



doi:10.1016/S0016-7037(03)00079-6

## Preservation of organic matter in mound-forming coral skeletons

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(Received August 22, 2001; accepted in revised form January 13, 2003)

**Abstract**—This study demonstrates that intracrystalline organic matter in coral skeletons is well preserved over century timescales. The extent of preservation of organic matter in coral skeletons was investigated by measuring total organic carbon (TOC), total hydrolyzable amino acid (THAA), chloropigment, and lipid concentrations in 0–300 year old annual growth bands from *Montastraea annularis* (Florida Keys) and *Porites lutea* (Red Sea). Organic matter intrinsic to the calcium carbonate mineral (intracrystalline) was analyzed separately from total skeletal organic matter. The Red Sea coral had less TOC (0.02–0.04 wt%) than the Florida Keys coral (0.04–0.11 wt%), but a higher percent of intracrystalline organic matter in all annual bands measured. Carbon in the form of THAA, most likely from mineral-precipitating proteins, contributed 30–45% of the TOC in both corals. Carbon in lipids represented about 3% of the TOC in the coral skeletons. Chlorophyll-a and b were present in annual bands where endolithic algae were present, but these compounds were minor contributors to TOC. The distribution of specific organic compounds showed that organic matter was well preserved throughout the time period sampled in both the total and intracrystalline pools. Variations in THAA were not correlated with TOC over time, suggesting that organic matter that is involved in biomineralization, like amino acids, may be deposited in response to different environmental factors than are other components of skeletal organic matter. Differences in the quantity and composition of organic matter between the two corals investigated here were assessed using principal components analysis and suggest that location, species and skeletal structure may all influence organic matter content and possibly the degree of physical protection of organic matter by the coral skeleton. Further, our study suggests that intracrystalline organic matter may be better protected from diagenesis than non-intracrystalline organic matter and may therefore be a more reliable source of organic matter for paleoceanographic studies than total skeletal organic matter. Copyright © 2003 Elsevier Science Ltd

### 1. INTRODUCTION

Investigations of the composition and stability of organic matter in biominerals suggest that carbonates and silicates can be important conveyers of relatively unaltered organic matter in the marine environment (e.g., Carter and Mitterer, 1978; Maita et al., 1982; Collins et al., 1992). As such, the isotopic composition of this organic matter has recently been employed in studies of paleoproductivity (e.g., Shemesh et al., 1993; Sigman et al., 1999; Rosenthal et al., 2000; Crosta et al., 2002). Organic matter in coral skeletons may also prove valuable in the development of tools for paleoceanographic investigations. Inorganic compounds in coral skeletons are presently used as paleoceanographic proxies due to the well-defined stratigraphy of the annual carbonate bands (e.g., Druffel, 1997). However, research on organic matter in coral skeletons is less extensive than that in other types of biominerals. Here we investigate the century-scale preservation of organic matter in the skeletons of two species of mound-forming scleractinian corals with the hope that understanding temporal variability in composition and concentration of coral skeletal organic matter will lead to the development of new paleoceanographic proxies in the future.

The concentration of total organic carbon in mound-forming

coral skeletons is less than 0.5 wt % (Wainwright, 1962; Swart, 1981; Bak and Laane, 1987) and derives from many sources. These include the organic matrix synthesized by the coral to control mineral precipitation (Abelson, 1955), material adsorbed from ambient seawater (Isdale, 1984), and organic matter from endolithic algae (Duerden, 1902), bacteria (DiSalvo, 1969) and fungi (Bak and Laane, 1987). The skeletal organic matrix that directs biomineralization is a major component of organic matter in skeletons (Mitterer, 1978; Lowenstam and Weiner, 1989; Cuif et al., 1999). Soluble proteins rich in acidic amino acids, and insoluble proteins rich in glycine, form the template on which CaCO<sub>3</sub> crystals are precipitated (Constantz and Weiner, 1988; Levi et al., 1998). Soluble polyanionic proteins, containing sulfated carbohydrate moieties, are thought to be directly responsible for both promoting and inhibiting CaCO<sub>3</sub> precipitation (Addadi et al., 1987; Dauphin, 2001).

Lipids are minor organic constituents of coral skeletons (Isa and Okazaki, 1987; Stern et al., 1999). Lipids, including *n*-alkanes, fatty acids, and cholest-5-en-3 $\beta$ -ol (cholesterol), may participate in calcification; however, much less is known about their composition and their role in biomineralization compared to amino acids. Shell lipids can record the stable isotopic signature of their source and can be preserved over time in the shell matrix (CoBabe and Pratt, 1995).

Pigmented bands are commonly found in coral skeletons. They originate from endolithic algae, usually *Ostreobium quekettii*, that bore through the skeleton and grow just beneath the coral tissue (Duerden, 1902; Lukas, 1969; LeCampion-Alsumard et al., 1995). The organic matter from these algae,

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including extremely labile compounds such as chlorophyll-a, can still be present in skeletal material that is hundreds of years old (Risk et al., 1987; LeCampion-Alsumard et al., 1995).

Chemical bonding between the numerous sources of organic matter and biominerals is not well understood. Organic matter in coral skeletons may be adsorbed onto the surface of minerals, in between mineral crystals, or occluded within mineral structures (Towe, 1980; Sykes et al., 1995). Methodologically these types of mineral associations are difficult to distinguish. The term intracrystalline refers to organic matter that is occluded within the mineral structure. It is typically operationally defined as any organic matter that is not removed from a finely powdered skeleton by exposure to a strong oxidant such as commercial bleach (Shen and Boyle, 1988; Robbins and Brew, 1990; Gaffey and Bronnimann, 1993; Sykes et al., 1995). Organic matter between mineral crystals is often referred to as intercrystalline; however, we will not use that term here to avoid any inference about association of the non-intracrystalline material. Non-intracrystalline organic constituents of coral skeleton, which are removed by bleach treatment, can also be well protected; however, they are more likely than intracrystalline organic matter to be degraded because they are more accessible to interstitial fluids and enzymes of endolithic organisms (DiSalvo, 1969; Highsmith, 1981; Bak and Laane, 1987). Water in coral skeletons may participate in the chemical alteration of intracrystalline and non-intracrystalline organic matter over long time-scales through hydrolysis reactions (Hudson, 1967; Gaffey, 1988).

Our study investigates the composition and diagenesis of intracrystalline and non-intracrystalline organic matter in two corals, *Porites lutea* and *Montastraea annularis*. Previous work has demonstrated that amino acids in coral skeletons are relatively well preserved on hundred year timescales (Goodfriend et al., 1992; Nyberg et al., 2001). We used changes over time in the concentration and composition of total organic carbon, amino acids, lipids, chloropigments and the C/N ratio of intracrystalline and total organic matter as organic diagenetic indicators. Diagenesis was quantitatively assessed using principal components analysis.

## 2. STUDY SITES AND SAMPLING

Two cores (TRII and TRIII) of *Montastraea annularis* were collected on two neighboring coral heads by H. Hudson in 1982 from The Rocks coral reef (24°57'N, 80°33'W) one mile offshore from Plantation Key, Florida, in four meters of water. Radiocarbon records for these cores are published (Druffel, 1989). Annual average sea surface temperatures were between 26.4–27.1°C (annual range, 22.5–30.0°C) during the years 1955 through 1982 (<http://ferret.wrc.noaa.gov/las/>, COADS data). TRII contained growth bands dating from 1642–1978. TRIII contained growth bands from 1939–1982. Annual bands from 1704, 1880 and 1939–1982 were analyzed here.

Two cores (AR2 and AR2B) of *Porites lutea* were collected on one coral head by D. Schrag and M. Moore in July, 1995, from Alternative Reef in Ras Mohammed National Park in the Red Sea (27°43'N, 34°07'E) in three meters of water. The  $\delta^{18}\text{O}$  record from this core is published, and sea surface temperatures ranged annually between 24.3–30.2°C for the years 1973–1995 (Eshel et al., 2000). Combined, AR2 and AR2B date from 1799

to 1995. Annual bands from 1802, 1880 and 1982–1995 were analyzed here.

## 3. MATERIAL AND METHODS

### 3.1. Coral Preparation

The Florida Keys coral was cut into slabs (1 cm thick), X-rayed and mapped as part of an earlier study (Druffel et al., 1989). Slabs were sectioned beneath each dense growth band using a band saw so that each section dated from July to June of the following year. Here, annual bands from 1939–1982 (TRIII) that had been stored since that earlier study were analyzed for total organic carbon (TOC), insoluble nitrogen, chloropigments and total hydrolyzable amino acids (THAA). Lipids were analyzed in the 1972 annual band. Annual bands from 1704 and 1880 (TRII) were also analyzed for TOC and THAA.

The Red Sea cores were cut lengthwise into quarters as part of an earlier study (Eshel et al., 2000). Here we analyzed annual bands (determined by counting visible high and low density bands) from 1802, 1843, 1885 and 1982–1995 for TOC, insoluble nitrogen and THAA. Chloropigments were analyzed in 1802 and 1843 bands; the 1843 band was also analyzed for lipids.

To clean samples, all exposed surfaces were ground away at low speed using a Dremel tool equipped with a diamond tip. Subsamples were obtained by grinding the coral at low speed with a Dremel tool until enough ground coral (~1 g) was collected from a given annual band.

### 3.2. Isolation of Intracrystalline Organic Matter

To isolate intracrystalline organic matter, bleach was used to oxidize non-intracrystalline organic matter that was physically exposed when samples were ground to a fine powder (<40 $\mu\text{m}$ ). This technique has been used previously on biogenic carbonates (Gaffey and Bronnimann, 1993). Ground samples (~1g) were placed in 12-mL combusted glass vials with 10 mL of 5% NaClO (commercial bleach solution) and shaken continuously for eight days.

The appropriate duration for bleach treatment was first determined by bleaching a coral sample (1957 annual band of Florida Keys coral) for up to 35 d. Samples of bleached coral were removed at 2–4 d intervals (in triplicate) for 13 d and again after 35 d. Bleach was changed every 3 d. Amino acid and TOC analyses indicated that after 8 d, no further loss in organic matter occurred (see results). Procedural blanks consisted of empty vials in which bleach was added. These blanks were carried through each step of the procedure and resulted in undetectable contamination. Five samples were ground using two different methods to determine if grain size was an important influence on the amount of organic matter removed by bleach. A Dremel tool was used to grind samples to a fine powder (~40 $\mu\text{m}$ ). A mortar and pestle was used to produce larger grain sizes (~40–300 $\mu\text{m}$ ). Grain size was determined by sieving the ground coral. There was no significant difference in the amount of organic matter or amino acids removed by bleach in the two sample sets.

### 3.3. Organic Carbon, Amino Acid, Lipid and Chloropigment Analysis

#### 3.3.1. Organic carbon

Total organic carbon and intracrystalline organic carbon were measured after dissolving unbleached or bleached coral (~1 g) in 6N HCl (Fig. 1a). When coral skeletons are dissolved in 6 N HCl, a portion of the organic matter is insoluble. Insoluble organic matter was removed from solutions of dissolved coral by filtration through a combusted Whatman GFF filter (0.7  $\mu\text{m}$  nominal pore size). The filter was rinsed with 1 mL of 1% HCl to remove any soluble organic matter left on the filter, and filtrates were combined.

HCl-insoluble organic matter (on filters) from bleached and unbleached samples was analyzed for C and N on a Carlo-Erba elemental analyzer. HCl-soluble organic matter (in filtrates) was analyzed for C using a Shimadzu dissolved organic carbon (DOC) analyzer. Soluble and insoluble fractions were summed to obtain TOC or intracrystalline OC.

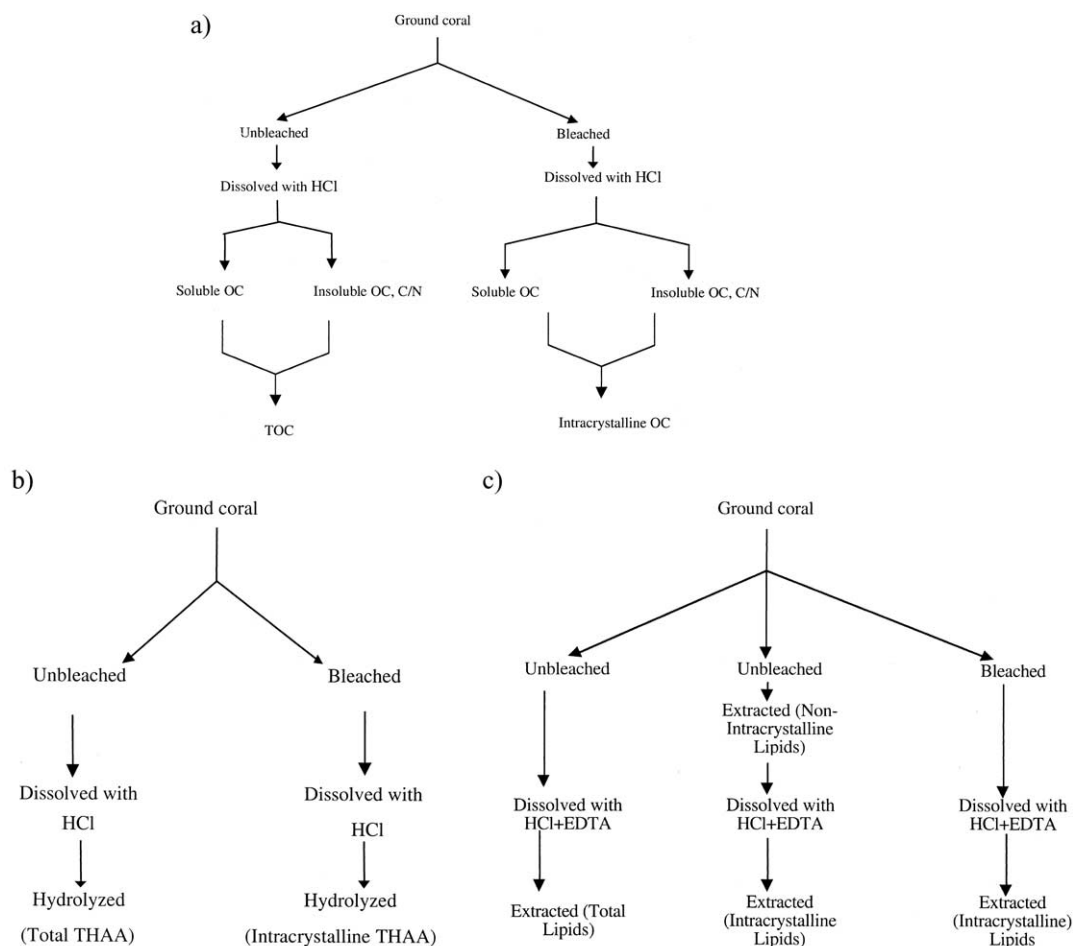


Fig. 1. Isolation of intracrystalline and non-intracrystalline organic components of the coral skeleton for measurements of a) organic carbon, b) amino acids, c) lipids.

### 3.3.2. Amino acids

For amino acid analyses, bleached or unbleached ground coral (~100mg) was dissolved in enough 12 N HCl to result in a 6 N HCl solution after dissolution (Fig. 1b). Additional 6 N HCl was added for the hydrolysis. Hydrolyses were carried out at 150°C for 90 min under N<sub>2</sub> atmosphere (Cowie and Hedges, 1992). Total hydrolyzable amino acids (THAA) were analyzed by high pressure liquid chromatography (HPLC) using precolumn OPA derivatization (Lindroth and Mopper, 1979; Lee et al., 2000). Replicates agreed within ±10–15% for each amino acid. A protein amino acid mixture (Pierce Chemical) was used as a standard. Known amounts of the non-protein amino acids β-alanine and γ-aminobutyric acid (Sigma Chemical) were added to Pierce standard H during dilution. Hydrochloric acid blanks were carried through the procedure, and values were subtracted from all data. Blank values were near the detection limit of the method.

### 3.3.3. Lipids

Skeletal lipid concentrations were measured using ~1g of powdered (<40 μm) coral. Strong acid dissolution is a harsh treatment for lipids, so methods for skeletal CaCO<sub>3</sub> dissolution were adapted from studies of intact skeletal organic matrices (Weiner and Erez, 1984) that result in minimal alteration of organic matter (Fig. 1c). To measure total skeletal lipids, a commercial 0.1N HCl/EDTA solution (Polysciences Inc.) was used to dissolve the CaCO<sub>3</sub>. Total lipids were extracted from the acid solution with 100% CH<sub>2</sub>Cl<sub>2</sub> and analyzed. Intracrystalline and non-intracrystalline lipids were distinguished using two methods. In the

first, samples were preextracted with CH<sub>2</sub>Cl<sub>2</sub> to remove non-intracrystalline lipids. In the second method, samples were bleached to remove non-intracrystalline lipids. In both cases, CaCO<sub>3</sub> was then dissolved in HCl/EDTA from which intracrystalline lipids were extracted and analyzed. Non-intracrystalline lipids were calculated by difference.

Lipid concentrations were quantified using methods described by Wakeham et al. (1997a). Neutral lipids (sterols, fatty alcohols, hydrocarbons and alkenones) and acidic lipids (hydrolysis products of wax ester, triacylglycerols, steryl esters and phospholipids) were derivatized as the trimethylsilyl and methyl esters, respectively, quantified by gas chromatography, and identified by gas chromatography-mass spectrometry. Analytical precision was usually approximately ±20%. Method blanks included CH<sub>2</sub>Cl<sub>2</sub> extracts of the HCl/EDTA solution and a CH<sub>2</sub>Cl<sub>2</sub> blank. Blank concentrations were subtracted and were <1% of the sample concentrations.

Chloropigments were extracted from 1 g of powdered (<40 μm) coral with 5 mL HPLC-grade 100% acetone, following the methods of Sun et al. (1993a). Two successive extracts were combined and filtered. Chloropigments were not extracted from dissolved coral samples, since acid would alter greatly the composition of the chloropigments. In any case, concentrations would likely be small given their presumed source from endolithic algae. We found very low or undetectable concentrations of intracrystalline phytopigments and phytol, suggesting that this assumption is correct. Phytopigments would have been produced during the analytical methods. Phytol, the esterified side chain of Chl-a would have been produced if Chl-a had degraded over time.

Chl-*a* and its degradation products (phaeophorbide, phaeophytin,

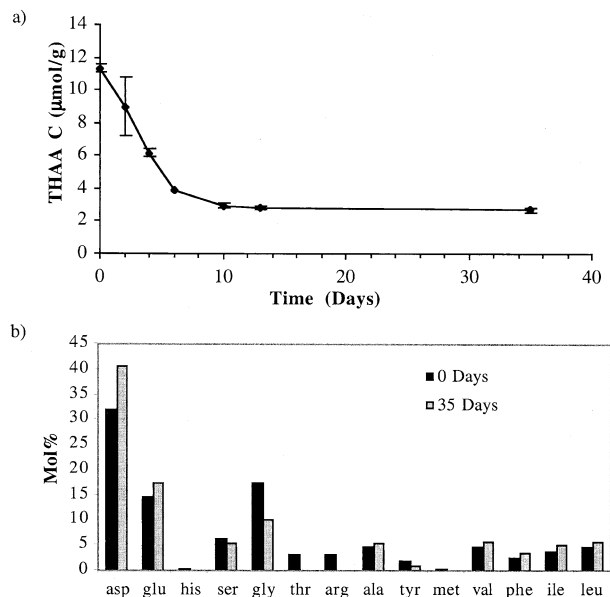


Fig. 2. a) Loss of total hydrolyzable amino acid carbon during experimental bleach treatment of the 1957 annual band of the Florida Keys coral. Error bars are based on results of triplicate samples for each time point. b) Amino acid composition before bleach treatment and after 35 d of bleach treatment.

chlorophyllide and pyropheophorbide) were determined by ion-pairing, reverse-phase HPLC. The precision for replicate samples extracted over a several month period was  $\pm 15\%$ . Chl-a of known concentration (Turner Designs) was used as the standard. Standards for Chl-a degradation products were produced in the lab from purified Chl-a following the methods of King (1993), and their concentrations determined spectrophotometrically using known extinction coefficients (King, 1993).

## 4. RESULTS

### 4.1. Bleach Treatment Exposure Time

Bleach treatment of the 1957 annual band from the Florida Keys coral for 8 d resulted in a reduction of total organic carbon from 60 to 16  $\mu\text{mol C/g coral}$ , a loss of 74%. Amino acid carbon was reduced from 11 to 2.8  $\mu\text{mol C/g coral}$ , a loss of 73% (Fig. 2a). Aspartic acid was enriched, and glycine was depleted in the remaining intracrystalline THAA (Fig. 2b). No further loss of organic carbon or amino acids was observed

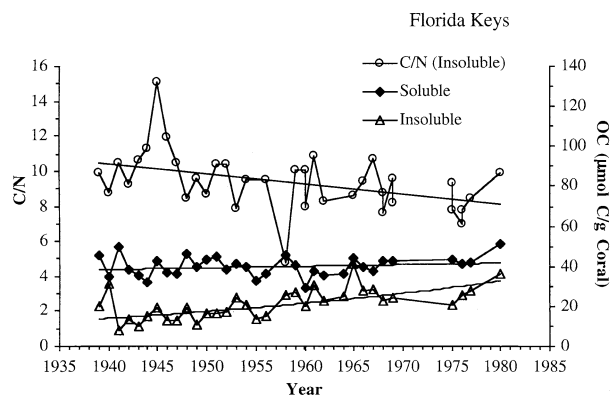


Fig. 3. Soluble and insoluble organic carbon concentrations and C/N ratio of Florida Keys *Montastraea annularis*. Soluble organic carbon  $r^2 = 0.038$ ,  $p = 0.28$ ; insoluble organic carbon  $r^2 = 0.41$ ,  $p = 0.00005$  and C/N ratio of insoluble organic matter  $r^2 = 0.18$ ,  $p = 0.01$  ( $n=36$ ).

after an additional 27 d of bleach treatment. Based on these results, all other samples were bleached for 8 d.

### 4.2. Organic Carbon

#### 4.2.1. Florida Keys coral (*Montastraea annularis*)

Between the years 1939 and 1982, Florida Keys coral TOC values ranged between 39–85  $\mu\text{mol C/g coral}$  (0.04–0.11 wt %; Table 1; Fig. 3), in good agreement with previously reported values of organic matter in other scleractinian coral skeletons (Wainwright, 1962; Swart, 1981; Constantz and Weiner, 1988). Bleach treatment of these samples revealed that on average from 1939–1982, the intracrystalline fraction of organic carbon represented  $\sim 35\%$  of the total carbon. In older annual bands, 1704 and 1880, TOC concentrations were 56 and 64  $\mu\text{mol C/g coral}$ , respectively. These values were within the range reported for the younger bands; however, the intracrystalline fractions were higher than in younger bands, 52 and 54% of the total, respectively (Table 1).

Organic carbon in corals includes both soluble and insoluble fractions. The average ratio of acid-soluble to acid-insoluble TOC was 2.1 from 1939–1982, 3.7 in 1880 and 4.3 in 1704 (Table 1). In the intracrystalline pool, the soluble OC to insoluble OC ratio was 2.9 in 1958 and  $\sim 7$  in 1704 and 1880. With the exception of two annual bands, C/N ratios of acid-insoluble

Table 1. Organic matter fractions of coral samples of different ages.

Location	Age	TOC $\mu\text{mol C/g coral}$	Intracrystalline OC/TOC	Total C/N	Intracrystalline C/N	Total Soluble/Insoluble OC	Intracrystalline Soluble/Insoluble OC
Florida	1939-1982	39-85	0.35	7-12	5.5-8.3	2.1 (average)	2.9 (1958)
Florida	1704, 1880	56, 64	0.52, 0.54	5.9, 6.2	5.0, 4.6	4.3, 3.7	7.5, 7.0
Red Sea	1995, Polyp	1710	0.02	9.4	8.3	0.56	1.7
Red Sea	1982-1994	26-60	0.44	4.3-6.5	4.6-8.0	3.0	3.3
Red Sea	1802, 1885	30, 28	0.79, 0.89	5.8, 5.4	4.2, 3.9	4.5, 4.3	8.6, 9.1

samples of different ages from the Florida Keys and Red Sea.

\*C/N values are of insoluble fraction only.

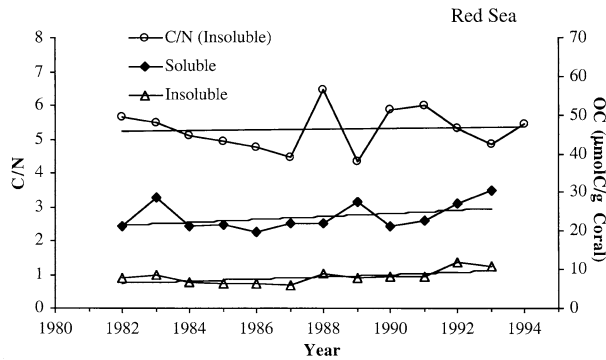


Fig. 4. Soluble and insoluble organic carbon concentrations and C/N ratio of Red Sea *Porites lutea*. Soluble organic carbon  $r^2 = 0.017$ ,  $p = 0.18$ ; insoluble organic carbon  $r^2 = 0.36$ ,  $p = 0.04$ , and C/N ratio of insoluble organic matter  $r^2 = 0.0029$ ,  $p = 0.9$  ( $n=12$ ).

total organic matter ranged between 7.0–12 (Fig. 3). C/N ratios of acid-insoluble intracrystalline organic matter were between 4.6–8.3 (Table 1).

#### 4.2.2. Red Sea coral (*Porites lutea*)

Between the years 1982 and 1994, Red Sea coral TOC values ranged between 26–60  $\mu\text{mol C/g coral}$  (0.02–0.04 wt %) (Table 1; Fig. 4). This range is somewhat higher than the 11–23  $\mu\text{mol C/g coral}$  found by Bak and Laane (1987) in samples of modern *Porites lutea* from the Indonesian Archipelago. On average, 44% of the TOC in Red Sea coral bands between 1982 and 1994 was intracrystalline. In older annual bands, 1802 and 1885, TOC concentrations (28 and 30  $\mu\text{mol C/g coral}$ ) were within the ranges reported for the more recent annual bands, and 89 and 79%, respectively, of TOC contents were intracrystalline (Table 1).

In both intracrystalline and total organic matter pools, acid-soluble OC was  $\sim 3$  times greater than acid-insoluble OC between 1982 and 1994, a larger difference than in the Florida Keys coral. In 1802 and 1885, the ratio of soluble to insoluble OC was  $\sim 4.5$  and  $\sim 9.0$  in the total and intracrystalline pools, respectively (Table 1). The C/N ratio of acid-insoluble total organic matter was 4.3–6.5 from 1982–1995 and was  $\sim 5.5$  in 1802 and 1885 (Table 1; Fig. 4). The C/N ratio of acid-insoluble organic matter in the intracrystalline pool was 4.6–8.0 in 1982–1995 and  $\sim 4.0$  in 1802 and 1885 (Table 1).

The surface polyp layer of the *Porites* core was analyzed for TOC. This layer includes the living tissue of the coral at the time the core was collected. The polyp layer contained 1.7 mmol C/g coral, or 30–70 times more TOC than in bands immediately beneath the polyp layer (Table 1). Intracrystalline TOC was 34  $\mu\text{mol C/g}$ , similar to the intracrystalline organic carbon concentration in other bands. In contrast to the underlying skeletal material, polyp tissue contained two times more insoluble than soluble TOC. This insoluble organic matter also had a higher C/N (9.4) than did any other annual bands in the Red Sea coral ( $\sim 4$ –7). The intracrystalline acid-insoluble organic matter in the polyp layer had a lower C/N of 8.3.

Table 2. Comparison of total and intracrystalline amino acids in samples of different ages from the Florida Keys and the Red Sea.

Location	Age	Total THAA $\mu\text{mol C/g coral}$	Intracrystalline THAA/Total THAA
Florida	1939–1982	10–30	0.44 (average)
Florida	1704, 1880	19, 23	0.36, 0.38
Red Sea	1995 (Polyp)	543	0.01
Red Sea	1994–1982	13–25	0.33 (average)
Red Sea	1802, 1885	13	0.7

### 4.3. Amino Acids

#### 4.3.1. Florida Keys (*Montastraea annularis*)

Total hydrolyzable amino acid (THAA) carbon in unbleached samples ranged from 10–30  $\mu\text{mol THAA C/g}$ , or 29–86 mg amino acid/100g of coral (Table 2). For comparison, total THAA concentrations of 79–120 mg/100g coral were reported for a Caribbean sample of *Montastraea faveolata* with 0–300 yr old bands (Nyberg et al., 2001). While we saw a slight decrease in total THAA with age between 1982 and 1940 ( $p = 0.01$ ,  $n = 33$ ), older annual bands (1704 and 1880) had total THAA values within the range of the younger bands (19.0 and 23.0  $\mu\text{mol THAA C/g coral}$ , respectively). Between 1940 and 1982, intracrystalline THAA concentrations were between 6.9 and 11  $\mu\text{mol C/g coral}$ , except for 1957, which contained 2.8  $\mu\text{mol THAA C/g coral}$  (Fig. 5). In 1704 and 1880, intracrystalline THAA concentrations were 6.5 and 8.5  $\mu\text{mol THAA C/g coral}$ , respectively. Thus, on average, intracrystalline THAA was about 40% of the total THAA in both young and old annual bands (Table 2).

#### 4.3.2. Red Sea (*Porites lutea*)

Total THAA concentrations ranged from 13–25  $\mu\text{mol THAA C/g coral}$  in unbleached Red Sea *Porites* (Fig. 6) and decreased slightly with age between 1994 and 1982 ( $p = 0.01$ ,  $n = 13$ ). These values are slightly higher than other reported values of 6.7  $\mu\text{mol THAA C/g coral}$  in *Porites lichen* (Abelson, 1955) and 1.5–3.0  $\mu\text{mol THAA C/g coral}$  of various species of

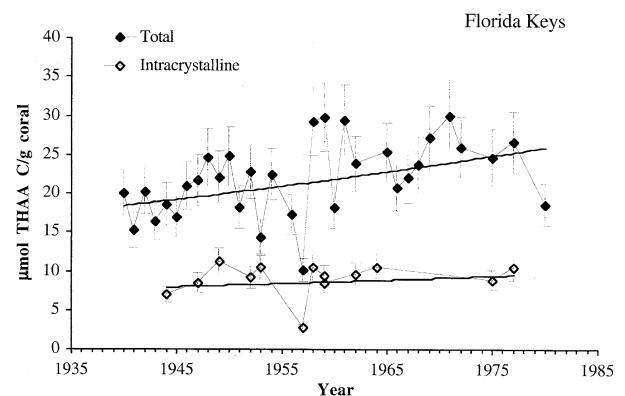


Fig. 5. Total hydrolyzable amino acid carbon in untreated (Total;  $r^2 = 0.19$ ,  $n = 33$ ,  $p = 0.01$ ) and bleached (Intracrystalline;  $r^2 = 0.03$ ,  $n = 13$ ,  $p = 0.6$ ) *Montastraea annularis* from Florida Keys (1939–1982).

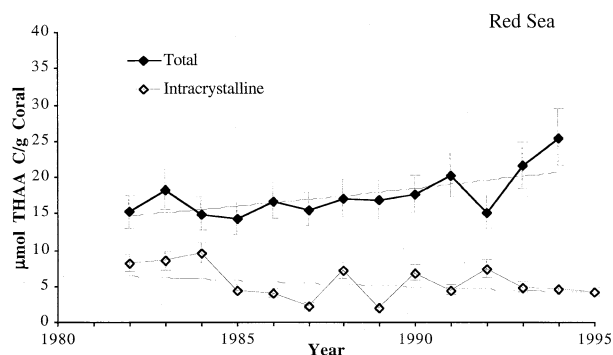


Fig. 6. Total hydrolyzable amino acid carbon in untreated (Total;  $r^2 = 0.45$ ,  $n = 13$ ,  $p = 0.01$ ) and bleached (Intracrystalline;  $r^2 = 0.17$ ,  $n = 14$ ,  $p = 0.14$ ) *Porites lutea* from Red Sea (1982–1995).

*Porites* (after cleaning with NaOH) (Mitterer, 1978). Older annual Red Sea coral bands, 1802 and 1885, contained 13  $\mu\text{mol}$  THAA C/g, not significantly different from the low end of the range reported for younger annual bands (Table 2). Intracrystalline THAA concentrations were 2.0–9.4  $\mu\text{mol}$  THAA C/g, an average of 33% of total THAA. There was no significant difference in the intracrystalline THAA concentration between young and old samples. In older bands (1802 and 1885), intracrystalline THAA concentrations were both 8.9  $\mu\text{mol}$  THAA C/g, about 70% of total THAA (Table 2).

Amino acids were analyzed in the polyp layer. Total THAA concentration was 543  $\mu\text{mol}$  C/g coral, 20–40 times higher than in other annual bands (Table 2). Only 1% of THAA carbon was intracrystalline in the polyp layer.

#### 4.3.3. Amino acid compositions

The amino acid compositions of all bands of the two corals (Fig. 2b and 7) were similar to previously published values for total THAA in coral, with high relative amounts of aspartic acid, glutamic acid, glycine and alanine (Wehmiller et al., 1976; Mitterer, 1978; Goodfriend et al., 1992). There was little or no histidine or methionine. The near absence of  $\beta$ -alanine and  $\gamma$ -aminobutyric acid indicates little or no enzymatic decarboxylation of aspartic and glutamic acid (Lee and Cronin, 1982). Very small quantities of these non-protein amino acids were occasionally seen in bleached samples (<0.3 mole %), possibly an artifact of the bleaching procedure. Chemical degradation of aspartic acid might produce fumaric acid, the deamination product of aspartic acid (Bada and Miller, 1968). However, we did not analyze for this compound. On average, in the Florida Keys coral, mole % aspartic acid, glutamic acid, arginine, alanine and tyrosine were lower, and serine, glycine, threonine, methionine, valine, phenylalanine, isoleucine and leucine higher in intracrystalline THAA than in total THAA (Fig. 7a). In the Red Sea coral, mole % aspartic acid and alanine were higher in intracrystalline THAA than in total THAA, and threonine, arginine, tyrosine, methionine, valine, phenylalanine, isoleucine and leucine were lower (Fig. 7b).

The amino acid composition of the coral polyp layer was different than that of adjacent bands (Fig. 7b), with aspartic acid (12 mole %) relatively depleted, and serine (13 mole %) enriched, in the polyp layer relative to older bands. The amino

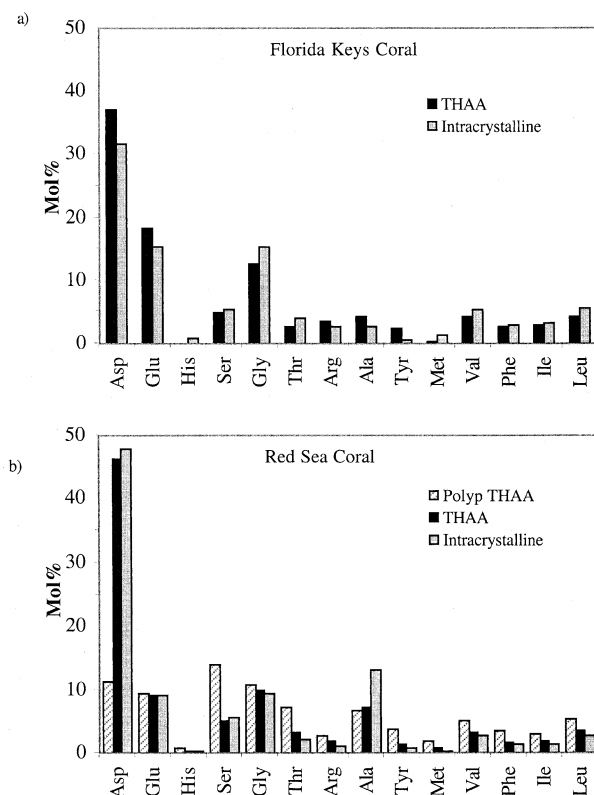


Fig. 7. a) Average composition (mole %) of amino acids in total THAA and intracrystalline amino acid in a) Red Sea polyp layer and bands from 1994–1982; b) Florida Keys coral from 1982–1940.

acid composition of the 1994 annual band just beneath the polyp layer was also depleted in aspartic acid and enriched in serine, although less so than in the polyp (data not shown). This indicates that a small amount of tissue was still present in this band. After bleach treatment, the amino acid composition of the polyp layer was similar to other bands (see Fig. 7b). This similarity in composition and concentration between the bleached polyp layer and other annual bands suggests that bleach was effective at removing polyp tissue amino acids.

#### 4.4. Lipids

Two extraction methods were compared to determine the most effective way to isolate intracrystalline lipids with maximum recovery (Fig. 1c). As a basis for comparison, the  $\text{CaCO}_3$  in an untreated sample of coral was dissolved, and total lipids extracted with solvent. In the first extraction method, pre-extraction of a sample with solvent removed non-intracrystalline lipids, as operationally defined here. When the preextracted coral was dissolved in acid and the resulting solution re-extracted to remove intracrystalline lipids, the total lipid concentration obtained (non-intracrystalline + intracrystalline) equaled that of the first untreated sample. In the second extraction method, samples were bleached to remove non-intracrystalline lipids. However, intracrystalline lipid concentrations in bleached corals were similar to total lipid concentrations after  $\text{CaCO}_3$  dissolution, suggesting that bleach did not remove

non-intracrystalline lipids. Therefore, only results where pre-extraction was used are presented here. Stern et al. (1999) have shown that saponification of powdered mollusk shells removes lipids that are not solvent extractable. Thus, some of the “intracrystalline” lipids we report here may be bound in intercrystalline spaces. Our operational definition of intracrystalline lipids is limited in this respect, and simply represents lipids that are not routinely extracted from CaCO<sub>3</sub>-rich samples using organic solvents.

Lipids were analyzed in the 1972 annual band of the Florida Keys coral (Fig. 8a,c). Total fatty acid concentration was 1.8  $\mu\text{mol carbon/g coral}$ , with the intracrystalline fraction accounting for  $\sim 30\%$  (0.52  $\mu\text{mol C/g coral}$ ) of the total. More than half of the non-intracrystalline carbon was in C18:0 fatty acid (0.76  $\mu\text{mol carbon/g coral}$ ). In the intracrystalline fraction, C18:0 had a slightly higher concentration than C16:0. Odd-chain-length fatty acids (iso 15:0, *anteiso* 15:0, iso 17:0 and 17:0) were found in small amounts in both the intracrystalline and non-intracrystalline pools (although less in the intracrystalline pool).

Non-intracrystalline neutral lipids were 10 times more abundant than intracrystalline neutral lipids in the Florida Keys coral (0.26 and 0.026  $\mu\text{mol carbon/g coral}$ , respectively). The sterol 24-ethylcholest-5-en-3 $\beta$ -ol (sitosterol) and C18:0 alcohol accounted for most of the neutral lipid carbon (Fig. 8c). Small quantities of phytol in the non-intracrystalline fraction suggest the presence of Chl-a in the coral at some point in its history, since one of the major sources of phytol is from the esterified side chain of Chl-a.

Lipids were also analyzed in the 1843 annual band of the Red Sea coral. The total fatty acid concentration was 0.36  $\mu\text{mol carbon/g coral}$ , with the intracrystalline fraction accounting for 47% (0.17  $\mu\text{mol carbon/g coral}$ ) of the total. C16:0 and C18:0 were present in approximately equal quantities in both the intracrystalline and non-intracrystalline fractions (Fig. 8b). Odd-chain fatty acids were mainly found in the non-intracrystalline pool.

Neutral lipid concentrations in the 1843 annual band of the Red Sea coral were very low (0.022  $\mu\text{mol carbon/g coral}$ ) with non-intracrystalline neutral lipids (0.013  $\mu\text{mol carbon/g coral}$ ) approximately equal to intracrystalline neutral lipids (0.0089  $\mu\text{mol carbon/g coral}$ ). C18-alcohol, cholest-5-en-3 $\beta$ -ol, and 24-methylcholesta-5,24(28)-dien-3 $\beta$ -ol were the most abundant neutral lipids (Fig. 8d). C16-alcohol, phytol, and 24-ethylcholest-5-en-3 $\beta$ -ol were not detectable in the Red Sea coral.

#### 4.5. Chloropigments

Chloropigment analyses of the non-intracrystalline pool of younger Florida Keys coral with visible green bands indicated that these bands contained Chl-a, Chl-b (Fig. 9), occasional Chl-a degradation products, and unidentified chloropigments. In some years Chl-a was dominant, while Chl-b dominated other years after 1959. Phaeophytin was present in the two growth bands with the highest concentration of Chl-a. Unidentified chloropigment peaks (data not shown) may include siphonein or siphonaxanthin, common chloropigments found in Siphonales algae (Jeffrey, 1968; Schlichter et al., 1997). No authenticated standards were available for these compounds.

Chloropigments were analyzed in one visible green band of the *Porites lutea* from the Red Sea. A very small but detectable amount of Chl-a was present in this annual band (1802). Green algal bands were also present in some younger annual bands of the Red Sea coral (1993, 1992, 1991, 1990, 1988, 1983), but the chloropigments were not analyzed as the sample size was too small. Although Chl-a concentrations from endolithic algae have been reported previously in coral skeletons (Risk et al., 1987; LeCampion-Alsumard et al., 1995; Schlichter et al., 1997), the values reported are in units (e.g., moles Chl-a/number of algal filaments) that are not directly comparable with those reported here.

#### 4.6. Mass Balance of Total Organic Carbon

Although the major purpose of this study was to look at organic carbon preservation, we can provide an estimate of how much skeletal organic matter is present in the major biochemical classes analyzed. Amino acids are the most abundant component of organic matter in the coral skeletons analyzed (Table 3). Total THAA was  $\sim 30\text{--}40\%$  of TOC, and approximately one half of total THAA was intracrystalline in both young and old Florida Keys coral bands. In all Red Sea bands, total THAA was  $\sim 45\%$  of TOC. In young bands, one third of total THAA was intracrystalline, while in older bands, 70% was intracrystalline.

In the 1972 band of the Florida Keys coral, total lipids were 3.5% of the TOC, and intracrystalline lipids were 0.91% of the TOC (Table 3). In the 1843 annual band of the Red Sea coral, total lipids were 1.3% of the TOC and intracrystalline lipids were 0.61% of TOC. The smaller proportion of lipids in the older Red Sea coral may be due to loss of lipids via diagenesis, or to differences in the amount of lipid initially present in the coral skeleton at the time of mineral precipitation. Chloropigments did not account for a significant fraction of total carbon.

Although carbohydrates were not measured here, they are a universal component of skeletal organic matrices (Addadi et al., 1987). For example, in the bivalve *Mercenaria mercenaria*, carbohydrates account for 20% of the soluble organic matrix (Crenshaw, 1972). In general, Scleractinian corals have a higher proportion of carbohydrate than do molluscs (Dauphin, 2001), suggesting that they could make up an even greater proportion of the soluble organic carbon in the samples analyzed here. Humic and fulvic acids as well as DOC are also incorporated into coral skeletons from ambient seawater (Isdale, 1984). The amount of carbon in these pools has not been quantified.

### 5. DISCUSSION

Occlusion of organic matter in biominerals may be an important pathway for the preservation of labile organic compounds in biomineral-rich environments (Carter and Mitterer, 1978; Collins et al., 1991; Ingalls et al., 2003) and may be an important source of organic matter for paleoceanographic studies. While much is known about the composition of organic matter in corals, studies of organic matter diagnosis in corals have primarily focused on the use of D/L amino acid ratios as a tool for dating coral annual bands (Goodfriend et al., 1992). Since bacteria are present (Nyberg et al., 2001) and metaboli-

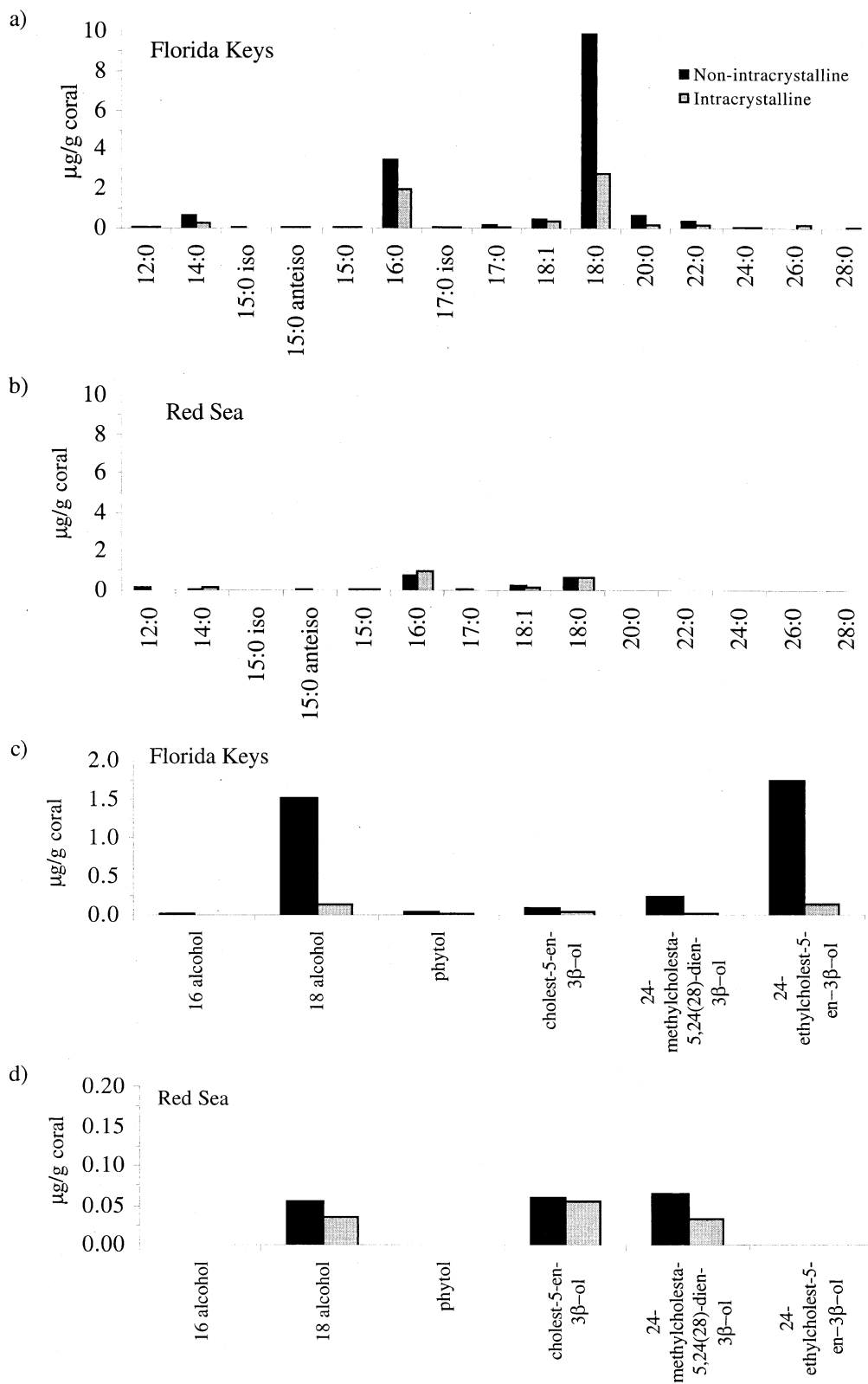


Fig. 8. Fatty acid composition of a) Florida Keys (1972) and b) Red Sea coral (1843). Neutral lipid composition of c) Florida Keys and d) Red Sea coral. Black bars are non-intracrystalline lipids and gray bars are intracrystalline lipids.

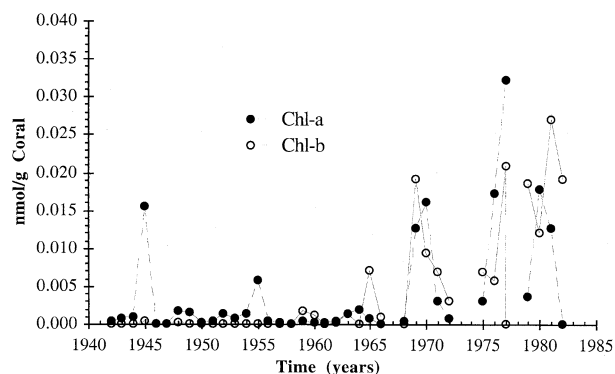


Fig. 9. Chlorophyll-a and b concentrations in coral from the Florida Keys. Peaks correspond to visible green algal bands.

cally active in coral skeletons (DiSalvo, 1969; Risk et al., 1987), some fraction of skeletal organic matter in coral skeletons is undergoing transformations and is available for bacterial consumption. In this study, we characterize the diagenetic status of organic matter in operationally defined intracrystalline and non-intracrystalline organic matter pools defined here. In this way, we relate the extent of organic matter preservation to the degree of organic matter association with the coral skeleton.

## 5.1. General Evidence for Preservation

### 5.1.1. Organic carbon and C/N

A decrease in TOC content with time in coral skeletons can indicate either organic matter remineralization or a decrease in carbon input over time. There was a significant decrease with age in the concentration of acid-insoluble OC among younger annual bands of both corals (1982–1939 in Florida Keys, Fig. 3 and 1994–1982 in Red Sea coral, Fig. 4). The concentration of acid-soluble OC remained relatively constant over these same time intervals in both corals (Figs. 3 and 4). Since  $\text{CaCO}_3$  crystal nucleation is thought to occur on the acid-soluble organic matrix (Levi et al., 1998), and these components resist chemical oxidation in intact carbonate biominerals, it is likely that this organic matter is well protected from degradation. Acid-insoluble organic matter, which alone does not initiate mineral precipitation (Addadi et al., 1991; Cuif et al., 1991; Dauphin, 2001), may more likely derive from sources that are not as tightly bound within the mineral. The apparent difference in reactivity of soluble and insoluble components of skeletal organic matter resulted in older bands containing a higher

proportion of intracrystalline organic carbon than younger bands (Table 1). The similarity in TOC concentrations in younger (<45y) and older bands (100–300 yr), suggests that the rate of degradation of organic matter must slow as coral skeletons age (Table 1), perhaps as increasingly more organic matter is protected by the mineral matrix.

In the marine environment, nitrogen-rich compounds such as proteins are thought to degrade preferentially, resulting in an increase in the C/N ratio of organic matter with age. For example, the C/N ratio of organic matter in fossilized mollusk shells is greater than in modern shells (Hudson, 1967). In the Florida Keys coral examined here, there was a slight increase in the C/N ratio of insoluble organic matter ( $p = 0.03$ ,  $n = 36$ ) with age between 1982 and 1939 in unbleached skeletons (Fig. 3). However, C/N ratios in older bands (1704 and 1880) were not significantly different from those in younger bands (Table 1). Likewise, there was no significant increase with age in the C/N ratio of insoluble organic matter of the Red Sea coral ( $p = 0.9$ ,  $n = 15$ ) or between 1802 and 1994 (Table 1; Fig. 4). These results suggest that while nitrogen-rich compounds may be lost from young annual bands (<45yr), this process may not be as important in bands older than 50 yr. The higher C/N ratio of 9.4 in the coral polyp layer (1995) indicates that the polyp has proportionally less protein than skeletal organic matter. This is not surprising since up to 12% of the dry weight of coral polyp tissues can be lipids, which are generally depleted in nitrogen (Harland et al., 1992).

Diagenetic dissolution and re-precipitation of  $\text{CaCO}_3$  in the skeleton may also be responsible for changes in the amount of organic carbon/g of coral skeleton. Dissolution could result in the release of organic carbon, while authigenic re-precipitation of previously dissolved  $\text{CaCO}_3$  might result in a small amount of organic matter being trapped in minerals, and thus preserved. Gautret (2000) suggested that the organic matrix in a coral skeleton serves to inhibit authigenic re-precipitation of  $\text{CaCO}_3$ . In addition, Enmar et al. (2000) found that less than 2% of coral pore space could be filled by re-precipitated  $\text{CaCO}_3$  within 100 yr. Thus, on the 40-yr time-scale of the younger corals analyzed here, only a small percent (<2%) of the skeleton is likely to be transformed and is therefore not a likely cause of changes observed in TOC/g coral. Variations over time in the relative concentrations of organic and inorganic carbon deposited during growth could also be responsible for changing TOC concentrations between annual bands. This possibility would best be addressed by studying corals in culture where growth conditions are well constrained. In general, our results suggest that intracrystalline organic matter in coral is well preserved.

Table 3. Percent of total and intracrystalline amino acids and lipids in the total organic carbon pool. Lipid values are for one annual band.

Location	Age	% Total THAA	% Intracrystalline THAA	% Total Lipid	% Intracrystalline Lipid
Florida	1939–1982	30–40	15 (average)	3.5 <sup>a</sup>	0.91 <sup>a</sup>
Florida	1704, 1880	35	13	nd	nd
Red Sea	1994–1982	40–50	15 (average)	nd	nd
Red Sea	1802, 1843	45	31	1.3 <sup>b</sup>	0.61 <sup>b</sup>

nd: No lipid data were collected for these samples.

<sup>a</sup> analysis of 1843 annual band.

<sup>b</sup> analysis of 1972 annual band.

### 5.1.2. Total hydrolyzable amino acids (THAA)

Amino acids are known to be stable over hundreds of years in intact coral skeletons (Wehmiller et al., 1976; Goodfriend et al., 1992; Nyberg et al., 2001). However, the extent of organic matter mineral association was not addressed in these studies. Processes such as chemical condensation (Collins et al., 1992), hydrolysis (Walton, 1998) and racemization (Goodfriend et al., 1992; Endo et al., 1995) alter the nature of amino acids in carbonate matrices over thousands to millions of years (Wehmiller et al., 1976).

In our study, linear regressions of amino acid data in Figure 5 and 6 suggest that there was a small decrease in total THAA of both the Florida Keys coral and the Red Sea coral. However, there was no significant loss of intracrystalline THAA in either coral over short or long time-scales (Fig. 5, 6; Table 2), suggesting that this material is better preserved. The higher intracrystalline/total THAA ratio in older Red Sea coral (Table 2) likely reflects a combination of the loss of non-intracrystalline amino acids with time and differences in the proportion of intracrystalline amino acids laid down during mineral precipitation in the past. A detailed discussion of the THAA composition appears below.

### 5.1.3. Lipids

Because lipids were analyzed in only one sample from each coral core in this study, trends over time cannot be assessed. In addition, heterotrophic feeding and algal photosynthesis influence fatty acid compositions in coral tissue, so that compositions can be variable (e.g., Harland et al., 1992). Previous reports of fatty acids in corals indicate that there is usually more C16:0 than C18:0 in coral tissue and skeletons (Meyers et al., 1974; Isa and Okazaki, 1987; Harland et al., 1993). These two compounds are also the major fatty acids in the corals we analyzed, although C18:0 was present in higher concentration than C16:0. Odd-chain-length fatty acids found in the intracrystalline and non-intracrystalline pools are typically associated with the presence of bacteria (Perry et al., 1979; Kaneda, 1991; Wakeham et al., 1997b). As mentioned earlier, bacteria can actively metabolize organic matter in coral skeletons. A bacterial source of these lipids would also agree with the finding by Nyberg et al. (2001) that some D-amino acids in coral originate from bacteria. However, previous studies of a zooxanthellae-bearing symbiotic anemone suggests that odd-chain fatty acids are also present in zooxanthellae (Harland et al., 1991) and in coral tissue (Harland et al., 1993), so their source in our samples is not clear. The presence of bacterially-derived lipids in coral skeletons could also be an indication that the solvent extraction used did not remove all non-intracrystalline lipids, as mentioned earlier.

### 5.1.4. Chloropigments

Green algal bands containing chlorophyll-a (Chl-a) are commonly observed in coral skeletons (Duerden, 1902; Golubic, 1969; Lukas, 1969; LeCampion-Alsumard et al., 1995). Since Chl-a is lost quickly from most environments (within months), its presence in old corals illustrates the preservation potential of coral skeletons. In anoxic sediments, where organic matter preservation can be extensive, Chl-a degrades to phaeophytin,

which can then remain undegraded for decades (Sun et al., 1993b). The 1945, 1965 and several bands between 1970 and 1982 of the Florida Keys coral contain Chl-a (Fig. 9), but very little or no phaeophytin. While it is not known what the original concentration of Chl-a was in these annual bands, it is clear that at least some Chl-a has been protected from degradation and that the concentration is variable. Even the 1802 Red Sea coral band still contained Chl-a. Some protection may be afforded by the ability of endolithic algae to bore into the skeleton and deposit Chl-a in the restricted space of the skeletal matrix. In anoxic sediment incubation experiments where solutes are not allowed to diffuse across the sediment water interface, Chl-a preservation is also extensive (Sun et al., 1993a, 1993b; Ingalls, 2002). Restricted diffusion and anoxia within coral heads (Sanzone et al., 1990) are likely to contribute to the preservation of non-intracrystalline organic matter. Precipitation of CaCO<sub>3</sub> onto algal filaments (Schroeder, 1972; Kobluk and Risk, 1977) and intact algal cell walls (Duerden, 1902; Lukas, 1969) could also protect chloropigments from degradation.

## 5.2. Quantitative Evidence for Preservation of Organic Matter in Coral Skeletons

### 5.2.1. Organic matter reactivity

Several approaches to quantifying the extent of organic matter diagenesis have recently been applied to marine systems (Middelburg, 1989; Wakeham et al., 1997b) and might be useful in assessing diagenesis in corals. For example, Middelburg (1989) developed a relationship between decomposition rate constants ( $k$ ) of sedimentary organic matter (calculated from sediment core profiles and laboratory incubation experiments) and the apparent age ( $t$ ) of organic matter such that  $\log k = -0.95 \log t - 0.81$ . If we use the slope of the log linear regression of organic carbon vs. age to represent a first-order decomposition rate constant for organic matter, we can apply the Middelburg equation to corals. In the Florida Keys coral, between 1982 and 1940, the degradation rate constant calculated for insoluble organic carbon ( $k = 0.02 \pm 0.01 \text{ yr}^{-1}$ ;  $r^2 = 0.41$ ) is ten times greater than for the soluble fraction ( $k = 0.002 \pm 0.003 \text{ yr}^{-1}$ ;  $r^2 = 0.04$ ). If these rate constants are used in the Middelburg equation, they yield apparent ages of 440 (insoluble) and 4700 (soluble) years. Since we know the coral bands are <45 yr old, these calculations indicate that coral organic matter is much less reactive than sedimentary organic matter of the same age. Thus, these data demonstrate that "freshness" of organic matter depends on the matrix in which it resides, and not necessarily on its intrinsic lability.

The apparent age calculation also suggests that the soluble component is afforded a greater degree of protection by the coral skeleton than is the insoluble fraction. In modern carbonate shells most organic matter is acid-insoluble, while in ancient samples, acid-soluble organic matter predominates (Weiner and Lowenstam, 1980). An increase in the importance of soluble organic matter with age, which was also observed here (Table 1), could be due to either hydrolysis of large insoluble proteins into smaller soluble peptides and amino acids, or, selective preservation of the soluble skeletal matrix components. Weiner and Lowenstam (1980) concluded that selective preservation of soluble components is more likely

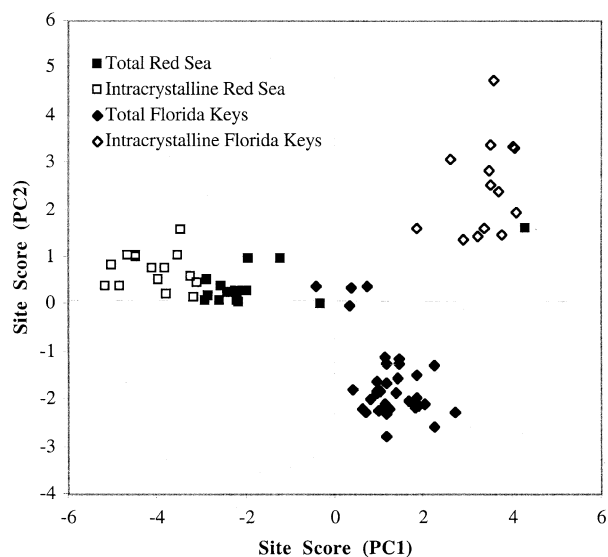


Fig. 10. Principal components analysis site scores for PC1 and PC2 for total and intracrystalline THAA samples analyzed.

because soluble proteins rich in acidic amino acids are thought to have a closer association with the mineral than insoluble components.

### 5.2.2. Amino acid degradation state

Amino acid compositions can vary with organic matter source and degradation state. To quantitatively assess differences in amino acid composition among samples, we carried out a principal components analysis (PCA) on a data set that included the mole% of each amino acid in both total and intracrystalline amino acid pools in all coral bands analyzed. Mole % values were standardized before carrying out the PCA by subtracting the mean and dividing by the standard deviation of all samples in the data set. The axis of the first principal component (PC1) explained 46.7% of the variance; the axis of the second principal component (PC2) explained 18.5% of the variance. In PCA, the “site score” for each sample indicates the distance of each data point from the axis of the first principal component. In previous studies, the site scores for the first principal component (PC1) have been related to the degradation state of organic matter (Dauwe et al., 1999). This interpretation of PC1 was possible because the authors chose a sample set with a broad range of degradation states, thus ensuring that degradation was the primary cause of variability in amino acid composition. While interpreting PC1 as a measure of degradation appears to be widely applicable to many environmental samples, the unusual composition and stability of coral skeletal amino acids suggests that PC1 may be influenced primarily by variations in the source of organic matter. We have found this to be true in other samples with unusual source material (Ingalls et al., 2003).

A comparison of PCA site scores shows that the amino acid source can be distinguished along the first principal component (Fig. 10). Plotting PC1 against PC2 shows that the samples within a given type cluster in distinct groups based on their

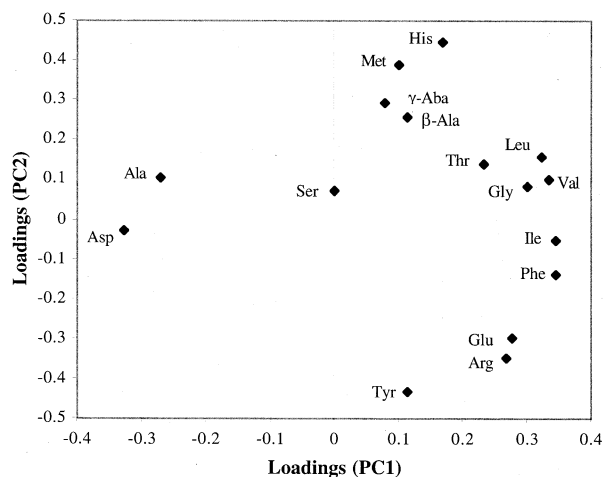


Fig. 11. PCA loadings for PC1 and PC2 of sample variables (amino acid mole%).

composition. Both species and matrix (intracrystalline) can be clearly distinguished. Aspartic acid and alanine had negative loadings for PC1 (Fig. 11), suggesting that these amino acids are enriched in samples with negative PC1 site scores. In the Florida Keys coral, intracrystalline amino acids are depleted in aspartic acid and alanine on average relative to THAA (Fig. 7a) and also have higher site scores (Fig. 12a). The opposite is true for the Red Sea coral (Fig. 7b, 12b).

Using PCA of amino acids to assess diagenesis may be more difficult in coral skeletons than in environments where diagenesis is more rapid. Qualitative changes in the composition of amino acids over time that are common in other environments are not found in coral skeletons. Assembling coral samples with known degradation states over a much longer timescale than investigated here might allow diagenetic trends to be assessed. Source appears to be a larger cause of variance in composition among samples analyzed here. For example, the intracrystalline amino acid composition is most likely genetically determined, while the total THAA pool may have additional non-indigenous sources, and may include THAA from DOC of surrounding waters or from endolithic organisms. As mentioned earlier, Nyberg et al. (2001) suggest that bacterial peptidoglycan may be a significant source of D-amino acids in coral skeletons.

Bacterial peptidoglycan is also rich in aspartic acid, glutamic acid, alanine and glycine, the major amino acids in coral skeletons. The contribution of these amino acids in marine particles and sediments usually increases with degradation, except for glutamic acid, which decreases with degradation (Lee and Cronin, 1984; Müller et al., 1986; Burdige and Martens, 1988; Lee et al., 2000). In the Florida Keys coral, mole % aspartic acid and glutamic acid in THAA increase with age, while mole % glycine and alanine decrease with age (Fig. 13). These trends are different from those found in other environments, and protection of aspartic acid and glutamic acid by the coral skeleton is the most likely explanation for this difference. Aspartic and glutamic acid degradation products ( $\beta$ -alanine and  $\gamma$ -aminobutyric acid, respectively) usually increase with age of organic matter (e.g., Lee and Cronin, 1982). These non-protein amino acids were only rarely present in coral samples, and only in trace concentrations, suggesting that enzymatic degradation

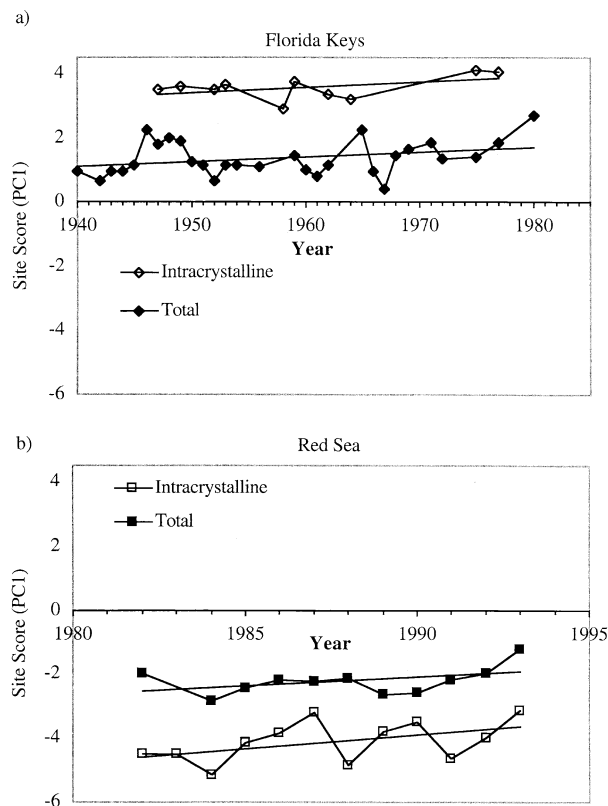


Fig. 12. PC1 site scores for Total and Intracrystalline THAA of a) Florida Keys coral (Total THAA  $r^2 = 0.11$ ,  $n = 29$ ,  $p = 0.08$ ; Intracrystalline THAA  $r^2 = 0.22$ ,  $n = 10$ ,  $p = 0.18$ ) and b) Red Sea coral (Total THAA  $r^2 = 0.2$ ,  $n = 11$ ,  $p = 0.17$ ; Intracrystalline THAA  $r^2 = 0.24$ ,  $n = 12$ ,  $p = 0.04$ ).

of these amino acids was minimal. In addition, mole% aspartic acid increased with age in the Florida Keys coral (Fig. 13). Principal components analysis suggests that there is no strong

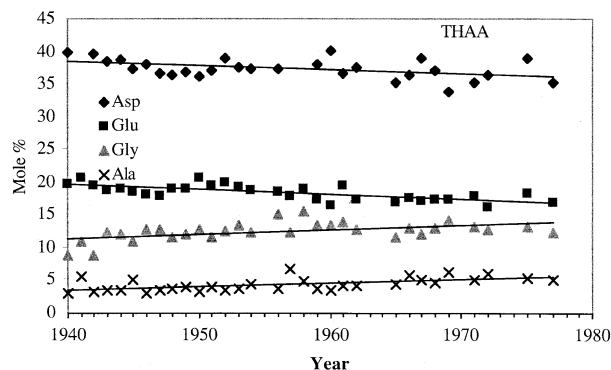


Fig. 13. Mole % of aspartic acid, glutamic acid, glycine and alanine in Total THAA of the Florida Keys coral from 1940 through 1977. Linear regressions are: aspartic acid,  $r^2 = 0.21$ ,  $n = 29$ ,  $p = 0.015$ ; glutamic acid  $r^2 = 0.49$ ,  $n = 31$ ,  $p = 0.00007$ ; glycine,  $r^2 = 0.26$ ,  $n = 31$ ,  $p = 0.005$ ; alanine,  $r^2 = 0.34$ ,  $n = 31$ ,  $p = 0.000001$ . Data from 1957 and 1958 was left out of the aspartic acid regression due to anomalous data in these samples. These bands contained winter stress bands (Druffel et al., 1989) that may have contributed to the unusual aspartic acid content of these bands.

overall trend in amino acid composition with time in either coral (Fig. 12).

### 5.2.3. Source effects on organic matter composition

As shown by the PCA of amino acids, the source of organic matter in corals appear to cause a greater variation in amino acid composition among different samples and sample pools than does degradation. However, sources of organic matter can also affect reactivity. Coral skeletons include non-indigenous sources of soluble and insoluble organic matter, such as dissolved or particulate organic carbon from surrounding waters (Isdale, 1984; Boto and Isdale, 1985; Susic et al., 1991). A portion of DOC may be less reactive than organic matter synthesized by the coral (Isdale, 1984). If this less reactive organic matter is a significant portion of coral TOC, a slower apparent degradation rate would be observed. Since most of this material is probably non-intracrystalline, this could, in addition to mineral associations, cause the observed slow degradation of non-intracrystalline organic matter.

In younger annual bands, the fraction of amino acids in the intracrystalline pool of *Montastraea annularis* ( $44 \pm 6\%$ ) was relatively similar to that in *Porites lutea* ( $33 \pm 5\%$ ). In older bands, however, the proportion of THAA in the intracrystalline fraction was much higher in *P. lutea* ( $70 \pm 6\%$ ) than in *M. annularis* ( $37 \pm 6\%$ ). One possible cause of this difference between the two species is skeletal structure, including the coral morphology at the time of skeletal deposition as well as changes after deposition. For example, while skeletal densities of both species range between 1 and 2 g/cm<sup>3</sup> (Dodge and Brass, 1984; Barnes and Devereux, 1988), their surface area and porosity probably differ. *P. lutea* has very small polyps (1.0–1.5 mm diameter) and septa that are perforated with small holes (Wood, 1983). This results in a porous skeletal structure, hence the name *Porites*. Polyps of *M. annularis* are larger (3 mm in diameter), and the septa are not perforate. These differences very likely influence how fast solutes can be transported through the skeleton and therefore the dissolution rate of the skeleton and degradation rate of organic matter. Also, skeletons with higher surface areas would tend to contain a larger fraction of non-intracrystalline organic matter relative to intracrystalline organic matter. Higher porosity and surface area in *Porites* would account for its higher proportion of intracrystalline amino acids in older samples.

Although the interannual variability of PCA site scores (Fig. 12) is small, differences suggest that sources of organic matter to the coral skeleton may vary over time. Alternatively, the composition of the organic matter laid down by the coral may change over time, possibly in response to environmental factors. Also, degradation may occur in some annual bands due to endolithic activity or variability in skeletal morphology over time. At no time do the variations with time obscure the basic differences in composition.

### 5.3. Paleoenvironmental Indicators

Numerous studies have used trace constituents of coral skeletons to infer past environmental conditions. Several of these

studies were based on the use of organic constituents of coral skeletons: e.g., extent of D/L amino acid racemization as a proxy for coral age on hundred year timescales (Goodfriend et al., 1992), and fluorescent organic constituents as a proxy for terrestrial runoff (Isdale, 1984). However, attempts to correlate green Chl-a bands, deposited by *Ostreobium*, with environmental factors have been wrought with problems due to seasonal variations in skeletal density (e.g., Macintyre and Smith, 1974; Highsmith, 1979; Bak and Laane, 1987; Risk et al., 1987; Pätzold, 1988). Clearly, we must learn more about the factors that control organic matter inclusion in coral skeletons to fully realize their potential. Still, because of the favorable conditions for organic carbon preservation in coral skeletons, organic constituents of corals appear well preserved and therefore may be useful as paleoenvironmental indicators. For example, the  $\delta^{13}\text{C}$  of polyp tissues from living coral appears to match that of skeletal organic matter ( $-16.7\%$  to  $-12.1\%$ ) in the most recent annual band (Land et al., 1975), suggesting that the skeletal organic material may be a record of coral tissue  $\delta^{13}\text{C}$  over time. Coral skeleton  $\delta^{15}\text{N}$  values may reflect changes over time in light availability (Heikoop et al., 1998) and possibly other environmental factors such as the coral's nutrient status and food source. Investigations of the isotopic composition of bulk organic matter and individual compounds from coral skeletons are likely to yield insights into past environmental changes. Also, a greater understanding of the uncharacterized organic matter may lead to new insights about dissolved and particulate organic matter incorporated from surrounding waters.

The use of coral skeleton organic compounds as paleoindicators will require better records of environmental parameters than are available for the cores we analyzed. However, we did have temperature records for the Florida Keys site and attempted to correlate THAA concentrations with temperature. For years between 1939 and 1982, winter sea surface temperatures (SST) lower than  $23.3^\circ\text{C}$  were present during 1951, 1957–58, 1960, 1964, 1970, 1977, and 1981 (<http://ferret.wrc.noaa.gov/las/>, COADS data). Of those years, we analyzed annual bands for 1951, 1957–58, 1960 and 1977 in our study. Except for 1970, we found total and intracrystalline THAA concentrations to be lower for those years than during years with higher winter SST. The 1957–58 winter low in SST is reflected by a winter stress band that is visible in the X-ray positive (Druffel and Linick, 1978). This band also has exceptionally low total and intracrystalline THAA concentrations. For the Red Sea coral, SST and  $\delta^{18}\text{O}$  records are available, and we attempted to correlate these records with our data. Green algal bands occurred in 1983, 1988, and 1990 through 1993. The years, 1983, 1989, 1992 and 1993 had winter low temperatures  $<25^\circ\text{C}$ , and 1988 had summer temperatures  $>30^\circ\text{C}$  (Eshel et al., 2000). Both of these temperature extremes would stress growing corals. In addition, several of these annual bands (1983, 1988, 1990 and 1992) contained elevated intracrystalline THAA as well as soluble OM (1983, 1988, 1992 and 1993). These preliminary data suggest that the organic matter content of coral skeletons is sensitive to environmental conditions and is therefore worth pursuing as a potential paleoenvironmental indicator.

## 6. CONCLUSIONS

This study describes the composition and concentration of a large fraction of the organic matter in coral skeletons of various ages. Unlike previous studies of organic matter in coral skeletons, we distinguished between the intracrystalline and non-intracrystalline pools of organic matter. We find that organic matter decomposition occurs more rapidly in the non-intracrystalline pool than in the intracrystalline pool, although decomposition in both pools is slow. This is consistent with current theories of the role of mineral association in protecting organic matter, although other factors such as skeleton porosity to interstitial fluids and oxygen may also be important. Intracrystalline organic matter within coral skeletons appears to be very well preserved, and this material could be an important addition to the current suite of coral-based tools presently used in paleoceanographic studies. In particular, examination of the concentrations of organic carbon and individual organic compounds in coral skeletons as well as the isotopic composition of this organic matter merit further development as paleoceanographic indicators.

Compositional changes that occurred over time were not typical of patterns seen during degradation in other environments. Principal components analysis of coral amino acid composition indicates that the source of amino acids is more important than degradation in explaining differences in composition.

*Acknowledgments*—We thank S. Griffin for advice in preparing Florida Keys coral samples, D. Schrag and M. Moore for supplying Red Sea coral samples, D. Hirschberg for TOC analyses and S. Wakeham for assistance with lipid analyses and the use of his lab. M. Collins, R. Aller, L. Mayer, K. Cochran and two anonymous reviewers helped improve the quality of this manuscript. Support for this research was provided by the NSF Chemical Oceanography Program. Acknowledgement is also made to the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. This is MSRC contribution number 1255.

*Associate editor:* D. Burdige

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