomer (‘13&lt;sup&gt;2&lt;/sup&gt;-hydroxy-PP-a’)</td>
<td>33.2</td>
<td>410, 502, 536, 610, 666</td>
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<td>Pyrobacteriopheophytin-a&lt;sub&gt;ag&lt;/sub&gt;</td>
<td>33.3</td>
<td>358, 526, 750</td>
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<td>Pyropheophytin-b</td>
<td>33.9</td>
<td>436, 528, 598, 656</td>
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<tr>
<td>Pyropheophytin-a</td>
<td>34.1</td>
<td>410, 502, 536, 610, 666</td>
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<tr>
<td>Pheophtin-a’ (epimer)</td>
<td>34.7</td>
<td>410, 502, 536, 610, 666</td>
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<tr>
<td>Purpurin-18-phytyl ester</td>
<td>36.0</td>
<td>360, 408, 546, 696</td>
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<tr>
<td>Pyropheophytin-a</td>
<td>36.4</td>
<td>410, 502, 536, 610, 666</td>
</tr>
<tr>
<td>Pheoporphide-a-steryl ester (tent)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.5</td>
<td>410, 502, 536, 610, 666</td>
</tr>
<tr>
<td>Pyropheophorbide-a-cholesteryl esters (tent)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39–42±</td>
<td>410, 502, 536, 610, 666</td>
</tr>
</tbody>
</table>

* See text.
Once the typical bay phytoplankton had been examined in culture, we next turned to the analyses of natural communities. Fig. 2d–f contains the 440 nm HPLC/PDA chromatograms for a Florida Bay transect (Fig. 1) from the SW (Twin Key Basin, “TKB”), through the central bay (Rabbit Key Basin, “RKB”), into the NW bay (Sandy Key Basin, “SKB”) bordering the Gulf of Mexico. Chromatograms collected at other wavelengths, specifically 410 nm, failed to show the presence of any ‘pheopigments’. Therefore, it was concluded that these populations were very viable, under very minor heterotrophic pressure, and the seston at time of collection contained little, if any, resuspended materials. ‘Pheopigments’ could not be shown using PDA detection at 410 nm. The central bay (RKB, Fig. 1) revealed a strong dominance of cyanobacteria (Fig. 2e), most likely the S. elongatus contributor as zeaxanthin was dominant and myxoxanthophyll was absent. Populations closer to either the Atlantic (TKB, Fig. 1) or Gulf of Mexico (SKB, Fig. 1) revealed mixed populations either co-dominated by cyanobacteria and diatoms (TKB, Fig. 2d) or dominated by diatoms (SKB, Fig. 2f). Sandy Key Basin (Figs. 1 and 2f) yielded a pigment distribution much more typical of the open ocean in which chrysophytes, dinoflagellates (Fig. 2f, “P” = peridinin), and cyanobacteria co-exist.

From analyses of the waters and the typical phytoplankton, we find that the chlorophylls-a and -c1/-c2 will be source material for underlying sediments.

3.1.2. Seagrass

The angiosperm Thalassia testudinum, commonly called turtle grass, is often the most apparent photoautotroph in Florida Bay (McIvor et al., 1994; Fourqurean and Frankovich, 1996; Landesberg et al., 1996). Thalassia blades were collected as healthy fresh green and separated dead brown specimens. Fresh blades were mechanically cleaned of all apparent epiphytes and either extracted as such or frozen prior to analyses. The blades which had first been frozen, one method reported to disrupt chlorophyllase activity (Holden, 1976), yielded a pigment distribution similar to what one would expect of any higher plant. That is, in molar ratio, Thalassia testudinum was found to contain; chlorophyll-a (CHLa: 1.00), chlorophyll-b (CHLb: 0.24), lutein (0.40), neo-/viola-xanthins (Neo/Viola: 0.11), antheraxanthin (Anthera: 0.06) and β-carotene (0.06). Extraction of fresh ‘never frozen’ Thalassia blades yielded the following; chlorophyllide-a (CHLide: 0.17), pyrochlorophyllide-a (pCHLide: 0.24), CHLa (1.00), CHLb (0.35), lutein (0.54), Neo/Viola (0.16), Anthera (0.09) and β-carotene (0.06). Disruption of cellular integrity of fresh blades released...
active chlorophyllase forming CHLidea and pCHLidea. Interestingly, when CHLa is summed with the chlorophyllides-a and the remaining pigments are normalized to that value, the pigment distribution is identical (mol ratio ±0.01) to that found for Thalassia blades which had been frozen prior to extraction. We, therefore, conclude that the quantitative hydrolysis of phytofl (CHLidea) or phytopl plus the 13-c-carbomethoxy moiety (pCHLidea) is the only change which freezing prior to extraction alters, in this case. Additionally, this does indicate that the disruption of cellular structure, admixing enzymes and substrates, can be an active method of initial pigment alteration. In the case of Thalassia, the production of chlorophyllides may be expected as a result of mechanical injury and direct heterotrophic processing. Future studies will include oxic and anoxic senescent/death studies (cf. Louda et al., 1998a) on Thalassia in order to clarify such initial routes of chlorophyll alteration.

Brown dead Thalassia blades, collected as jetsam within grass beds and in barren basins, yielded pigment distributions quite characteristic of the Chrysophyta, especially diatoms (Bacillariophyceae). In this case, the relationship of fucoxanthin and chlorophyll-a in a 1.1:1 molar ratio, was very similar (0.9–1.2:1) to a wide variety of diatoms (cf. Millie et al., 1993; Wright et al. 1996; Louda et al., 1998a), plus the presence of chlorophylls-c1/c2, chemotaxonomically identifies the main source as chrysophytes, most likely diatoms, living upon and within the dead turtle grass blades. Lutein could derive from the original seagrass or from chlorophylls on the grass blades. The extremely low amount of chlorophyll-b found in the dead Thalassia blades, relative to the amount of lutein present, is taken as indicating that the corresponding lutein is in fact a remnant from the host grass blade and not due to living periphyton, chrysophyte or other. That is, in fresh Thalassia we found lutein/CHLb = 1.6±1, whereas the dead leaves yielded 1.8±0.1 lutein to CHLb ratio of over 38,500:1. Thus, selective destruction or preservation of CHLb or lutein, respectively, must have occurred. The CHLidea and pCHLidea found present a dilemma. They could certainly arise from the seagrass itself, as shown above. However, the propensity for diatoms to produce these pigments, and the pheophorides-a (Louda et al., 1998a), obviates a definite conclusion. No matter what the source, it can still be stated that dead Thalassia blades, upon burial, will contain CHLa, CHLidea, pCHLidea and some pheophorbide-a (PBIDA).

From the analyses of fresh and dead Thalassia blades, we find that the chlorophylls-a and -c1/c2 will be sourced to the sediments. Chlorophyll-b, very prevalent in fresh viable Thalassia blades, disappears extremely rapidly and will form an extremely small source to underlying sediments. The fates of chlorophyll-b from turtle grass in Florida Bay; be they total destruction, alteration into a CHLa-like derivative, incorporation into intractable early geopolymers, other, or all are unknown.

3.1.3. Drift alga-Sargassum sp. (Phaeophyta)

Benthic macrophytes besides Thalassia are indeed widespread in Florida Bay. As examples, the following are common residents; Halodule wrightii (shoal grass), Syringodium filiforme (Manatee grass), Batophora oesteri (Chlorophyta), Caulerpa sp. (Chlorophyta), Penicillus sp. (Chlorophyta), Laurencia sp. (Rhodophyta) inter alia. Many of these species require a hard bottom and are common only in the northeastern portion of the bay, and other sediment sourcing but not accumulating areas. Some are drift algae and occur throughout the bay (see Zieman et al., 1989; Lapointe et al., 1994; Thayer et al., 1994). During sampling in Whipray Basin, we encountered accumulations of Sargassum sp., a common drift alga in the bay (Zieman et al., 1989). Though not identified to species, it was easily differentiated from the pelagic Atlantic and Gulf species (S. fluitans, S. natans) common along the east and west coasts of Florida. The species collected and analyzed had a much softer texture and translucent golden brown appearance. Unlike the more familiar Sargassum species one encounters in the pelagic and coastal/littoral zones, the present form lacked sufficient buoyancy to remain at the surface and was nearly neutral with a tendency to sink in quiescent waters. Samples of Sargassum sp. were collected from the sediment surface both totally above the sediment and partially covered. Thus, complete incorporation into the flocculent surficial marls and subsequent total burial, forming a source of chlorophyll nuclei, is easily envisioned.

A typical analysis of this Sargassum sp. gave the following mol percentage pigment distribution; CHLa (48.0%), CHLa’ (7.2%), PTINA (2.2%), PBIDA (2.1%), chlorophyll-c (CHLc: 3.0%), fucoxanthin (Fuco: 16.2%), fucoxanthinol (Fuco: 1.4%), diadinoxanthin (Diadino: 1.7%), diatoxanthin (Diato: 5.0%), zeaxanthin (Zea: 5.6%), and β-carotene (βCar: 7.6%). This resulted in overall CHLa/Fuco and CHLc/CHLb ratios of 18.1:1 and 3.2:1. Respectively. Sporadically, trace amounts (<1%mol) of chlorophyll-b and lutein were detected and may indicate minor epiphytic colonization by chlorophytes.

3.1.4. Surficial sediments/biofilms.

The surficial sediments of Florida Bay, in the Whipray Basin area particularly, form a loose floc which is easily resuspended to form a cloudy benthic boundary layer of several centimeters on a ‘normal’ non-stormy day. Wind driven turbulence can indeed extend this floc throughout the (0.5–1.5 m) water column. The underlying sediments appear to be stabilized by organic gelification as there is a direct linear relationship between
%C$_{\text{org}}$ and % water, above 1.6% C$_{\text{org}}$ (Louda et al., unpubl. The ‘live’ pigments of a viable microphytobenthos were found to be admixed with sedimentary pigments in this surficial floc. Thus, a biofilm to true mat formation likely exists and is ‘contaminated’ by sedimenting primary and reworked OM. In certain parts of the bay, these biofilms have indeed evolved into well formed benthic mats and stabilize this floc (Fla. Bay. Conf. 1996, 1998).

Analyses of several surficial ‘flocs’ (Table 4) revealed the presence of a host of pigments including: CHLs-c$_1$/c$_2$, fucoxanthinol, fucoxanthin, pheophorbid-a (PBIDa), pPBIDa, diadinoxanthin, diatoxanthin, several (8–12) unidentified carotenols, lutein, zeaxanthin, BCHLa, cyclophorbide-a-enol (CYCLO), spirilloxanthin, CHLa, echinenone, BPTI$a$, PTI$a$, trans- and cis-ß-carotene, plus several steryl esters of PBIDa and pPBIDa. Based on chemotaxonomic estimation via first order regression formulae (Louda et al., 1998b, 1999), we calculated relative abundances of algal divisions and phototrophic bacteria and concluded that the surficial ‘biofilm’ portion of this surficial floc consisted of a diatomaceous cyanobacterial community closely underlain by purple sulfur bacteria. Discussed below is an example of the chromatograms of surficial sediments (0–5 cm bsf) and these, except for lowered amounts of the ‘chlorophylls’ per se, can serve to represent the surficial distributions, as well.

From the analyses of the surficial flocs and biofilms, we find that the chlorophylls-a, -c$_1$/c$_2$, and bacteriochlorophyll-a serve as source nuclei for tetrapyrrole diagenesis. Again, as with dead Thalassia, chlorophyll-b was found in exceedingly short supply or to be totally absent. Overall, it is found that cyanobacteria, diatoms and purple sulfur bacteria serve as the major chlorophyll nucleus sources to the carbonate marls of central Florida Bay. Seagrasses are a likely major contributor of bulk OM to the sediments, in spite of losing about 40% of seagrass OM is lost in the first 2 or so cm of burial (Orem, W. R., personal communication). However, among the lipophilic pigments, it appears that only a minor amount of Thalassia lutein and perhaps some derivatives of the ‘xanthophyll-cycle’ pigments (neo-/viola-xanthin, anthaxanthin) survive. There is a possibility that some turtle grass chlorophyll-a nuclei survive in the sediments as PBIDa and pPBIDa. That is, we reported above that the chlorophyllase in turtle grass blades will form CHLidea and pCHLidea. Should this occur in nature, via injury and/or senescence, then one could expect the subsequent loss of Mg and the production of the corresponding pheophorbides. Alternately, all chlorophyll -a and -b could be destroyed by enzymatic pathways (Mg-dechelatase, chlorophyllase, pheophorbide-oxides), akin to many higher plants (see Ziegler et al., 1988; Iturraspe et al., 1994; Gossauer and Engel, 1996; Matile et al., 1996).

### 3.2. Sediment cores

#### 3.2.1. Organic carbon, depth/age correlations, and pigment yield index (PYI)

Overlaid within Fig. 3a are the downhole profiles of organic carbon at Pass-Eagle Keys Bank (PEKB), Jim Foot Key (JFK) and Whipray Basin Sites No. 1 (WRB1) and No. 2 (WRB No. 2: Fig. 1). On the right-hand y-axis, radiochemical dates are indicated for Whipray Basin Sites No. 1 and No. 2. Jim Foot Key sediments, deposited in the NW corner of Whipray Basin, were not dated. The deepest sample at PEKB was deposited about 1960, indicating a much more rapid sedimentation rate (~1.8 cm/year) relative to the Whipray Basin Sites (~0.4 cm/year) for the period 1960 to present (see Fig. 3a).

Given that the bulk carbonate carbon dates by $^{14}$C to about 4000 yBP (Wanless and Taggett, 1989) and that the bottom of WRB2, hitting the Pleistocene reef ‘bedrock’ at 120 cm bsf, dates to only 1655 AD, a disparity may seem evident. However, since the marine transgression which last buried the basal peats (ca. 4015 yBP: Davies, 1980), a period of active carbonate sedimentation, thought to be derived primarily from calcareous algae, occurred and this, now ‘old’, carbonate is periodically redistributed about the bay into the anastomotic mud banks we see today (Swart et al., 1989; Wanless and Taggett, 1989). As these older carbonates are repositioned, newer organic matter and radionuclides, such as $^{210}$Pb and $^{137}$Cs, are entrained. Thus, the downhole profiles we see now, at WRB No. 2 for example, are in fact only for the concluded 340± years of sediment stability. Pre-existing OM, being re-exposed to oxygenated conditions and filter feeding heterotrophy during catastrophic sediment relocation events, probably became more and more refractory such that labile compounds, such as the pigments, were destroyed. This hypothesis stems from trends in pigment diagenesis discussed below.

During our studies on Deep Sea Drilling Project (DSDP) core samples, we required a way to ‘normalize’ pigment trends without interference by sediment compaction and between samples of different organic richness. Therefore, we defined a pigment yield index or PYI (Louda et al., 1980; Baker and Louda, 1982). PYI is taken as the yield of pigment given in $\mu$g pigment/g sediment, dry weight divided by the percentage dry weight organic carbon. This index allows a quick visual and also a quantitative estimation of the relative preservation of pigments as a portion of the bulk OM. As points of reference, PYI values of about 14 were found for the Upper Miocene olive gray clayey nanno chalks recovered at 588 m bsf in the San Miguel Gap of the California Borderlands (Louda and Baker, 1981). PYI values were found to decrease logarithmically from about 15 to 0.5 within 400 m of burial of the olive
Table 4
Percent composition of chlorophyll-a and bacteriochlorophyll-a derivatives in 2 surficial flocs and a 1.2 m core from Whipray Basin, site no 2

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<tr>
<td>PBIDa</td>
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<td>60.53</td>
<td>27.36</td>
<td>12.43</td>
<td>6.27</td>
<td>7.08</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPBIDa + CYCLO</td>
<td>48.90</td>
<td>53.70</td>
<td>80.54</td>
<td>49.87</td>
<td>0.00</td>
<td>53.74</td>
<td>0.00</td>
<td>35.25</td>
<td>0.00</td>
<td>14.71</td>
<td>61.46</td>
<td>0.00</td>
<td>71.39</td>
<td>0.00</td>
</tr>
<tr>
<td>SUMPTINA&lt;sub&gt;b&lt;/sub&gt;</td>
<td>25.60</td>
<td>15.90</td>
<td>11.46</td>
<td>27.75</td>
<td>72.83</td>
<td>38.80</td>
<td>100.02</td>
<td>62.23</td>
<td>99.96</td>
<td>85.29</td>
<td>32.38</td>
<td>99.98</td>
<td>25.63</td>
<td>100.04</td>
</tr>
<tr>
<td>PBID &amp; pPBIDa-SEs</td>
<td>0.80</td>
<td>0.90</td>
<td>0.00</td>
<td>1.62</td>
<td>0.00</td>
<td>0.00</td>
<td>2.52</td>
<td>0.00</td>
<td>0.00</td>
<td>6.15</td>
<td>0.00</td>
<td>2.98</td>
<td>0.00</td>
<td>17.66</td>
</tr>
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(continued overpage)
brown muddy diatomaceous oozes in the Guaymas Basin of the Gulf of California (Baker and Louda, 1982).

PEKB is an area of rapid accumulation of redeposited sediment, as it occurs within the sediment “migrational” zone as defined by Wanless and Tagett (1989). Organic carbon here is at about 2% (Fig. 3a) and changes little downhole. PYI values at PEKB start at about 3.5 and decreases rapidly to 1.5 and remain at that value for the remainder of the core.

The SE shoal of Jim Foot Key forms the leeward of Whipray Basin proper and receives much wave action during the majority of the year as the trade wind blow mainly SE to NW in the area. Here, organic carbon is about 4% at the surface and decreases rapidly to about 2%, similar to PEKB, by about 10 cm bsf. This top 10 cm at JFK, also a rapid accumulating site, could be reflecting the increased water column productivity of the basin during the recent algal blooms (see Robblee et al., 1991; Philips and Badylak, 1996; Philips et al., 1999). However, low PYI values (1–1.5) at the surface, and throughout the core, argue against the increased OM at the surface being due to pigment rich autochthonous production. Rather, redeposition likely explains the higher organic carbon in the upper 10 cm at the Jim Foot Key site. There is one stratum of increased preservation (PYI=2.5) which occurred at 24 cm bsf.

The NE (WR1) and SW (WR2) sites in Whipray Basin (Fig. 1) parallel each other downhole in age and organic carbon profiles, at least for the 75 cm which they have in common. WR no. 2 has a slightly higher Corg (6%) over the first 20 cm, as compared to WR no.1 (5%).

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### 3.2.2. Chlorophyll diagenesis, downhole trends

Cores from PEKB, WR#1 and JFK were sectioned only in 0–4 cm intervals and, aside from anecdotal
comments, will not be discussed in detail. However, it can be stated that downhole trends in pigment diagenesis (-Mg, pPBIΔa→CYCLO, etc.) for those cores paralleled those found for WR no.2. The core from Whipray Basin Site no.2 (WR no.2: Fig. 1) was sampled in 2 cm sections and, as this was the only core which went to the Pleistocene ‘bedrock’, was analyzed in the most detail.

Table 4 contains the percent composition of chlorophyll-a, bacteriochlorophyll-a, and their derivatives, as well as selected internal ratios, for 2 representative surficial biofilms/flocs and the 18 sections from WR no. 2 studied to date.

The chlorophylls-c₁/c₂ were found only within the very surficial sediment flocs and are therefore absent from this table. Whether the chlorophylls-c are destroyed or incorporated into early geopolymers and are therefore hidden from bitumen analysis cannot be ascertained from the available data. The early diagenetic fate of chlorophyll-c remains an enigma. The pyropheoporphyrins-c₁ and -c₂ have been reported as being present in the surface sediments of Priest Pot, a small eutrophic lake in Cumbria, U.K., and their formation was concluded as being due to herbivory (Harradine and Maxwell, 1998). Though no quantities were reported, those authors did conclude that the chlorophylls-c were possibly less stable than chlorophyll-a.

Many trends became evident upon dissection of these data (Table 4). Prior to covering chlorophyll-a and bacteriochlorophyll-a early diagenesis, we reiterate that we have determined by pigment based chemotaxonomy (Louda et al., 1998b, 1999), the main sources of pigments to this sedimentary column to derive from the activities of a diatomaceous-cyanobacterial biofilm underlain by purple-sulfur bacteria. Added to these main sources are the pigments which settle out of the water column, either directly or in fecal pellets. Heterotrophy at the sediment surface and sediment bioturbation is known throughout various portions of the bay (Wanless and Tagett, 1989). At WR no.2, very strong anoxia, as evidence by elevated H₂S, goes at least to the sediment surface and during quiescent periods likely extends off the bottom.

The largest drop in the ratio (~8→3.6:1; Table 4) of CHL-a to BCHL-a occurs at about 2–4 cm sub-bottom. This, we suggest, is due to the combined phenomena of rapid CHL-a destruction, which eventually (6–8 cm) slows as more stable derivatives become dominant, and the production of BCHL-a in these layers. The intense solar radiation in this area, coupled with a shallow (~0.5m) water column assuredly allows the light required for bacterial photosynthesis to penetrate the few cm indicated here. Light measurements were not, however, collected.
Fig. 4 contains the downhole profiles of the losses of Mg (Fig. 4a) from chlorophyll-a, forming pheophytin-a, and of the 13\(^{2}\)-carbomethoxy moiety (Fig. 4b) from pheophytin-a, forming pyropheophytin-a. Near the surface of these sediments, CHLa/PTINa is only about 2:1, indicating considerable CHLa reworking prior to sediment stabilization. However, once incorporated, the profile of Mg removal versus depth is close to that of a first order reaction which completes its first half-life (viz. CHLa/PTINa=2:1\rightarrow0.2:1) in the uppermost 10 cm and is essentially completed by 20 cm. The slight presence of intact CHLa at 66 and 92 cm bsf is taken as a reflection of the increased PYI values (Fig. 3b) in these strata. Likewise, rapid conversion of PTINa to pPTINa (Fig. 4b) occurs within this sediment column. That is, PTINa is halved (2.0:1\rightarrow1.0:1), with production of pPTINa, between 0 and 12 cm bsf and again (1.0:1\rightarrow0.5:1) from 12 to 66± cm bsf. Therefore, of the phytylated pigments, pyropheophytin-a dominates below about 35 cm bsf. It has also been reported that the “anaerobic bacterial degradation of the marine prymnesiophyte *Emiliania huxleyi*” produces pPTINa as the major CHLa derivative after 77 days incubation during which bacterial numbers increase in concert with the production of sulfide from sulfate (Spooner et al., 1995). A similar situation exists in these sediments except that strong sulfide, including much H\(_2\)S, exists at ‘t\(_0\)’ (depth=0 cm bsf) and that pPTINa does not exceed PHtin\(_a\) until about 6–8 cm bsf, or for about 3–4 years (Table 4, Fig. 3a). However, the surficial floc from barren portions of Whipray Basin, likely containing much more resuspended materials, has pPTINa at about a five-fold dominance over PTINa. As the so-called ‘carbomethoxy’ group can also be thought of as being the methyl ester of formic acid, the requisite ‘pyro’ (demethylation/decarboxylation) reactions are not difficult to envision and could include methanogenic bacteria. That is, the elements of CH\(_4\) and CO\(_2\) are present.

Fig. 5 is the chromatogram of the non-polar pigments from the extract of the 0-4 cm bsf section of the core from PEKB (Fig. 1). This partial chromatogram reveals many of the pigments typical of the Florida Bay sediments. All forms of pheophytin-a are present, including; PTINa per se, its allomer (13\(^{2}\)-hydroxy), epimer, and pyro (‘13\(^{2}\)-decarbomethoxy’) derivatives. All *trans* and (15-?) *cis* \(\beta\)-carotene are evident. A series of peaks follow pPTINa (\(t=36.4\) min. cf. Table 3) which we tentatively identify as a pheophorbide-a (37.7 min) and a series of pyropheophorbide-a steryl esters (\(t=40–42\) min). This tentative identification is based upon their reported elution orders (cf. Eckardt et al., 1991; Pearce et al., 1993, 1998; Harradine et al., 1996; King and Wakeham, 1996; Riffé-Chalard et al., 1999) and the fact that their UV/vis spectra were indistinguishable from those of all forms of pheophorbide-a and pheophytin-a, synthetic or geochemical. These phorbin steryl esters were quantified using \(E=1%/1\) cm=713 at 410 nm, for pheophytin-a (cf. Mantoura and Llewellyn, 1983) and an approximate molecular weight of 924 Da. The resultant data for the PBIDA- plus pPBIDA-‘SEs’ are

![Fig. 4. Downhole trends in the ratios (a) sum of chlorophylls-a (= a+epimer+allomer) over the sum of pheophytins-a (= a+epimer+allomer), and (b) pheophytin-a / pyropheophytin-a versus depth of burial at Whipray Basin site no. 2.](image-url)
given in Table 3. These pigments, strong indicators for zooplankton, and possibly other microcrustacean, herbivory (Pearce et al., 1998 and references cited), exhibit sporadic occurrences within the downhole sequence at Whipray Basin site no. 2. The abundances of the combination of the PBIDa- and pPBIDa- steryl esters are plotted versus depth within Fig. 6. Eight of the sixteen samples analyzed from the WRB2 core contained quantifiable amounts of the PSEs. Aside from minor occurrences of the PSEs in surficial sediments at PEKB and WR1/WR2 (0–2 cm), the highest abundance was found at 66–92 cm bsf. These sediments correspond to a period of lowered PYI-values following a downhole maximum at 96–100 cm bsf. The sample at 92 cm bsf is still within the elevated PYI profile but is within a trend towards lowered pigment preservation following the downhole organic carbon maximum around 1740 AD (see Fig. 3a–b).

$^{13}$C, $^{17}$Cyclo(pyro)pheophorbide-a-enol (‘CYCLO’) emerged as the most abundant chlorophyll-a derivative in most of the strata from the WR no. 1 and 2 sites. Additionally, CYCLO was quite apparent in the surface sample (0–5 cm bsf) from PEKB (Fig. 1).

During the separation of the pigments in the Florida Bay marls, it became apparent that CYCLO was a very important component and that it, unfortunately, partially co-eluted with spirilloxanthin, the major bacterial carotenoid in these sediments. In order to quantify these pigments to the best accuracy possible, we choose 686 and 530 nm, respectively. This allowed selective integration and reproducible quantitation for CYCLO and spirilloxanthin. It must be noted that the peak containing CYCLO tended to tail. This occurred in spite of the
fact that all other peaks generated by this system were entirely Gaussian. This phenomenon may be due to keto-enol changes during chromatography but is beyond the scope of the present study.

In the surface sediments studied from Florida Bay, pyropheophorbide-a (pPBIDA) dominated over CYCLO (Table 4). Several downhole plots serve to illustrate the diagenesis of the dephytylated CHL-a derivatives. Fig. 7a is a plot of pPBIDA and CYCLO when their sum is taken as unity. That is, this pair is ‘self-normalized’. Here, pPBIDA is found to decrease rapidly and is essentially absent after 18cm bsf (Fig. 3a, Table 4). Thus, CYCLO appears to be generated at the expense of pPBIDA. It could be argued that pPBIDA is being destroyed and CYCLO remains unscathed. This, we feel, is not the case and claim that this does in fact represent a diagenetic Dieckmann-like cyclization of pPBIDA to give CYCLO as the product within these strongly anoxic highly sulfidic carbonates. Further, in several strata, CYCLO is the most abundant readily identifiable CHL-a derivative in the bitumen. The structural relationship of pPBIDA and CYCLO, as well as the reaction and resultant changes in the UV/Vis spectrum, is given as Fig. 8.

Returning to Fig. 7, much can be inferred about the pre-/syn-depositional paleoenvironment which leads to the presence or absence of CYCLO in these sediments. Fig. 7b is a co-plot of the amounts of CYCLO and the pheophytins-a versus depth of burial. They exhibit opposite trends. This is actually as should be expected since they form subsets of one set, CHL-a derivatives. In this case, CYCLO derives from the dephytylated route and the PTINsa from the route which retains the phytol ester. CYCLO derives directly from pPBIDA which forms in part from senescence/death processes (Louda et al., 1998a) but mainly from metazoan heterotrophy (cf. Head and Harris, 1992, 1996; Bianchi et al., 1998; and references in each), obviously an oxic to dysoxic endeavor. If we now plot CYCLO versus BCHLa plus derivatives (Fig. 7c), as an indicator of anoxia (cf. Eckardt et al., 1991), we find that these 2 source non-related pigments also vary in opposite directions. Therefore, it is concluded that weakly anoxic to oxic conditions at the sediment water interface allows heterotrophy by benthic and burrowing organisms to generate mainly pPBIDA which, following burial, is then converted within these anoxic carbonates to CYCLO. Conversely, strong anoxia at the sediment surface, possibly even extending above the bottom, decreases heterotrophic activity enough that mainly the senescent/death products, dominated by PTINsa (cf. Louda et al., 1998a), to predominate with the concurrent production of significant amounts of bacteriochlorophyll-a by the purple-sulfur bacteria. The correlation of oxic conditions yielding pPBIDA extends not only to the post-depositional diagenetic generation of CYCLO but also to the pre-/syn-depositional generation of the steryl esters of PBIDA and pPBIDA, which also tend to be higher in strata with elevated

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Fig. 7. Downhole profiles in various pigments as pertaining to the diagenetic generation of cyclo (pyro) pheophorbide-a-enol (‘‘CYCLO’’). (a) Trend in pyropheophorbide-a and ‘‘CYCLO’’ when normalized to their summed value, (b) pheophytin-a (solid trace) compared to ‘‘CYCLO’’ (open circles, dashed trace), and (c) sum of bacteriochlorophyll-a and bacteriopheophytin-a (solid trace) compared to ‘‘CYCLO’’ plus pPBIDA (open circles, dashed trace).
levels of CYCLO (see Table 4). Strata with very low to non-detectable levels of PSEs generally have increased levels of PTINa and BPTINa, indicating strong anoxia and chlorophyll diagenesis which follows the senescence/death routes rather than starting with herbivory induced alterations.

132,173-Cyclophorphoride-a-enol ("CYCLO": lower/right). The dehydration involved in the Dieckmann-like cyclization reaction is indicated.

now know it, following reduction with NaBH4 and it gave a spectrum identical to any 'pheopigment' of the "a" series which had been so treated with borohydride (see Baker and Louda, 1986).

"CYCLO" was first isolated, and correctly identified, from the sponge *Darwinella oxeata* (Karuso et al., 1986). Since that time, a variety of "antioxidants" related to CYCLO have been identified in extracts of filter feeders and surficial sediments (Sakata et al., 1990; Montforts et al., 1994). In these cases, the main compound identified has been "chlorophyllone" (= "hydroxychlorophyllone"), an oxidation product of CYCLO, and in each case chromatography over normal phase silica gel was reported. The importance of noting 'normal phase silica gel', as thin-layer (TLC) or column (CC) chromatography, here stems from an observation which we made during the present study. In an attempt to concentrate CYCLO from the extract of a bulked sediment samples known to have high percentages of this compound, we ran the extract through a bed of conventional silica gel (Merck silica gel-60, 40-63 μm, P/N 9385), eluting with increasing acetone in petroleum ether. We recovered all other pigments (e.g. β-carotene, pheophytins-a, zeaxanthin etc.) in the ratios which we knew existed prior to the silica gel CC. However, we could not recover any CYCLO and it was replaced by a compound with UV/vis spectrum identical to that reported by Ma and Dolphin (1996) for the hydroxychlorophyllones-a (λSoret = 408, λ4 = 666 nm). Upon RP-HPLC/PDA determination on the system used in the present studies, the retention time was found to have shortened by about 2 min, indicating a compound which was more polar relative to CYCLO. Given that extraction with 90% aqueous acetone and direct RP-HPLC separation of the native extract is a very mild method, we can only conclude that CYCLO is the main natural derivative and that the many of 'chlorophyllones' reported in the literature may be analytical artifacts, if exposed to 'normal' phase silica in the presence of oxygen. Whatever the exact in situ form of CYCLO, the fact that a 132,173-cyclophorphoride nucleus forms in nature does explain the existence of 'cyclobutano' and certain bicycloalkanoporphyrins (BiCAPs) in oil shales and petroleum crudes (see Chicarelli and Maxwell, 1986; Callot, 1991). The direct link from the oxygen free bicycloalkano 'chlorin' (= 7,8-dihydroporphyrin) derivative of CYCLO to the C33 BiCAP was provided by the isolation and identification of these in the same sample, an immature Pliocene clay, by Keely and Maxwell (1990).

4. Conclusions

The extraction of lipophilic pigments from the carbonate marl sediments of upper central Florida bay was to
be best performed using 100% tetrahydrofuran, as opposed to the more common solvents such as acetone, methanol or aqueous mixture of each.

The 2 µm picoplankter common to central Florida Bay was shown to be a cyanobacterium through chemotaxonomy including the identification of myxoxanthophyll, zeaxanthin, echinenone, and β-carotene in the presence of only chlorophyll-a.

Analyses of various potential source biota (phytoplankton, drift algae, seagrasses, microphytobenthos) revealed that the chlorophylls-a, -b, -c1/-c2 and bacteriochlorophyll-a were all in good pre depositional supply. The chlorophylls-b and -c1/-c2 were found to have totally disappeared from the bitumen within the first 2-4 cm of burial. CHLb was concluded as being destroyed within senescent /dead Thalassia leaves. The CHLs-c1/-c2 may or may not be destroyed and/or incorporated into geopolymers. After about 10 years of burial, the only identifiable tetrapyrroles were CHL-a and BChL-a and their derivatives.

Elevated pigment preservation (PYI values) were found at the Whirpray Basin sites at 0–26, 60–70 and 90–100 cm bsf, roughly correlating with 1945-present, 1800–1820 and 1730–1740 AD. An elevated signal, either as organic carbon or as pigment preservation, could not be shown for the cyanobacterial blooms which have occurred within the past 15 years.

The microphytobenthos of upper central Florida Bay is concluded as being composed of a diatomaceous-cyanobacterial biofilm/mat underlain by purple sulfur bacteria. The structure of this community appears to fluctuate only to the extent that BChL-a increases in relative abundance during periods of strong anoxia and that the productivity of the entire community, as witnessed by organic carbon and pigment preservation (PYI-values), reacts to water column nutrient supplies.

The diagenesis of chlorophyll-a was found to include a smooth (first order?) loss of Mg to yield pheophytin-a and the time-effected loss of both C-15 and C-14 to yield pheophytin-b and b-carotene in the 1561–1580

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