Lipid biomarkers in ooids from different locations and ages: evidence for a common bacterial flora


1Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA
2Department of Geosciences and Penn State Astrobiology Research Center, The Pennsylvania State University, University Park, PA, USA
3Department of Geosciences, Smith College, Northampton, MA, USA
4NOSAMS, Woods Hole Oceanographic Institution, Woods Hole, MA, USA
5Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA, USA

ABSTRACT

Ooids are one of the common constituents of ancient carbonate rocks, yet the role that microbial communities may or may not play in their formation remains unresolved. To search for evidence of microbial activity in modern and Holocene ooids, samples collected from intertidal waters, beaches and outcrops in the Bahamas and in Shark Bay in Western Australia were examined for their contents of lipid biomarkers. Modern samples from Cat and Andros islands in the Bahamas and from Carbla Beach in Hamelin Pool, Western Australia, showed abundant and notably similar distributions of hydrocarbons, fatty acids (FAs) and alcohols. A large fraction of these lipids were bound into the carbonate matrix and only released on acid dissolution, which suggests that these lipids were being incorporated continuously during ooid growth. The distributions of hydrocarbons, and their disparate carbon isotopic signatures, were consistent with mixed input from cyanobacteria together with small and variable amounts of vascular plant leaf wax (C_{27}-C_{35}; \delta^{13}C = -25 to -32‰, Vienna Pee Dee Belemnite (VPDB)). The FAs comprised a complex mixture of C_{12}-C_{18} normal and branched short-chain compounds with the predominant straight-chain components attributable to bacteria and/or cyanobacteria. Branched FA, especially 10-MeC_{16} and 10-MeC_{17}, together with the prevalence of elemental sulfur in the extracts, indicate an origin from sulfate-reducing bacteria. The iso- and anteiso-FA were quite variable in their \(^{13}\)C contents suggesting that they come from organisms with diverse physiologies. Hydrogen isotopic compositions provide further insight into this issue. FAs in each sample show disparate \(\delta^D\) values consistent with inputs from autotrophs and heterotrophs. The most enigmatic lipid assemblage is an homologous series of long-chain (C_{24}-C_{32}) FA with pronounced even carbon number preference. Typically, such long-chain FA are thought to come from land plant leaf wax, but in this case, their \(^{13}\)C-enriched isotopic signatures compared to co-occurring n-alkanes (e.g., Hamelin Pool TLE FA C_{24}-C_{32}; \delta^{13}C = -20 to -24.2‰, VPDB; TLE n-alkanes \delta^{13}C = -24.1 to -26.2‰, VPDB) indicate a microbial origin, possibly sulfate-reducing bacteria. Lastly, we identified homohopanoic acid and bishomohopanol as the primary degradation products of bacterial hopanoids. The distributions of lipids isolated from Holocene oolites from the Rice Bay Formation of Cat Island, Bahamas were very similar to the beach ooids described above and, in total, these modern and fossil biomarker data lead us to hypothesize that ooids are colonized by a defined microbial community and that these microbes possibly mediate calcification.

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Corresponding author: R. E. Summons. Tel.: 617 452 2791; fax: 617 253 8630; e-mail: rsummons@mit.edu

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INTRODUCTION

Ooids, small spherical to ellipsoidal coated grains characterized by concentric layers of calcium carbonate, are among the most prevalent components of carbonate sediments known from the geological record (e.g., Rodgers, 1954; Flügel, 1982; Richter, 1983) and are thought to form in agitated settings, such as shallow, carbonate environments like those in the Bahamas today. Differences concerning the definition of these grains (e.g., Peryt, 1983) led to one of the most fundamentally debated questions of their origin: Are ooids biogenically produced grains? Nonetheless, some consensus surrounds the classification of ooids, which is based largely on microfabric and mineralogy (Tucker & Wright, 1990), and they are distinguished from oncoids by the regular, even nature of laminae that surround the nucleus. Ooids are also generally 2 mm in size or smaller, although ‘giant’ ooids (pisoids) are reported from the Proterozoic, and rarely in the Phanerozoic (see Trower & Wright, 1990), and they are distinguished from oncoids by the regular, even nature of laminae that surround the nucleus. Ooids are also generally 2 mm in size or smaller, although ‘giant’ ooids (pisoids) are reported from the Proterozoic, and rarely in the Phanerozoic (see Trower & Wright, 1990) for a review). These descriptive characteristics of ooids do not require a common mode of formation, so these grains are generally thought to represent a polygenetic grouping that can form from biogenic and/or chemical precipitation, with the relative importance of each process being unclear. However, despite the long-recognized association of ooids and organic matter (e.g., Břehm et al., 2006; Davies et al., 1978; Folk & Lynch, 2001; Reitner et al., 1997; Płée et al., 2008), recent work has called into question the role of microbes in the accretion of ooid laminae (Duguid et al., 2010). Endolithic borings, common features of modern ooids, suggest a prominent effect from endolithic micro-organisms that can lead to micritization of ooid cortices and cement precipitation in these borings (Harris, 1977; Duguid et al., 2010); what remains debated is whether micro-organisms, locally and directly, foster the precipitation of carbonate layers around the cortex.

Despite the pervasive nature of ooids as grains in carbonate strata throughout geological time, they occur in only a handful of places in the modern ocean. Two of these regions were selected for analysis in this work: the Bahamas and Western Australia (Fig. 1). These two areas provide a suitable comparison of the microbial constituents of modern ooids from contrasting depositional environments.

Ooids are common components of carbonate sand in several areas of the Bahamian Archipelago, including the northwestern part of Cat Island, for example, (Kindler, 1992; Kindler & Hearty, 1996; Myroie et al., 2006; Glumac et al., 2011) where ooids comprise beach sand and make up much of the exposed Holocene eolianite, both of which were sampled here. Similarly, at Joulters Cays to the northwest of Cat Island (see Fig. 1), ooid dunes line tidal channels, which can be as large 200 m wide (Harris, 1979; Shinn et al., 1993).

Hamelin Pool in Western Australia (see Fig. 1) is a hypersaline basin that is well known for its microbial mats and stromatolites colonized by cyanobacteria, coccolid and filamentous algae, and diatoms (Burne & Moore, 1987). At Carbla beach, on the eastern shore of Hamelin Pool and in intimate association with the well-documented stromatolites, ooids are also abundant. Unlike most occurrences in the Bahamas, these particular ooids are forming in hypersaline waters thereby providing an important environmental variant for the present work.

Lipid biomarker studies of sedimentary organic matter are one means to access clues about processes involved in formation and diagenesis of sedimentary rocks and their component minerals. Carbonates are particularly suitable for this approach as they are shown to trap lipids characteristic of the microbes present when they formed. Methane seep limestones, for example, bear the distinctive biomarker and isotopic signatures of both aerobic and anaerobic methane-oxidizing bacteria and archaea (Michaelis et al., 2002; Birgel et al., 2006, 2008; Birgel & Peckmann, 2008). Massive carbonate towers at the active vents of Lost City Hydrothermal Field bear molecular evidence, in the way of DNA and biomarker lipids, for both methanogens and sulfate-reducing bacteria (Bradley et al., 2009). However, to date, there are relatively few detailed studies of lipids associated with ooids or oolites. Reitner et al. (1997) investigated the organic matrices of Great Salt Lake ooids. Jenkins et al. (2008) detected lipids from methanotrophic archaea in ooid-like coated grains found in a Cretaceous methane seep deposit in Japan. Edgcomb et al. (2013) investigated the nature and diversity of the bacterial community colonizing Highborne Cay ooids by comparing the patterns of solvent-extractable lipids with clone libraries of small subunit ribosomal RNA gene fragments.

Here, we report an analysis of the distributions of FAs, hydrocarbons, and triterpenoids present in modern and Holocene ooids from different localities. The FA data for each sample, including their stable isotopic compositions, show remarkable parallels between localities that indicate that the ooids have been colonized by a common microbial community throughout their formation and further suggests that this community contributed, at least in part, to calcification of the ooid cortex. Our results are interpreted in the light of the report by Edgcomb et al. (2013) whose 16S ribosomal RNA clone library data for ooids from Highborne Cay showed that cyanobacteria were the most diverse taxonomic group detected, followed by Alphaproteobacteria, Gammaproteobacteria, Planctomycetes, Deltaproteobacteria, and several other groups. These authors also observed that the overall bacterial diversity within ooids is comparable to that found within thrombolites and stromatolites at the same locality.
SAMPLES AND METHODS

Sampling locations are illustrated in Fig. 1. Modern ooid samples were obtained from the intertidal zones of beaches in the Bahamas and from Shark Bay in Western Australia. Bahamian samples were collected from Alligator Point on the leeward side of the Exuma Sound coast of northern Cat Island (intertidal) and from Joulters Cays (subtidal), Andros Island in the Bahamas (Harris, 1977). A third sample of modern ooids was obtained from 1 m deep subtidal waters at the north end of Carbla Beach in Hamelin Pool, Shark Bay, Western Australia. These samples were kept frozen, or refrigerated at <4°C, prior to analysis.

Holocene oolites were collected from outcrops of the North Point and Hanna Bay members (1–4 and 5 ka, respectively; Carew & Mylroie, 1985; Boardman et al., 1987, 1989) of the Rice Bay Formation on Cat Island (Mylroie et al., 2006; Glumac et al., 2011). Eolian oolites comprise the Holocene North Point and overlying Hanna Bay members on northern Cat Island; both units consist of spherical to elliptical, fine to medium sand-size ooids (Glumac et al., 2011). Bulk samples were collected and stored in the dark at room temperature until processing.

The outer edges of the North Point and Hanna Bay oolite samples were pigmented dark green and thus inferred to be colonized by viable micro-organisms. All
pigmented surfaces were removed using a rock saw and the inner white sediment crushed with a mortar and pestle. The Cat Island beach ooid sample was extracted intact while the subtidal Carbla Point and Joulters Cays ooid samples were first powdered in a puck mill. The Joulters Cays, Cat Island, and Carbla Point samples were also analyzed petrographically; they were embedded in epoxy and thin sectioned to examine their internal fabrics.

Small-scale extraction by sonication

Approximately 15 g of each sample was divided into three 60-mL centrifuge tubes. A mixture (40 mL) of 9:1 dichloromethane (DCM)/methanol was added to each centrifuge tube followed by sonication for 15 min and centrifugation at 2200 rpm for 40 min or until the finest particulates had settled completely. After repeating the procedure, the clarified solvent extracts were transferred to glass vials, gently evaporated under a stream of dry nitrogen, and weighed. All extracts, denoted TLE, were faintly colored.

Large-scale extraction by accelerated solvent extraction

To obtain some additional lipid for stable isotope analysis, extra sample was subjected to Accelerated Solvent Extraction (Dionex ASE 350). Each sample (~100 g) was weighed and placed in a large-capacity stainless steel cell with a layer of baked sand above the sediment. They were extracted with 9:1 DCM/methanol at 100°C, with the system pressure set to 1000 psi. Clean sand blanks were prepared and run at the beginning and end of the ASE cycle. These extracts were clear and treated as above. The Joulters Cays ooids and North Point extracts from the ASE were both faintly colored.

Carbonate-bound lipids

The solid carbonate residue from the 15 g sonication extraction was placed in a 1000 mL beaker and dissolved by gradual addition of hydrochloric acid (2N). A viscous light brown foam formed during sample digestion and this was captured with minimal loss. After dissolution was complete, the entire residue was diluted with HPLC-pure water and extracted five times with DCM. As before, these extracts were concentrated under a stream of dry nitrogen, filtered through a sodium sulfate column to remove any residual water, and then dried and weighed. Lipids released by acid dissolution are denoted TLE2. Extract weights are given in Table 1.

Removal of elemental sulfur

Joulters Cays ooids contained a substantial amount of elemental sulfur evident as crystals forming when the saturated hydrocarbon fractions were taken to dryness. Other ooid TLE and TLE2 samples contained sulfur in trace amounts. Elemental sulfur was removed by standing the extracts, dissolved in DCM, in contact with freshly activated copper shot followed by filtration.

Bulk δ13C

Samples for analysis of bulk organic carbon isotopes were decalcified using 6N HCl until no further reaction was observed. The residues were ground to a fine powder and weighed in triplicate into tin cups. The δ13C values were determined using a Fisons (Carlo Erba) NA 1500 elemental analyzer fitted with a Costech Zero Blank Autosampler, coupled to a Thermo Finnigan Delta Plus XP isotope ratio mass spectrometer. The data were calibrated against a CO2 working standard and normalized to acetanilide, sucrose, and urea isotopic standards.

Derivatization

Internal standards (2 μg each of epiandrosterone, 5α-androstane, and β-methyltricosane) were added to aliquots of each sample prior to derivatization. These aliquots were first treated with 200 mL dry methanolic HCl (prepared by adding acetyl chloride to cool, dry methanol) with heating 60°C for 24 h in order to convert glycrides into fatty acid methyl esters (FAME). The samples were taken to

Table 1 Gravimetric yields of total lipids and fatty acid methyl esters from modern and Holocene ooid samples by two extraction methods

<table>
<thead>
<tr>
<th></th>
<th>North Carbla</th>
<th>Joulters Cays</th>
<th>Cat Island</th>
<th>North Point</th>
<th>Hanna Bay</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC (LO I) %</td>
<td>3.1</td>
<td>2.1</td>
<td>1.9</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>δ13Corg</td>
<td>−15.8 ± 1.3</td>
<td>−17.7 ± 1.6</td>
<td>−17.4 ± 0.2</td>
<td>−20.7 ± 2.0</td>
<td>−15.5 ± 2.5</td>
</tr>
<tr>
<td>Lipid % TOC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASE TLE</td>
<td>0.68</td>
<td>0.05</td>
<td>0.05</td>
<td>0.22</td>
<td>0.09</td>
</tr>
<tr>
<td>Sonication TLE</td>
<td>3.70</td>
<td>0.47</td>
<td>0.28</td>
<td>1.03</td>
<td>4.30</td>
</tr>
<tr>
<td>Sonication TLE2</td>
<td>1.09</td>
<td>0.66</td>
<td>0.42</td>
<td>0.74</td>
<td>2.13</td>
</tr>
<tr>
<td>Sonication TLE2 FAME</td>
<td>0.005</td>
<td>0.002</td>
<td>0.002</td>
<td>0.012</td>
<td>0.023</td>
</tr>
<tr>
<td>Sonication TLE2 FAME</td>
<td>0.032</td>
<td>0.004</td>
<td>0.018</td>
<td>0.011</td>
<td>0.015</td>
</tr>
</tbody>
</table>

FAME, fatty acid methyl esters.

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dryness, transferred to gas chromatography-mass spectrometry (GC-MS) vials and then further derivatized by adding 50 μL BSTFA and 50 μL pyridine with further heating to 60°C for 30 min prior to analysis.

Gas chromatography-mass spectrometry analysis

Samples were analyzed using an Agilent 7890A GC equipped with a Gerstel programmable temperature vaporizing (PTV) injector and interfaced to an Agilent 5975 Mass Selective Detector. The GC was fitted with a J&W DB5-HT (30 m length, 0.25 mm inner diameter, 0.25-μm film thickness) capillary column using helium as the carrier gas. After a pulsed splitless injection with the oven held at 60°C for 5 min, the column ramped to 340°C at 20°C per min and then held for 20 min. For quantification of FAME, the analyses were repeated using an Agilent 7890A GC fitted with a flame ionization detector and operated as above. FAME were identified by a combination of their mass spectra and GC retention times relative to authentic normal and branched FAME mixtures obtained from SupeLco Analytical.

Gas chromatography-isotope ratio mass spectrometry (GC-IRMS) analysis

Prior to isotope analysis, large aliquots of the TLE and TLE2, constituting 50% of the total were methylated with acidic methanol, as above, and subject to liquid chromatography over ca. 10 cm columns of silica gel in a Pasteur pipette. Five fractions were obtained using an elution sequence of solvents of increasing polarity. Aliphatic hydrocarbons were eluted in the first fraction with hexane [1/8 column dead volume (DV) determined empirically for each silica bed] followed by aromatic hydrocarbons in 2 DV 4:1 hexane/DCM, ketones, and FAME in 2DV DCM, alcohols in 2 DV 4:1 DCM/ethyl acetate and diols 2 in DV 7:3 DCM/methanol. Stable carbon isotope analysis on the hydrocarbon and FAME fractions was performed using a Thermo Trace GC fitted with a PTV injector and equipped with a J&W DB-1 (60 m length, 0.32 mm inner diameter, 0.25 μm film thickness)-fused silica capillary column and coupled to a ThermoFinnigan Deltaplus XL isotope ratio mass spectrometer via a combustion interface operated at 850°C. Column temperatures were programmed from 60°C at a constant flow of 2.5 mL per min and a temperature gradient of 10°C per min to 100°C, followed by a temperature gradient of 4°C per min to 320°C then isothermal for 20 min. Stable carbon isotope ratios were determined relative to an external CO2 standard that was cross-calibrated relative to a reference mixture of n-alkane (Mixture B) provided by Arndt Schimmelmann (Indiana University). Data are presented in the conventional δ13C notation as part-per-thousand (‰) deviations from VPDB. No corrections were made for the carbon or hydrogen added by methylation. Analytical uncertainties under these conditions are conservatively estimated as ±0.4‰.

Due to limited abundance, hydrogen isotopic analyses were only feasible for the FAME fraction, and then only for the most abundant compounds. These analyses were performed at Caltech on a Thermo Trace GC coupled to a Delta+ XP IRMS via a GC/TC pyrolysis interface operated at 1440°C, as described by Zhang et al. (2009). GC conditions were similar to those for δ13C analyses. D/H ratios of analytes were measured relative to CH4 reference gas added to the GC effluent via an external loop and are presented in the conventional δD notation as part-per-thousand (‰) deviations from Vienna Standard Mean Ocean Water (VSMOW). No further normalization to the SMOW/SLAP scale was attempted. FAME δD values were not corrected for the hydrogen added by the methyl ester derivatives. This correction typically adjusts all δD values in the same direction by <1‰. Squalene was added to every sample as an internal standard and yielded a δD value of −169.2 ± 1.4‰ as compared to its value determined off-line combustion/reduction of −168.9 ± 1.9‰ (A. Schimmelmann, Indiana University). Analytical uncertainties for hydrogen isotopic analyses are conservatively estimated as ±5‰.

The H-isotopic composition of Hamelin Pool water was determined using a spectroscopic Water Isotope Analyser (Los Gatos Research, Inc., Mountain View, CA, USA). The sole sample was analyzed five times, with six replicate injections for each analysis, against two separate water standards with δD values near 0‰. The resulting standard error (σ/νn) for the pooled data was 0.6‰.

Radiocarbon analysis

Four samples of TLE were submitted to NOSAMS for AMS radiocarbon analyses. These were combusted in evacuated quartz tubes with 100 mg CuO and the resultant carbon dioxide reacted with Fe catalyst to form graphite. The graphite was then analyzed for radiocarbon content on NOSAMS’s 500 kilovolt AMS system using NBS oxalic acid I (NIST-SRM-4990) as the normalizing standard. As per convention, data were corrected to a δ13CVPDB value of −25‰ using 13C/12C ratios measured concurrently on the AMS system and are reported using the Δ14C nomenclature.

Bulk composition by loss on ignition

Bulk weight percent organic matter, weight percent carbonate, and weight percent ash of each sample were estimated using loss on ignition (LOI) techniques. 300–400 mg of rock powder was weighed into a small ceramic crucible and combusted in a Barnstead/Thermolyne.
30 400 furnace at 550°C for 4 h. Once cool, the powders were weighed, and mass loss at this step taken as the organic matter content. The powders were then recombusted at 950°C for 2 h, and the further mass loss taken as weight percent carbonate. Material remaining after the final combustion (primarily silicate, oxide, and sulfide minerals) was considered ash.

RESULTS

Petrography

Ooids from Joulter’s Cays and Cat Island, Bahamas, and North Carbla, Hamelin Pool, Australia were examined in thin section in order to evaluate their relative preservation states. The Joulter’s Cays sample contained ooids with variable preservation; some are heavily micritized from endolithic borings (Fig. 2A,B) while others preserve relatively undisrupted cortical layers (Fig. 2A). The nuclei of ooids at these localities are typically peloidal and only rarely skeletal. The outer layers of many ooids are well preserved, but the inner part of the cortex is commonly bored and/or micritized. Cat Island ooids show a similar preservational style to those analyzed from Joulter’s Cays (Fig. 2C,D). They are also occasionally cemented together to form grapestone (Fig. 2D), and skeletal grains are rare. Hamelin Pool ooids preserve exceptional outer cortical fabrics and show little evidence for endolithic boring in their outermost laminae (Fig. 2E,F). These thin sections demonstrate that other grains are generally rare in the ooid populations selected for biomarker analysis, presumably as a result of sorting in the depositional environment (Glumac et al., 2011), and demonstrate that there is variability in the preservation of ooid cortices between localities.

Bulk compositions

The total organic carbon contents, as determined by loss on ignition, were between 3.1 and 1.6% (Table 1). The three modern samples all had slightly more organic carbon than the Holocene oolites. Bulk δ13Corg values, determined after decalcification, were variable from −15.5 to −20.7‰ VPDB. The decalcified residues themselves, which contained a significant proportion of inorganic material, were isotopically heterogeneous based on a dispersion in the EA data.

Fig. 2 Photomicrograph of ooids embedded in epoxy from Joulter’s Cays and Cat Island, Bahamas, and Carbla Beach, Shark Bay, Australia. (A) Joulter’s Cays ooids. Arrow 1 shows ooids with well-preserved outer cortex and Arrow 2 shows micritized ooid; (B) Joulter’s Cays ooids, one with round endolithic boring (arrow); (C) Cat Island ooids, with well-preserved cortical fabrics (arrows); (D) Cat Island ooids, two of which show micritic envelope around ooids (arrow) resulting in grapestone formation; (E and F) Carbla ooids showing well-preserved cortical fabrics and little endolithic boring and/or micritization of laminae.
Total lipid extracts

As shown in Table 1, and relative to the weights of ooids extracted, the extraction by sonication appeared to afford significantly higher total lipid extract yields (by a factor of \(\sim 5\)) than was produced by the ASE extraction. In contrast to the total lipid extracts, the FA yields from the ASE extraction were broadly similar to those obtained by sonication despite there being minor differences in relative abundance. Given the compositional similarities, we focus the remainder of the results and discussion on the composition of the lipids extracted by the sonication procedure. One point of note is that the yields of alcohols, including sterols, which were a very minor fraction of the total TLE, were slightly higher in the ASE extracts. The reason for the large weight discrepancy between TLE obtained by the sonication protocol vs. that obtained by ASE remains unknown. However, it is possible that there was some very fine-grained inorganic material in the ASE extracts that was not removed by simple filtration.

Fatty acid methyl esters

Fatty acids were abundant and diverse in structure as evident in the total ion chromatograms of the FAME fraction of the TLE from Carbla ooid sample (Fig. 3). Individual compounds were initially identified using mass chromatograms of the characteristic 74 Da McLafferty rearrangement ion of the FAME. Identifications were confirmed, where possible, by comparison of the mass spectra and retention times of the FAME with those of authentic standards. The relative abundances of FAME in the Holocene and Modern samples, as quantified from the GC-FID chromatograms, are presented in the form of histograms in Figs 4 and 5. Saturated, normal (straight-chain) FAME with chain lengths from C\(_{10}\) to C\(_{32}\) were accompanied by branched FAME with chain lengths of C\(_{12}\) to C\(_{18}\). Monounsaturated C\(_{16}\) and C\(_{18}\) FAME were minor or trace compounds in most of the samples, and they were more often identified in the TLE than in TLE2 (Fig. 3). Traces of a C\(_{16:2}\) FAME were detected in the Carbla and Cat Island ooids, but the loci of unsaturation were not determined due to the low abundances of these components.

With two exceptions, n-C\(_{16}\) and n-C\(_{18}\) were the most abundant FA in all samples, and their concentrations were in the ranges 3–11 \(\mu g^{-1}\) TOC and 2.5–46 \(\mu g^{-1}\) TOC, respectively. The exceptions were iso-C\(_{16}\) FA (85 \(\mu g^{-1}\) TOC) being dominant in the Hanna Bay TLE and a C\(_{18:2}\) FA (6.7 \(\mu g^{-1}\) TOC) in the Cat Island sample. The C\(_{16:0}\) and C\(_{18:0}\) FAME showed similar patterns in the TLE and TLE2 samples with the C\(_{16:0}\) FAs generally being more abundant. The one exception was the Hanna Bay TLE2 sample that shows a slightly higher C\(_{18:0}\) abundance. For
FA larger than C_{18}, there was an overt even-over-odd carbon number preference, and their overall abundances decreased progressively with chain length. These long-chain fatty acids (LCFA) ranged from C_{22} to C_{36}, generally with a maximum at C_{22} or C_{24}, and were accompanied by very low amounts of iso, anteiso, and mid-chain methyl analogs as can be seen in the chromatogram of the North Carbla TLE FAME (Fig. 3A). The concentration of n-C_{24:0} FAME ranged from 0.8 to 3.9 g g^{-1} TOC in the TLE to 2–22 g g^{-1} TOC in TLE2. Of all the samples, the ooids from Carbla Beach had the highest concentrations of LCFA.

A common feature of all the samples analyzed was a relatively high abundance of branched FAME, with C_{14}–C_{18} iso- and anteiso FAME the most commonly identified. The iso-C_{16:0} FAME was present in all the samples (0.8–85 g g^{-1} TOC in TLE and 1.0–20 g g^{-1} TOC in TLE2) and, as mentioned above, the most abundant FA in the carbonate-bound fraction of the Holocene oolite from Hanna Bay (85 mg g^{-1}). Iso- and anteiso-C_{15:0} FAME were also present in all samples. Another common feature was the presence of a mid-chain monomethyl FAME with 17 carbon atoms. Comparison with a mixture of authentic standards confirmed that the predominant isomer was 10-methylhexadecanoic acid (10-methyl-C_{16:0}). In fact, for the North Point sample, the 10-methyl-C_{16:0} and n-C_{17:0} FAME were present in almost equal concentrations. Some samples also contained trace amounts of a FAME that, on the basis of relative retention time, was probably 10-methyl C_{17:0}.

In addition to the FAME that were formed by transesterification of complex ester-linked lipids, we also identified low abundances of TMS FA derivatives in most samples. These were likely derived from free FAs that were incompletely methylated in the first step of our procedure. All the TLE samples contain TMS C_{16:0} and C_{18:0} as well as traces of other FA. We also observed that, compared to TLE, the TLE2 samples contain more TMS VLCFA with an even-over odd carbon number preference.

Other lipids

Trace amounts of n-alcohols, identified as their trimethylsilyl derivatives, and n-alkanes are present in both Modern and Holocene ooids. Their low abundances, however, precluded a detailed comparison across the sample set. The

Fig. 4 Histograms showing relative abundances of fatty acids in two Holocene ooid grainstones from Cat Island, Bahamas. The columns labeled TLE and TLE2 compare the compositions of freely extractable lipids with those released by acid dissolution. The data are all for sample that were extracted using sonication.
Holocene samples contained saturated alcohols from \( n-C_{10} \) to \( n-C_{32} \) while, in the modern ones, we could only detect these compounds as low as \( n-C_{14} \), and the abundances of alcohols were greater in the TLE compared to the TLE2. Samples that contained longer chain length alcohols exhibited a distinct even-chain length preference as was the case with the FA. This could be seen most clearly in the TLE samples and the Joulters Cays ASE-extracted sample (data not shown). The fossil oolite samples from North Point and Hanna Bay had larger amounts of \( C_{16} \) and \( C_{18} \), whereas the Modern samples were more enriched in \( C_{24} \) and \( C_{26} \). Many of the samples contained more than one isomer for the \( C_{15-17} \) alcohols as shown by the presence of multiple peaks with identical mass spectra but slightly different retention times. The low abundances of alcohols in the extracts precluded formal identification of these isomers.

Steroids were detected in low abundance in most of the samples. In contrast to the FAs, the distributions of sterols differed markedly across the sample set. Cholesterol (\( \sim 900 \text{ ng g}^{-1} \text{ TOC} \)) and cholestanol (\( \sim 600 \text{ ng g}^{-1} \text{ TOC} \)) were the most prominent sterols in the Joulters Cays TLE,
and the same compounds were present in TLE2. In the North Point sample sitosterol, stigmasterol, and campesterol were identified in trace amounts. Sitosterol, stigmasterol, and cholesterol along with stigmasta-3,5-diene were identified as trace components in the North Carbla sample. Apart from the detection of traces of cholesterol in all the samples, there did not appear to be any clear trend in the sterol contents of the ooids.

In contrast to the variable patterns of sterols, several hopanoids were consistently present in the samples. The most abundant were methyl esters of the C_{31}–C_{33} hopanoic acids and the TMS ether of bishomohopanol. Of these compounds, bishomohopanoic acid methyl ester was the most abundant. The methyl ester of bishomohopanoic acid has a prominent molecular ion at 484 Da and is accompanied by fragment ions at 469 (M–15), 369 (pentacyclic ring system), 263 (base peak; ring D cleavage with side chain), and 191 Da. In the particular case of the North Carbla TLE and TLE2, bishomohopanoic acid methyl ester was visible in the total ion chromatograms (Fig. 3) and was estimated to be present at a level of ~0.4 μg g⁻¹ TOC based on a comparison of peak areas with those of the FAME. Further, three isomers were present with the predominant one identified as having the biological 17β, 21β-22R configuration based on its position as the last eluting isomer and the relative intensities of the 191 (A+B ring fragment) and 263 (D+E+ side chain) Da fragment ions. Smaller amounts of the 17α, 21β and 17β, 21α isomers were also identified on the basis of elution order and the relative intensities of the 191 and 263 Da fragment ions (Peters et al., 2005).

### Carbon isotopic compositions of FAME

The C-isotopic compositions of ooid FAs vary widely although some consistent trends can be discerned (Table 5). In particular, the FAME from the freely extractable lipid (TLE) were distinct from those in the carbonate-bound fraction (TLE2). In almost all cases, the same compound in the bound fraction was more enriched in ¹³C than the one in the freely extractable fraction. FAME from the North Carbla sample were more enriched in ¹³C compared to those from the Bahamian Archipelago and, as well, they varied over a narrower range than those of the other samples. The C-isotopic compositions of the branched FAs show greatest disparity with, for example, the iso-C₁₆ FAME having a δ¹³C value as low as −39.1‰ VPDB in the Hanna Bay TLE and as high as −15.5‰ in the North Carbla TLE2. Four measurements were possible for the 10-methyl-C₁₆ FAME and they too varied widely from −36.9 to −20.0‰. The most abundant n-C₁₆:0 and n-C₁₈:0 FAME varied within a narrower range of values from −19.2 to −24.5‰ (C₁₆:0) and −18.8 to −26.5‰ (C₁₈:0). The long-chain C₂₀:0–C₃₂:0 FAME followed a similar trend of narrow isotopic dispersion within each sample of TLE and TLE2.

### Hydrogen isotopic compositions of FAME

Hydrogen isotopic measurements were possible for a few compounds in each sample with the North Carbla TLE sample providing greatest coverage of FAME (Table 2). Here, the prominent n-C₁₆:0 and n-C₁₈:0 FAME had δD values of −129.6 and −121.6‰ VSMOW, respectively. The even carbon number long-chain FAME varied over a similar range while the C₂₃ and above, odd carbon number counterparts were slightly different and mostly a little enriched in D. As with carbon isotopes, the North Carbla FAME were more D-enriched than those of the Hanna Bay and North Point samples. The greatest contrast was in the H-isotopic compositions of the short-chain branched FAME that were as heavy as +4.8‰ VSMOW in the case of the iso-C₁₅ from North Carbla TLE. The anteiso-C₁₅:0 and iso-C₁₅:0 were also significantly more enriched than co-occurring n-C₁₆:0 and n-C₁₈:0 FAME. Short-chain branched FAME in the North Point and Hanna Bay samples were 60–100‰ more D-enriched than n-C₁₆:0 and n-C₁₈:0 FAME, echoing the trend in the North Carbla ooid sample. The hydrogen isotopic composition of the

<table>
<thead>
<tr>
<th>Carbon #</th>
<th>Isomer</th>
<th>Hanna Bay TLE</th>
<th>North Point TLE2</th>
<th>North Carbla TLE</th>
<th>North Carbla TLE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>n</td>
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<td></td>
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<td>18</td>
<td>n</td>
<td>−101.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Iso</td>
<td>−134.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>n</td>
<td>−121.6</td>
<td>−114.4</td>
<td></td>
<td></td>
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<tr>
<td>22</td>
<td>n</td>
<td>−128.0</td>
<td>−96.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>n</td>
<td>−135.2</td>
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<tr>
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<td>n</td>
<td>−116.2</td>
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<td></td>
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<tr>
<td>25</td>
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<tr>
<td>29</td>
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<td>−107.3</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>n</td>
<td>−123.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The North Point and Hanna Bay samples are Holocene oolites while the North Carbla sample is from contemporary beach ooids. The increased sample demands of H-isotopic measurement precluded analysis of more compounds.
water at the North Carbla ooid collection site was +14.2‰ VSMOW.

**Carbon isotopic compositions of hydrocarbons**

Normal alkanes are only a trace component of the organic matter that was freely extractable from the ooids. On the other hand, they are easily isolated in a clean fraction by liquid chromatography that renders them amenable to isotopic analysis (Table 3).

**Radiocarbon**

Radiocarbon analyses of the North Carbla total lipid extracts (Table 4) revealed that the carbon was roughly contemporaneous with modern seawater. The carbonate-bound lipids were, however, significantly older. A similar contemporaneous distribution is expected that individual grains in any sample will have spent variable proportions of their existence in, above, and beneath seawater. Thus, it seems quite remarkable that the distributions of FAs recovered from the three samples of contemporary ooids are quite similar and so distinctive. As such, they provide insights into the nature of the biological communities that have colonized them over 1000 year timescales. The close similarity of the FAME distribution patterns of freely extractable lipids in TLE and carbonate-bound lipids in TLE2 indicates that a biosignature for the microbial communities that have colonized the ooids during their existence are recorded in the carbonate matrix. While the prominent n-C16:0 and n-C18:0 FA are ubiquitous in bacteria and eukaryotes and do not allow us to identify specific sources, the short-chain, odd carbon numbered, and branched FA can be confidently attributed to bacteria (Kaneda, 1991) and suggest that specific kinds of bacteria have colonized the ooids during and/or after their formation.

The nature of the short-chain branched FAME identified here in TLE and TLE2 specifically suggest an association between sulfate-reducing bacteria and ooids. Sulfate-reducing bacteria have been shown to possess diverse cellular FAs that range from C12 to C19 (Taylor & Parkes, 1983; Dowling et al., 1986, 1988; Kuever et al., 2001) including branched FAs, present in their phospholipids, that are quite distinctive. The 13-methyl C15:0 (iso-C15:0) and 12-methyl C18:0 (anteiso-C15:0) FAs identified in these ooid samples have been previously identified in *Desulfobulbus* and *Desulfobacter* sp. (Taylor & Parkes, 1983), for example. Even more diagnostic is the 10-methyl C16:0 FA that is identifiable in all the samples and that is commonly found in members of the Deltaproteobacteria. It has been found in cultures of *Desulfobacter* sp. (Taylor & Parkes, 1983; Dowling et al., 1986, 1988) and in closely related genera *Desulfobulba* and *Desulfotignum* (Kuever et al., 2001). The 10-methyl C16:0 FA is also prevalent in environmental samples where sulfate-reducing bacteria are prominent (Hinrichs et al., 2000; Labrenz et al., 2000; Elvert et al., 2003). 10-Methyl C16:0 has also been identified in *Geobacter metallireducens* (Lovley et al., 1993), but unlike the aforementioned sulfate-reducing species, and the ooid FAME, it is accompanied by significant amounts of unsaturated C16 and C18 acids. Some samples also contained trace amounts of 10-methyl C17:0 which, along

---

**Table 3** Carbon isotopic compositions of hydrocarbons isolated from the freely extractable total lipids for four samples of ooids

<table>
<thead>
<tr>
<th>n-alkane Carbon #</th>
<th>Hanna Bay TLE</th>
<th>North Point TLE</th>
<th>Cat Island TLE</th>
<th>North Carbla TLE</th>
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</thead>
<tbody>
<tr>
<td>17</td>
<td>-14.1</td>
<td>-26.6</td>
<td>-21.7</td>
<td>-18.8</td>
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</table>
with 10-methyl C\textsubscript{15.0} and 10-methyl C\textsubscript{18.0}, has also been reported in Desulfobacter spp. (Dowling \textit{et al.}, 1986). Based on these data, we suggest the short-chain branched FAs identified here are primarily sourced from sulfate-reducing bacteria.

Very long-chain FA with a pronounced even-over-odd carbon number preference are often attributed to an origin from vascular plants due to their widespread occurrence in leaf waxes and prevalence in sediments with significant terrigenous plant inputs (Eglinton & Hamilton, 1967; Rezanka, 1989; Prasad \textit{et al.}, 1990; Rielley \textit{et al.}, 1991). However, the tropical shallow marine environments where these ooids were collected do not favor vascular plants being an important source of sedimentary organic matter. While eolian processes can deliver plant organic matter, an absence of significant riverine drainage to Hamelin Pool (Logan & Cebulski, 1970) and these Bahamian environments places a limit on the deposition of terrigenous organic matter. Moreover, in the present sample set, another major leaf wax component, long-chain hydrocarbons with an odd-over-even preference are only present in trace amounts and are isotopically distinct, in both C and H, from the co-occurring FA. Thus, the very long-chain FA and long-chain \textit{n}-alkanes appear to have distinctly different sources. Besides the ubiquitous vascular plants, very long-chain FA have been detected broadly across the eukaryote domain (Rezanka, 1989; Rezanka & Sigler, 2009).

Comparable observations of abundant medium- to long-chain FAs have been made in other marine settings, most recently in studies of the lipids preserved in sediments in the Santa Monica Basin (Gong & Hollander, 1997; Pearson \textit{et al.}, 2001) and in the Santa Barbara Basin (Li \textit{et al.}, 2009). Pearson \textit{et al.}, 2001, in their study of Santa Monica Basin sediments, observed a series of even-preference C\textsubscript{20}, FA, with a maximum abundance at C\textsubscript{24}. Among other characteristics, Pearson & Eglinton (2000) reported that the C\textsubscript{24} FA had \(\Delta^{13}C\) values near to the end-member DIC values determined for post-bomb and pre-bomb sediments from which the FA were isolated. Co-occurring odd carbon numbered \textit{n}-alkanes had more depleted \(\Delta^{14}C\) values. Where they could be measured in the ooid samples, \(\delta^{13}C\) values of the \textit{n}-alkanes were more depleted than the FA by 4–5\%\textsubscript{o} (Tables 3 and 5). These data suggested the C\textsubscript{20}–C\textsubscript{30} FA sources were distinct from the co-occurring odd carbon numbered \textit{n}-alkanes with the latter originating from a mixed fossil carbon and contemporary terrigenous higher plant sources (Pearson & Eglinton, 2000). Their observations are also consistent with those of an earlier study by Gong & Hollander (1997) who reported a similar C\textsubscript{24} predominance in the long-chain FAs and \(\delta^{13}C\) values that could not be reconciled with sources from vascular plants. Rather, they hypothesized mixed input from plants and bacteria to explain their data. In the Santa Barbara Basin study of Li \textit{et al.} (2009), hydrogen isotopic values for C\textsubscript{22}–C\textsubscript{26} FAs varied from –49 to –138\%\textsubscript{o} VSMOW and were also distinct from co-occurring leaf wax components. The authors tentatively attributed these compounds to aquatic macrophytes, but they were among the most D-enriched compounds detected in this study. There was also significant overlap with the \(\delta D\) values of the odd carbon number and branched FAs attributed to bacteria. When all these results are considered, it seems likely that there can be a primary bacterial source for C\textsubscript{22}–C\textsubscript{26} FAs with an even carbon number preference in the marine environment. Based on their detection in a cultured strain of spore-forming Desulfotomaculum sp. (Rezanka \textit{et al.}, 1990), we hypothesize that sulfate-reducing Fimicute bacteria are a candidate source for the long-chain FA that are prevalent in ooids.

### Hopanoic acids and hopanols

Hopanoic acids and hopanols are among the common diagenetic products of bacteriohopanepolys (BHP). All the ooid extracts examined here contained 17\β, 21\β-bishomohopanol, identified in the GC-MS analyses of the silylated TLE, and attributable to bacteria. Because the potential sources of BHP are so diverse, no specific statements can be made regarding the origin(s) of these biomarkers other than to note that their abundance was much higher than sterols. The most abundant hopanoid in all samples was 17\β, 21\β-bishomohopanoic acid that appears as a late-eluting peak in the GC traces of all FAME samples. Overall, the distributions of FAs and hopanoids combined with the low abundances of sterols and their diverse and inconsistent compositions suggest that the organic matter preserved in the ooids and oolites is largely of bacterial origin.

### Carbon and hydrogen isotopic compositions of the short-chain and branched FAME

A key inference that can be drawn from the carbon and hydrogen isotopic data for ooid TLE and TLE2 FAME is...
that environmental conditions have varied during ooid formation in the different locations represented in this study. Although the same compounds are present in the freely extractable and carbonate-bound lipids of all the samples, their isotopic compositions differ. In the case of the North Carbla ooid sample, the carbonate-bound lipids are, on average, about 350 years older based on $\Delta^{14}C$ values. Further, the $\delta^{13}C$ and $\deltaD$ values for carbonate-bound FA are consistently enriched by $\sim0.5$ to $3.0$ and $\sim10$ to $30\%$, respectively. Hamelin Pool lies in an arid region of Western Australia and is an evaporitic basin by virtue of its connection to the open ocean being restricted by a shallow sill, the Faure Sill (Logan, 1961; Playford & Cockbain, 1976). This high salinity seawater carries distinctive distribution of isotopic signatures for the water and carbon as shown by $^{13}C$ and $^{18}O$ enrichment of the endemic organisms from microbes (Des Marais et al., 1992) to metazoa (Edmonds et al., 1999).

The C- and H-isotope data for Hamelin Pool ooid lipids suggest that organic matter entrapped in the carbonate matrix incorporated C and H, which was isotopically different compared to the most easily extracted lipids, or that some fractionation process operated during entrapment of the organics in the carbonate. In the one case where we could measure $\Delta^{14}C$ values, the carbonate-bound fraction was, on average, older than the freely extractable fraction by ca. 350 years. This older $^{14}C$ age for the carbonate-bound lipids in the North Carbla sample suggests the $^{13}C$ and D differences carry a signal for earlier paleoenvironmental conditions. Similar patterns are seen in the C-isotopic compositions of free and carbonate-bound lipids in the Cat Island ooids and the Hanna Bay and North Point oolites. In all cases, the carbonate-bound FA are more enriched in $^{13}C$ by, on average 1–3%. A more detailed isotopic analysis, including $^{14}C$ ages of the FAs released during sequential dissolution of ooid matrix may reveal more about the processes leading to this offset and the timescales of their emplacement.
The hydrogen isotopic compositions of FAs can be informative about the physiology of organisms contributing these compounds to marine sediments. This is because the hydrogen isotopic fractionation between lipids and source water is in part a function of the way NADPH is formed in different metabolic pathways (Zhang et al., 2009). Photoautotrophs, for example, typically exhibit a lipid/water fractionation of $180-200$, while chemoheterotrophs often show even larger fractionations. In aerobic heterotrophs, the fractionation is more variable and spans $-150$ to $+200$. Using these guidelines, we can rationalize some of the hydrogen isotopic data for ooid FA and hydrocarbons. In Hanna Bay and North Point samples, where ooids formed in open ocean waters, the $\delta D$ values of C$_{16}$ and C$_{18}$ FAs vary from $-171$ to $-203$ VSMOW suggesting that photoautotrophs were important sources for these compounds in the Holocene Bahamian ooids. In comparison, $\delta D$ values for the same FA from the Carbla sample were significantly $D$-enriched at $-120$ to $-130$ VSMOW, yet the H isotopic composition of Hamelin Pool waters at the ooid collection site was $+14.2$ VSMOW. The $D$-enrichment in the TLE2 FA was even more extreme. These smaller fractionations may reflect a different balance of autotrophic vs. heterotrophic contributions to these FA. Alternatively, they may reflect the higher salinity waters in Hamelin Pool, or a combination of both factors.

Sample availability restricted measurement of $\delta D$ values of branched FA in the North Point sample to iso-C$_{16}$, iso-C$_{18}$, and 10-Me C$_{16}$ FA. All were $D$-enriched compared to the straight-chain compounds with 10-Me C$_{16}$ FA having a $\delta D$ of $-79.6$ VSMOW, consistent with a source distinct from photoautotrophs. Branched FAs in the North Carbla sample are even more enriched and have divergent $\delta D$ values suggesting they represent differential inputs from bacteria with heterotrophic physiologies. On the other hand, the VLCFA in the freely extractable lipid have $\delta D$ values in the same range as n-C$_{16}$ and n-C$_{18}$ FA in the same sample consistent with photoautotrophic or heterotrophic sources, but not to the exclusion of other possibilities.

**Overall similarities and origins of the ooid geochemical signatures**

Lipids that are readily extracted from ooids and oolites are very similar in composition to those released after carbonate dissolution. This suggests that the microbial community colonizing the ooids has varied little over time during their formation. The notable characteristics of the lipid distribution include a predominance of n-C$_{16}$ and n-C$_{18}$ saturated FA that, although ubiquitous in nature, likely represent a predominant source from photoautotrophic microbes such as cyanobacteria. These are accompanied by significant amounts of branched and saturated FA that likely originate from heterotrophic bacteria including sulfate-reducing bacteria. Very long-chain normal FA, with a pronounced even-over odd carbon number preference, together with smaller amounts of iso- and anteiso- counterparts may also originate from bacteria, possibly firmicutes, although we cannot unambiguously ascribe a particular source at this time. However, a recent molecular study of Bahamian ooids from Highborne Cay sheds some light on this. Small subunit (16S) ribosomal DNA data for a sample of ooids from the intertidal zone, generated from the living community via analyses of cDNA generated from RNA, indicate that the overall bacterial diversity was comparable to that found within thrombolites and stromatolites at the same location (Edgcomb et al., 2013). Cyanobacteria were the most diverse taxonomic group detected in the ooids, followed by Alphaproteobacteria, Gammaproteobacteria, Planctomycetes, and Deltaproteobacteria including diverse sulfate reducers. Sequences for the firmicute genus Moorella were also identified. These ooids had lipid signatures that were very similar to those observed in the present study. FAs were dominated by C$_{16.0}$ and C$_{18.0}$, each of which was accompanied by lower abundances of their mono- and diunsaturated analogs. Odd carbon number and branched C$_{14}$–C$_{17}$ FA were present, the most abundant of which was 10-Me C$_{16}$. Also prominent was the homologous series of even carbon numbered C$_{22}$–C$_{30}$ FA together with the methyl esters of bis(homohopanoic) acid and phytanic acid. A diverse assemblage of BHP was identified in LC-MS analyses of the acetate derivatives. Bacteriohopanetetrol (BHT) was the most abundant of these along with the corresponding 2-methyl and 3-methyl analogs and BHT cyclitol ether. Bacteriohopanetetrol was accompanied by a corresponding 3-methyl analog. These data, together with IPL precursors of the FAME are consistent with a diverse and predominantly bacterial microbial community (Edgcomb et al., 2013). In particular, the molecular evidence for combinations of primary producing cyanobacteria and heterotrophs that include sulfate reducers provides both organic matter and sources of alkalinity that can drive active carbonate precipitation as observed in other lithifying organosedimentary biofilms (Dupraz & Visscher, 2005; Dupraz et al., 2009).

**CONCLUSIONS**

Lipid distributions within the contemporary beach ooids from Western Australia and from the Bahamas, and Holocene oolites from the Bahamas, are remarkably similar. They suggest that a specific community of microbes has colonized these ooids wherever they form and possibly also contributed to ooid cortex growth. Studies of other, and more ancient, fossil oolites will be needed to verify this observation. Despite being limited in the number of measurements, the C- and H-isotopic data set for FAME and
hydrocarbons does firmly establish that the lipids we have isolated originate from microbes with diverse physiologies that include photoautotrophs, heterotrophs, and sulfate-reducing bacteria. By analogy with studies of the microbial communities forming stromatolites and thrombolites in similar environments, we hypothesize that colonizing microbes exist in the form of a bacterial biofilm that allows these microbes to operate syntrophically. The identification of sulfate-reducing bacteria biomarkers is particularly significant in this regard because they can provide an alkalinity pump in support of calcification (Riding, 1991; Visscher et al., 1998; Paterson et al., 2008; Dupraz et al., 2009).

This is an important consideration as it is known that ooid occurrences are exceptionally rare in environments, and particularly in the Pacific Ocean and some parts of the Atlantic, where pH and total alkalinity are significantly below values that allow facile carbonate precipitation (Rankey & Reeder, 2009).

The data presented here provide evidence suggestive of a specific microbiota inhabiting ooids in environments where they are actively forming. Although our data do not prove the involvement of microbial biofilms in driving ooid formation, we propose that there is substantial circumstantial evidence that they do. Specifically, the microbial community inhabiting ooids appears very similar to those inhabiting other environments where microbial mats, including stromatolites and thrombolites (Dupraz & Visscher, 2005) are undergoing active lithification while a photosynthetically powered community of biofilm-forming microbes is observed to play an active role in ooid formation in a freshwater environment (Pléc et al., 2008). Moreover, the presence and metabolic activity of sulfate-reducing bacteria in the marine biofilm communities serves to increase local alkalinity and so provides a plausible mechanism for enhancing rates of carbonate precipitation. Thus, – in contrast to the recent conclusions of Duguid et al. (2010) – we feel there is substantial evidence that microbes do play an active role in ooid formation, and that this potential role deserves further geobiological investigation.

ACKNOWLEDGMENTS

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