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D/H ratios of fatty acids from marine particulate organic matter in the California Borderland Basins

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Abstract

We report the molecular and hydrogen–isotopic compositions of fatty acids extracted from suspended particulate organic matter (POM) and surface sediments at three stations off the southern California coast: Santa Barbara Basin, Santa Monica Basin, and the Gulf of Santa Catalina. Values of δD for individual fatty acids ranged from −237‰ to −73‰ in POM and from −216‰ to −124‰ in sediments. For most fatty acids, there are no significant differences in δD between sampling locations, with depth at each location, or between POM and sediments. Two fatty acids of likely bacterial origin (*i*-15:0, 15:0) are strongly D enriched in all samples, while a third (*cyc*-17:0) is not. The origins of that enrichment are uncertain, and could reflect either an anomalous D/H fractionation in certain marine bacteria, or a significant terrestrial source for those fatty acids, or both. In surface POM and sediments, even carbon numbered fatty acids become slightly D enriched as chain length increases. This isotopic ordering is similar to that observed in living organisms, and is presumably biosynthetic in origin. In contrast, all POM samples from below the mixed layer show a consistent pattern of D depletion with increasing chain length. The order of D enrichment in these fatty acids is well correlated with their solubility, and may be caused by fractionations accompanying dissolution or degradation by microbes.

1. Introduction

The availability of compound specific methods for determining $^{2}H/^{1}H$ (D/H) ratios of individual lipids has prompted much interest in understanding the environmental and biogeochemical information recorded by these compounds. In plants and other photoautotrophs, the primary control over lipid D/H ratios appears to be the isotopic composition of environmental water, which ultimately supplies all hydrogen for biosynthesis (Sternberg, 1988; Sauer et al., 2001). As a result, studies to date have focused primarily on developing terrestrial paleoclimate proxies (Xie et al., 2000; Sauer et al., 2001; Huang et al., 2002, 2004; Sachse et al., 2006) and

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on inferring paleoenvironmental conditions from ancient sediments (Dawson et al., 2004; Sessions et al., 2004; Schefuss et al., 2005; Sachse et al., 2004). Studies exploring the H isotopic variability in individual plants have also dealt mainly with terrestrial species (Chikaraishi and Naraoka, 2001, 2007; Chikaraishi et al., 2004a; Bi et al., 2005; Sessions, 2006; Smith and Freeman, 2006). Consequently, relatively little is known about H isotopic variability in the lipid products of marine organisms, or about potential changes in the D/H composition of labile lipids accompanying degradation. To investigate these issues, we analyzed the δD values of fatty acids extracted from suspended particulate organic matter (POM) in depth transects from three stations located off the coast of southern California, as well as from core-top sediments underlying those stations.

Given that the isotopic composition of water provides first order control over lipid D/H ratios (Sternberg, 1988; Sauer et al., 2001; Huang et al., 2002), and that seawater is nearly homogeneous in its isotopic composition, a basic question that we seek to answer is whether there are any useful variations in the δD values of marine lipids. Several studies of marine organisms have suggested that there is significant variability both within and between individual species. For example, Sessions et al. (1999) observed variations in fatty acid δD values from −181‰ to −232‰ in two cultured marine phytoplankton, *Alexandrium fundyense* and *Isochrysis galbana*. Three specimens of marine macroalgae from the western Pacific produced fatty acids with δD values ranging from −189‰ to +48‰ (Chikaraishi et al., 2004c). Several studies of terrestrial plants and aquatic macrophytes also suggest that interspecies variability is common (Chikaraishi et al., 2004a,b; Sessions, 2006; Zhang and Sachs, 2007). Nevertheless, the question of whether such variability is manifest in marine organic matter – which integrates over many species and large spatial and temporal scales – remains unanswered. We show here that significant δD variations between fatty acids do persist in marine biomass, suspended POM, and sediments. There is significant potential for using these variations as tracers of a variety of marine biogeochemical processes, particularly with respect to microbial activities.

A second key question regards the influence of degradation on lipid δD values. Significant microbial degradation of fatty acids occurs in the water column and sediments, and the presence or absence of D/H fractionations associated with this process is unknown. Several studies have shown that there is no analogous fractionation of carbon-isotopes (Hayes et al., 1990; Huang et al., 1997), though Dai et al. (2005) have observed changes in lipid $\delta^{13}C$ of differing magnitudes and directions for different lipids in laboratory incubations. Since many enzymatic processes discriminate much more strongly against D than against ^{13}C as a result of strong hydrogen bonding interactions during substrate binding (Northrop, 1975), a larger signal in δD might be expected. One study of long chain leaf wax components (*n*-alkanes, *n*-alcohols, and *n*-acids) preserved in terrestrial sediments and rocks found no indications of diagenetic D/H fractionation (Yang and Huang, 2003). However, C_{14}–C_{20} fatty acids are much more labile (Lee et al., 2004) and hence subject to rapid turnover. Any fractionations associated with microbial degradation should thus be amplified in these compounds. To study these issues, we measured fatty acid δD values in water column depth transects, and compared those values to fatty acids from surficial sediments accumulating directly beneath.

A third question is whether water column oxygen content might influence lipid δD values. Control could be exerted via shifts in the microbial communities inhabiting high- versus low-O_2 waters, or through metabolic changes brought about by low O_2. For example, Valentine et al. (2004) observed strongly D depleted fatty acids produced by the obligately anaerobic bacterium *Sporomusa*, a result they attributed to *Sporomusa*'s utilization of H_2. Alternatively, influence over fatty acid δD values might be applied through enhanced preservation of organic matter in low O_2 waters. Two of the stations in this study (Santa Monica and Santa Barbara Basins) exhibit seasonal suboxia to anoxia at depth, while the third (Gulf of Santa Catalina) is open to the Pacific and presumed to be permanently oxygenated down to the sediment-water interface. These sites provide a basis for quantitative comparison.

2. Methods

2.1. Sample collection

POM and surficial sediments were collected at three stations (Fig. 1) off coastal southern California during the BASIN (Biogeochemistry of Anoxic Systems and Stable Isotopes in Nature) cruise of the

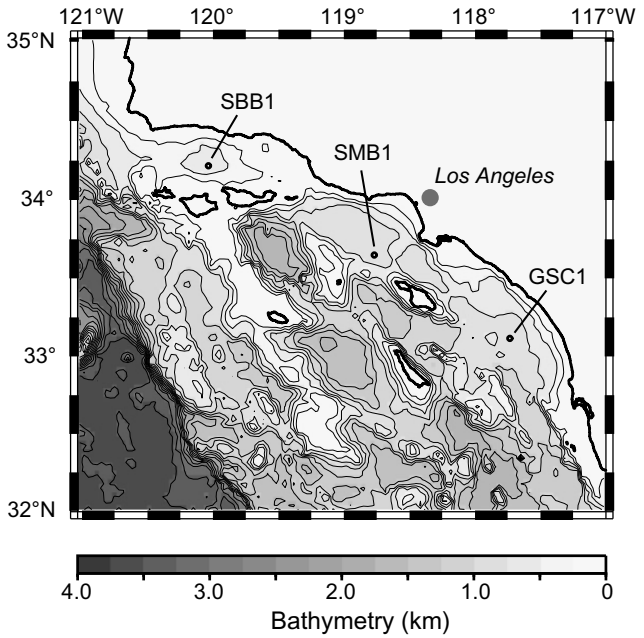

Fig. 1. Locus map for sampling stations: Santa Barbara Basin (SBB 1), Santa Monica Basin (SMB 1), and the Gulf of Santa Catalina (GSC 1).

R/V *New Horizon* in June, 2004. Stations included Santa Barbara Basin (SBB1; 34°13′N, 120°2′W, 583 m water depth), Santa Monica Basin (SMB1; 33°39′N, 118°46′W, 898 m water depth), and the Gulf of Santa Catalina (GSC1; 33°7′N, 117°44′W, 816 m water depth). SBB1 and SMB1 are located near the deepest points of the respective basins. Both Santa Barbara and Santa Monica Basins have sills which restrict circulation below depths of 470 m and 740 m, respectively. As a result, bottom waters in these basins become seasonally suboxic to anoxic. O_2 concentrations in these basins typically rise during spring upwelling events when dense, O_2 rich waters spill over the sills into the basins, resulting in a seasonal pattern of oscillating O_2 concentrations (Reimers et al., 1990; Sholkovitz and Gieskes, 1971). Dissolved O_2 concentrations at our sampling stations were measured by modified Winkler titration at the time of sample collection. These data indicate average O_2 contents of bottom waters around 0.04 ml/l at SBB1 and 0.06 ml/l at SMB1 (Fig. 2), near the lower end of typical O_2 ranges

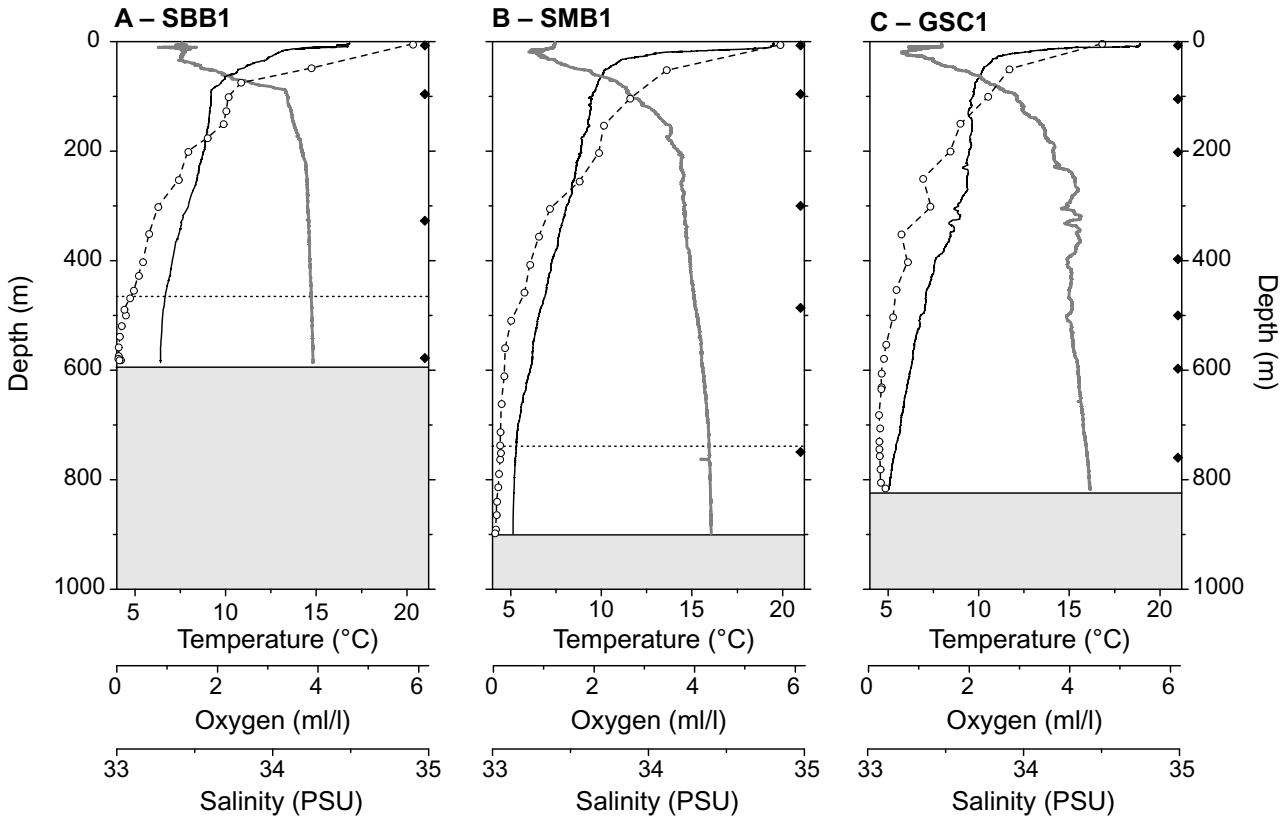

Fig. 2. Vertical profiles of temperature (black), salinity (gray), and dissolved oxygen (dashed line with circles) measured at the time of sample collection. O_2 was measured for Niskin bottle samples using a modified Winkler titration. Horizontal dashed lines indicate the sill depth in SBB1 and SMB1. Black diamonds mark sampling depths for POM at each station.

reported by the CALCOFI time series measurements (http://www-mlrg.ucsd.edu/calcofi.html). In contrast, bottom water dissolved O_2 concentrations at GSC1 averaged 0.2 ml/l, nearly an order of magnitude higher than at SBB1. Temperature and salinity profiles were comparable at all three sites at the time of sampling, with a mixed layer depth of 60–70 m (Fig. 2).

Depth profiles of suspended POM were collected by in situ filtration using a pair of battery powered pumps (WTS 6-1-142LV; McLane Research Laboratories, Falmouth, MA). Sampling depths are provided in Table 1. Pumps were fitted with a Nitex screen (45 μm nominal poresize) above a precombusted glass fiber filter (1.0 μm nominal poresize). Seawater was filtered for 1–3 h at each depth, corresponding to volumes of 350–880 l. Sampling volumes were determined independently by the pump control software and via a mechanical flowmeter affixed to the pump outlet. Both measurements typically agreed within 10%. After deployment, filters were wrapped in precombusted aluminum foil and immediately frozen to −15 °C.

Surficial sediments were collected at each station via multicore (Ocean Instruments, San Diego, CA). Multicores from all three stations preserved the sediment–water interface, with visible *Beggiatoa*-like mats present at SBB1. Multicores were immediately sub-cored using 2.5 cm lexan tubes, which were then extruded and sectioned into 5 cm depth intervals. Interstitial fluids were removed using modified Reeburgh squeezers under N_2 (Reeburgh, 1967), and the remaining sediments were transferred to precombusted glass jars and immediately frozen. All samples were transported on ice to Caltech and were kept frozen until processing. Only surface sediment samples (0–5 cm) from each station were analyzed for this study. Analyses of deeper sediments from these same stations will be reported elsewhere.

Table 1
Sampling depths and total fatty acid concentrations

Sample ID[a]	Sample depth[b]	Total fatty acids[c]	Volume filtered (l)
Santa Barbara Basin (34° 13′ N, 120° 46′ W)			
SBB1-B1	7	9.4	352
SBB1-B6	96	3.9	528
SBB1-A6	327	1.1	456
SBB1-B3	578	2.3	500
SBB1-MC3	0–5	19.8	–
Santa Monica Basin (33° 39′N, 118° 46′W)			
SMB1-B3	7	8.2	616
SMB1-B6	96	1.5	678
SMB1-A6	300	0.2	667
SMB1-B5	486	0.3	704
SMB1-A5	749	1.4	677
SMB1-MC1	0–5	46.5	–
Gulf of Santa Catalina (33° 7′N, 117° 44′W)			
GSC1-B5	7	8.4	756
GSC1-A5	105	1.6	713
GSC1-B4	202	1.8	880
GSC1-A4	397	1.3	810
GSC1-B2	500	1.4	880
GSC1-A2	597	0.8	874
GSC1-A6	760	1.4	798
GSC1-MC1	0–5	25.0	–

[a] Samples ending in "MC" are multicore sediment samples, all others are POM filters.
[b] Units are meters below sea surface (POM); centimeters below seafloor (sediment).
[c] Units are ng/l seawater filtered (POM); μg/g dry weight (sediment).

2.2. Lipid extraction and derivatization

One quarter of each glass fiber filter was lyophilized and then extracted using a modified Bligh and Dyer (1959) procedure. The dried filter was cut into small (~1 cm^2) pieces, covered with a solution of 1:2:0.9 dichloromethane (DCM)/methanol (MeOH)/water, and sonicated for 10 min. The extraction was repeated and the collected extract was separated into two phases by addition of DCM and water. The organic phase was isolated, and lipids were saponified with addition of 1 ml of 0.5 M NaOH in MeOH at 70 °C for 30 min. The organic phase was recovered and dried, redissolved in a small (~1 ml) volume of hexane, and then separated into fractions on a solid phase extraction (SPE) column containing 0.5 g of DSC–NH_2 stationary phase (Supelco) following the methodology of Sessions (2006). Fatty acids were eluted with 8 ml of 2% formic acid in DCM. Fatty acids were then derivatized as methyl esters (FAME) with BF_3/MeOH. Palmitic acid isobutyl ester (1 μg) was added as an internal standard to all samples. This fatty acid isolation procedure was adapted from Budge and Parrish (2003) specifically to ensure recovery of polyunsaturated fatty acids (PUFA).

Sediments were lyophilized, ground gently with mortar and pestle, and saponified with addition of 0.5 M NaOH in water at 70 °C for 4 h. Samples were then extracted by shaking four times with 15 ml methyl *t*-butyl ether (MTBE) after adjusting pH to 2. The extracts were filtered, evaporated to dryness under N_2, and redissolved in 1 ml hexane.

Separation by SPE and methylation of fatty acids then followed as for the POM samples.

2.3. GC/MS analyses

FAME extracts were analyzed by GC/MS using a ThermoFinnigan Trace GC equipped with a HP-5MS column (30 m × 0.250 mm × 0.25 μm), with the column effluent split between a flame ionization detector (FID) and ThermoFinnigan DSQ mass spectrometer. The GC oven was held at 80 °C for 1 min, ramped at 20 °C/min to 130 °C, and ramped at 5 °C/min to a final temperature of 320 °C. FAMEs were identified by comparison of their mass spectra and retention times to an authentic standard (37 component FAME mixture, Supelco), and were quantified using FID peak areas calibrated against the internal standard. Detection limits for this quantitation were ~2 ng/μl of injected FAME. Concentrations were then calculated relative to the total volume of filtered seawater, accounting for the 1:4 split of filter samples. Analytical uncertainties for FAME quantitation were estimated from replicate extractions of several filters. For palmitic acid (16:0), the most abundant fatty acid in all filters, the relative standard deviation of concentration ranged from 15% to 62%. While the precise reasons for this significant variability are not known, it is much larger than for similar extractions of homogenized sediments. The observed variability may thus reflect a heterogeneous distribution of sample material on the filter itself.

2.4. D/H analyses

FAME δD values were measured using a ThermoFinnigan Trace GC equipped with an EC-1 column (Alltech Associates; 30 m × 0.320 mm × 1.0 μm) coupled to a Delta^{+}XP isotope ratio mass spectrometer via a pyrolysis interface operated at 1440 °C. The GC oven temperature was ramped at 4 °C/min from 140 °C to 260 °C. The H_3^+ factor for this instrument was determined daily (Sessions et al., 2001) and ranged from 3.6 to 4.5. A mixture of 16 *n*-alkanes was analyzed repeatedly with the samples in order to establish scale normalization (Sessions et al., 2001) and to estimate analytical accuracy. The δD values of individual FAMEs were determined by comparison with two coinjected *n*-alkanes of known δD values. H added during derivatization of fatty acids as methyl esters was subtracted by mass balance, with the δD value of added methyl H determined from analysis of derivatized phthalic acid (Sessions, 2006). All δD values are reported relative to the V-SMOW standard in permil (‰) units, and are normalized to the SMOW–SLAP scale through the use of independently calibrated *n*-alkane standards.

Precision and accuracy of the FAME isotopic measurements are a function of both analyte concentration and crowding of the GC chromatogram. The standard deviation ($n = 6$) for replicate analyses of the most abundant fatty acid (16:0) in a single sample was 6‰, and represents the best attainable precision for this study. For the less abundant 14:0 fatty acid, the standard deviation ($n = 6$) was 17‰, representing a significant decrease in performance due to small peak size. In some cases, FAME peaks coeluted with other minor components in the chromatogram or were located at a position in the chromatogram with variable baseline. These instances are noted in the tables, and typical uncertainty for δD values of these peaks is ±15‰.

3. Results and discussion

3.1. Fatty acid concentrations

Twelve different fatty acids were quantified in the POM and surface sediment samples for this study. Other fatty acids were present in all samples (e.g., Fig. S-1 in the supplementary materials), but of insufficient quantity for D/H analysis and so are not reported here. Quantified fatty acids were: 14:0, *i*-15:0, 15:0, 16:0, 16:1, *cyc*-17:0, 18:0, 18:1, 18:2, 18:3, 20:5, and 22:6 (numbers refer to carbon atoms:double bonds; '*i*-15:0' is tentatively identified as 13-methyltetradecanoic acid; '*cyc*-17:0' is tentatively identified as *cis*-9,10-methylenehexadecanoic acid; '16:1' and '18:1' each contain coelutions of both ω-7 and ω-9 isomers). Complete compositional data for all samples is given in the online supplementary material.

Total fatty acid concentrations are reported in Table 1. Concentrations in sediments ranged from 19.3 to 46.5 μg/g and are typical for sediments along productive continental margins (Schulte et al., 2000; Niggemann and Schubert, 2006). For POM samples, concentrations were relatively high in the surface samples (9.4 ng/l at SBB, 8.2 ng/l at SMB, and 8.4 ng/l at GSC) and decreased rapidly in the upper ~100 m. Surface samples (7 m depth) were greenish-brown and presumably represent mainly living biomass. At all stations, fatty acid concentrations

reach a mid-depth minimum and exhibit a slight increase in the deepest samples. This increase could reflect a contribution from resuspended sediments, derived from either directly below the sampling station or from the basin slopes. However, as discussed below the isotopic compositions of some fatty acids in these deep POM samples do not match those of the underlying sediments or of sediments from the basin flank (C. Li, unpublished data). We also find no evidence (such as long chain fatty acids) for increased terrestrial inputs to these deepest samples, as might result from dense hyperpycnal stream discharge during storm events (Warrick and Milliman, 2003). Seasonal anoxia in SMB and SBB could contribute to the observed concentration increase in those basins by enhancing lipid preservation, but this mechanism is not applicable to GSC. Thus we cannot currently offer a satisfactory explanation for the observed patterns of increasing fatty acid concentration deep in the water column.

The molecular compositions of fatty acids as a function of depth are summarized in Fig. 3. In all samples, saturated even carbon number fatty acids (14:0, 16:0, and 18:0) comprised the largest fraction of total fatty acids. The most abundant fatty acid detected was 16:0, typically accounting for 25–30% of total fatty acids and in one case (GSC-A2) up to 44% of total fatty acids. Mono- and di-unsaturated fatty acids (16:1, 18:1, 18:2) contained roughly equal proportions of those three compounds, and decreased with depth at approximately the same rate as the saturated fatty acids.

Polyunsaturated fatty acids (PUFA; 18:3, 20:5, and 22:6) are abundant in surface POM samples from all three stations, accounting for 10–27% of total fatty acids. At SBB1 and SMB1, PUFA concentrations drop rapidly below the mixed layer, accounting for <2% of fatty acids in the deepest samples. This is consistent with previous observations showing that PUFA are preferentially degraded relative to their saturated counterparts (Wakeham, 1995; Harvey and Macko, 1997; Wakeham et al., 1997). However, at station GSC1 PUFA remain an appreciable fraction (typically 7–10%) of the total fatty acids even in the deepest samples, possibly indicating production of PUFA at depth. High PUFA concentrations at depth have previ-

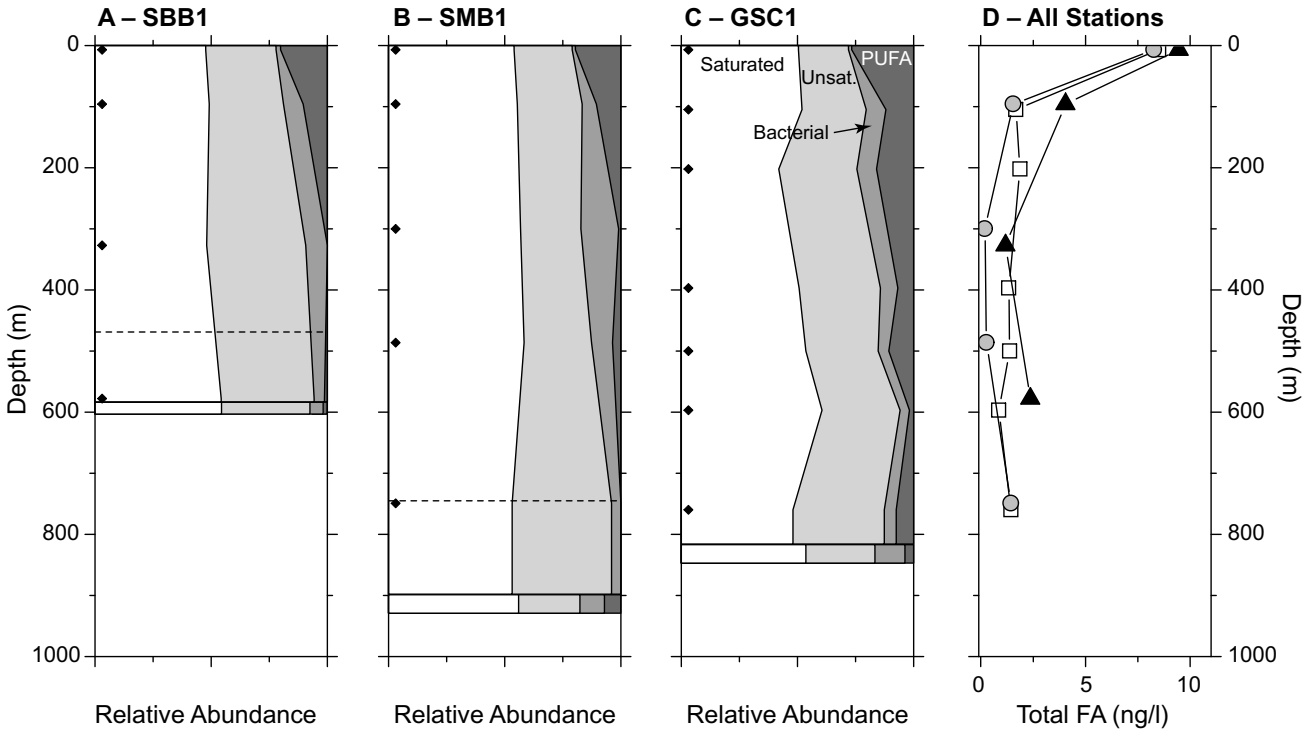

Fig. 3. Abundance and distribution of fatty acids. Panels A–C plot the relative abundance of saturated fatty acids (14:0, 16:0, 18:0; white), mono- and di-unsaturated fatty acids (16:1, 18:1, 18:2; light gray), bacterial fatty acids (*i*-15:0, 15:0, *cyc*-17:0; medium gray), and PUFA (18:3, 20:5, 22:6; darkest gray). Black diamonds mark sampling depths for POM. Surface sediment data are shown in the bar at the bottom of each graph. Panel D plots the concentration of total fatty acids (ng/l) at the three stations: SBB1 (black triangles), SMB1 (gray circles), GSC1 (white squares).

ously been reported in the tropical North Pacific (Wakeham and Canuel, 1988).

Fatty acids presumed to be of bacterial origin (*i*-15:0, 15:0, and *cyc*-17:0, Perry et al. (1979)) account for only 1–2% of total fatty acids in surface POM samples, but rise in relative abundance below the mixed layer at all three stations, accounting for 7–16% of total fatty acids at a depth of ~300 m. *Cyc*-17:0 increased in absolute abundance by 3- to 9-fold between 7 m and 100 m depth at all stations, whereas *i*-15:0 and 15:0 decreased slightly in absolute concentration with depth. These data are consistent with the idea that *cyc*-17:0 is produced in part by heterotrophic bacteria living below the thermocline (De Baar et al., 1983), whereas *i*-15:0 and 15:0 apparently have a stronger source near the surface.

The molecular composition of fatty acids in surficial sediments was generally similar to that of deep POM samples (Fig. 3), typically with slightly more bacterial and saturated fatty acids and slightly less unsaturated fatty acids in the sediments. These distributions are comparable to those observed for SMB sediments by Gong and Hollander (1997) and Pearson et al. (2001), with the notable exception that PUFA within Santa Monica sediments were not reported by either study. This omission may be attributed to the harsh saponification conditions employed by those studies.

3.2. Fatty acid D/H ratios

Fatty acid δD values are reported in Table 2 and Fig. 4. The positional isomers of 16:1 FAME partially coeluted during D/H analyses, so a single composite δD value is reported. Also, because 18:1, 18:2, and 18:3 FAMEs were not sufficiently resolved, a single composite δD value is reported for the corresponding fatty acids as '18:*x*'.

Large ranges in δD values of fatty acids from POM were observed at all three stations, presumably reflecting the range of potential sources and processes contributing to fatty acids in the marine environment. Values of δD for all fatty acids range between −73‰ and −244‰, a span of 171‰, while values for individual fatty acid structures (across all samples) span as much as 76‰. Isotopic differences were the most pronounced between differing

Table 2
Fatty acid δD values (‰)

Sample ID[a]	14:0	*i*-15:0	15:0	16:1	16:0	*cyc*-17:0	18:x	18:0	20:5	22:6
Santa Barbara Basin (34° 13′N, 120° 46′ W)										
SBB1-B1	−210 (12)[b]			−168 (9)	−190 (10)		−165 (5)	−210 (10)	−196	−193
SBB1-B6	−189 (10)	−73 (6)	−114 (23)	−184 (3)	−196 (17)	−167 (7)	−169 (9)	−212 (15)	−187 (12)	−159 (16)
SBB1-A6	−158 (2)[c]	−80 (12)[d]		−171 (3)	−207 (17)	−173 (3)	−189 (11)	−233 (23)[c]		
SBB1-B3	−144 (7)[c]	−96 (13)[d]		−168 (7)	−205		−144 (13)	−229 (15)[c]		
SBB1-MC3	−201 (7)	−138 (3)	−145 (17)	−204 (4)	−192 (12)		−204 (6)	−165 (1)	−216 (2)[c]	−176 (4)
Santa Monica Basin (33° 39′N, 118° 46′W)										
SMB1-B3	−212	−132		−186[d]	−185		−159		−192	−181[c]
SMB1-B6	−146			−162	−180		−170	−204		
SMB1-A6	−182 (4)			−170 (9)	−218 (9)		−203 (1)	−229 (8)		
SMB1-B5	−197 (1)			−187 (4)	−226 (29)	−189	−232 (9)	−237 (18)[c]		
SMB1-A5	−166			−160	−196		−168[d]	−231[c]		
SMB1-MC3	−201 (5)			−191 (3)	−184 (5)		−161 (4)	−171 (3)		
Gulf of Santa Catalina (33° 7′N, 117° 44′W)										
GSC1-B5	−220			−201[d]	−197		−179		−207[c]	−200
GSC1-A5	−181	−92	−161[d]	−164	−195		−170	−229[c]	−188[d]	−176
GSC1-B4	−199[c]	−98[c]	−120[c]	−172	−219	−169[d]	−215[d]		−175[d]	−149[d]
GSC1-A4	−214 (2)	−105 (2)[d]	−143[c]	−197 (5)	−211 (5)	−173[d]	−210 (2)		−188 (9)	−170 (3)
GSC1-B2	−208 (11)[c]	−105[d]	−126 (10)	−196 (12)	−226 (4)	−183 (11)[d]	−222 (3)	−236 (1)[c]	−191[d]	−171[d]
GSC1-A2	−174[c]		−154[c]	−191	−188	−179[d]	−216	−223[c]		
GSC1-A6	−204 (3)[c]	−107[d]	−153[d]	−195 (3)	−232 (5)	−165[d]	−229 (2)	−236 (3)[c]		
GSC1-MC3	−198 (2)	−107 (3)	−136 (7)	−175 (4)	−180 (2)	−177 (7)[d]	−147 (4)	−166 (2)		−124 (18)

[a] Samples ending in "MC" are multicore sediment samples, all others are POM filters.
[b] Standard deviation of multiple analyses is given in parentheses. Where no value is shown, only one analysis was possible.
[c] Peak coeluted with another, less abundant (<10% peak area) compound.
[d] Irregular background around peak; uncertainty is estimated at 15‰.

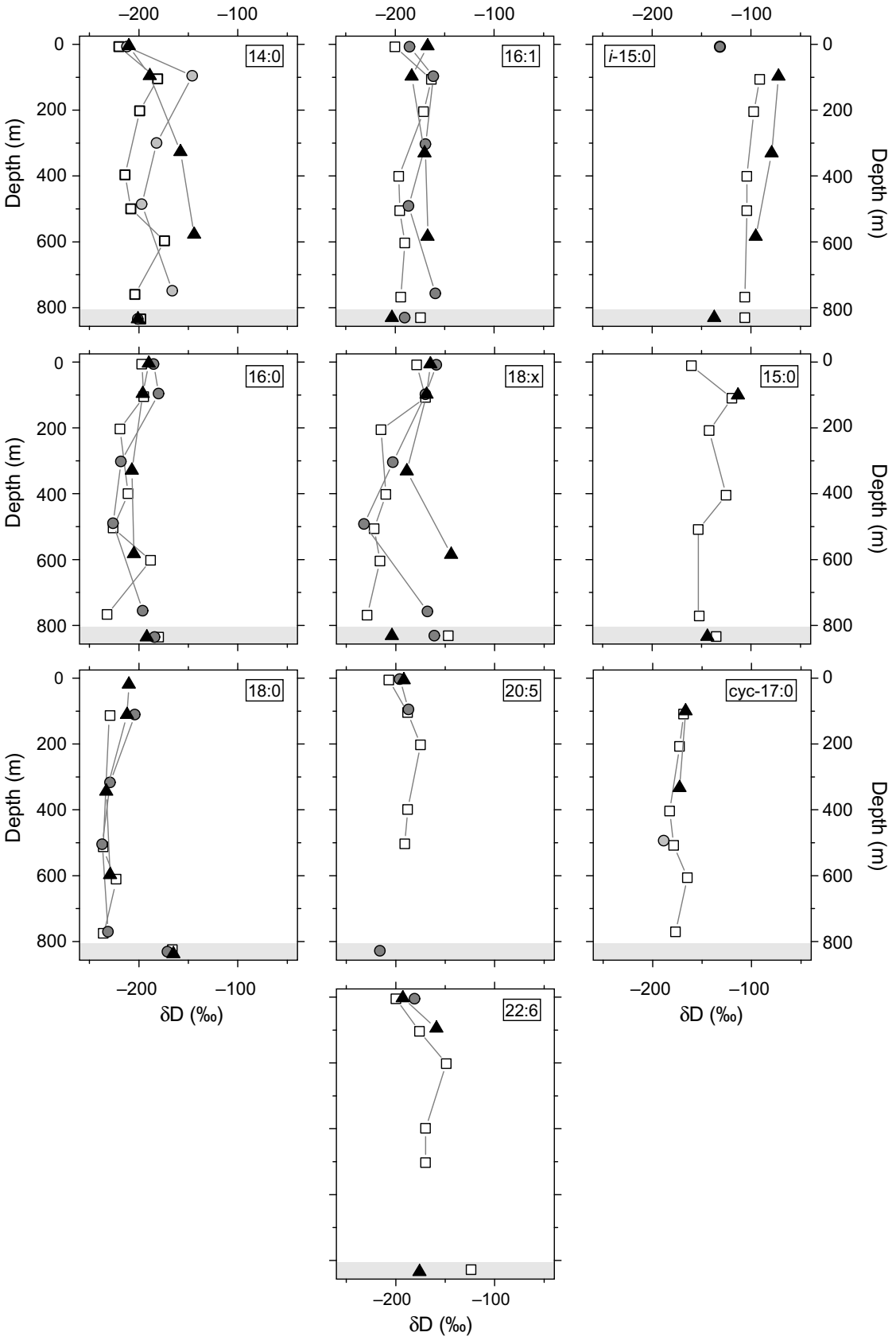

Fig. 4. Fatty acid δD values versus depth in SBB1 (black triangles), SMB1 (gray circles), and GSC1 (white squares). Each panel represents the data from all three sample locations for a specific molecular structure. Surface sediment data are shown in the gray bar at the bottom of each graph. Error bars are about the same size as symbols.

molecular structures, with relatively smaller changes apparent between sampling locations and depths. *i*-15:0 was consistently the most D enriched compound, with δD values ranging from −73‰ to −132‰, while 18:0 was consistently the most D depleted, with δD values ranging from −204‰ to −232‰ (Table 2). Other fatty acids fall between these two extremes. Values of δD for sedimentary fatty acids generally followed a similar pattern as for POM, with large differences between different molecular structures (total range −102‰ to −205‰) but only minor differences between sampling locations.

Such enormous variability among a class of closely related compounds is surprising, particularly given that none of it can be attributed to variations in source water. Our fundamental conclusion therefore is that large H isotopic signals do indeed exist in marine particle associated fatty acids, and should be a useful tool for studying biogeochemical processes. Which processes lead to this variability? Differing biosynthetic fractionations, physical mixing of sources, degradation, dissolution, and other potential mechanisms are all possible, and we examine these next.

3.3. D/H changes with depth

The in-situ filtration technique used here collects primarily suspended rather than sinking particles. It is thus strictly incorrect to interpret the depth profiles in Fig. 4 as equivalent to age profiles. The composition of POM at any given depth will be influenced by lateral advection, microbial production and processing, exchange between sinking and suspended matter, and many other factors (Wakeham and Canuel, 1988; Volkman and Tanoue, 2002; Lee et al., 2004). This point was emphasized by Williams et al. (1992), who showed that sinking and suspended POM isolated from SMB have very different $\delta^{13}C$, $\Delta^{14}C$, and $\delta^{15}N$ values. Nevertheless, bulk suspended POM does get older with depth, ranging from $\Delta^{14}C = +110‰$ at 80 m to −46‰ at 700 m depth (Williams et al., 1992). These particles should therefore reflect changes associated with particle aging, albeit with effects from other processes potentially superimposed. It remains a possibility that specific fatty acids (e.g., *cyc*-17:0) are derived almost entirely from bacteria living on the particles, turn over rapidly, and thus do not reflect the aging of the associated particles. Nevertheless, that caveat is unlikely to apply to the most abundant fatty acids (e.g., 16:0, 18:0), which almost certainly have their origins in the surface mixed layer.

To quantitatively explore isotopic changes with depth, we regressed δD values on depth for each fatty acid in each basin. Surface samples were not included in this analysis because they represent live biomass rather than detrital organic matter. Of the 21 fatty acid profiles with 3 or more data points, the slope of the regression was significantly different than zero at the 95% confidence level only for 15:0, 16:0, 16:1, and 18:*x* in GSC1. Average rates of change in δD for these 4 fatty acids were −5.9‰, −2.7‰, −4.7‰, and −8.5‰ per 100 m, respectively. It remains uncertain whether these trends reflect true biogeochemical phenomena that are hidden within the other profiles (which contained fewer sampling depths), or whether they represent statistical outliers in a more general trend of no significant change.

Regression of δD values against temperature and salinity for each station yielded only two significant correlations: 18:*x* and *i*-15:0 at station GSC1 are both correlated with salinity (in practical salinity units) at the 95% confidence interval. δD values for 18:*x* and *i*-15:0 exhibit a trend of −136‰/PSU and −41‰/PSU, respectively, over a total salinity range of 0.4 PSU. A correlation between alkenone δD and growth media salinity has been recently observed (Schouten et al., 2006) for the algae *Emiliania huxleyi* and *Gephyrocapsa oceanica*, but that trend was only ∼3‰/PSU over a range of ∼10 PSU and in the opposite direction to the trends observed in our data. It seems unlikely that salinity induced changes in biosynthetic D fractionation can explain our observations per se. More likely, salinity simply serves as a tracer for distinct sources of fatty acids associated with different water masses.

A third useful comparison is that of δD values from surface (7 m) samples versus those just below the thermocline (∼100 m). This depth interval spans the largest change in fatty acid concentration (Fig. 3), accompanied by the decomposition of living organisms and packaging of their lipids into POM. It is also the depth interval least susceptible to the effects of lateral advection of POM, and to the exchange of organic matter between sinking and suspended particles. For the 18 instances in which a specific fatty acid was measured at both 7 m and 100 m depths at a single station, the mean difference in δD values (100 m–7 m) was 16 ± 22‰ with deeper samples tending to be D enriched. Only one fatty acid, 14:0, exhibited a consistent,

significant enrichment at all three sampling stations (mean: $42 \pm 22‰$, $n = 3$).

Finally, as a test of the potential influence of low O_2 bottom waters on lipid D/H ratios, we compared δD values for the deepest sample depth at each station to the 100 m depth at the same station. For the 19 instances in which individual fatty acid δD measurements were made at both 100 m and the deepest sample in each basin, the mean difference in δD values (100 m – bottom) was $12 \pm 23‰$, with bottom samples tending to be D depleted. The trends in D depletion were inconsistent across basins and specific fatty acids, and so we find no evidence to support the hypothesis that water oxygen levels influence fatty acid δD values.

From this analysis we conclude that isotopic changes in particle associated fatty acids with depth in the water column are small or nonexistent. There is a general trend towards slight D depletion with depth in most of the fatty acids, though this trend is statistically significant in only 4 of the 21 individual lipid profiles. Changes in δD between surface waters and the base of the mixed layer are more variable. In instances where changes across the mixed layer are evident, they tend to reflect small D enrichments with depth. There is no evidence for a systematic change in fatty acid D/H associated with low O_2 bottom waters, including among the bacterial fatty acids.

3.4. Patterns of intermolecular fractionation

The δD values of fatty acids associated with POM span a range of 171‰. To facilitate comparison of variations between different molecular structures, three average δD values were computed for each fatty acid: (i) surface POM samples, (ii) 'deep' POM samples, including all depths below the mixed layer, and (iii) surface sediment samples. Each average includes data from all three sampling locations. These depth averaged values exhibit systematic isotopic ordering with several remarkable features (Fig. 5). First, two fatty acids of likely bacterial origin (*i*-15:0 and 15:0) are always D enriched relative to fatty acids of likely eukaryotic origin (14:0, 16:0, 16:1, 18:0, 18:*x*), in some cases by more than 100‰ (Fig. 5). A third bacterial fatty acid (*cyc*-17:0) is not D enriched, and was similar in δD value to 14:0 and 16:1 in most samples. Second, even carbon numbered fatty acids from deep POM show a consistent pattern of D depletion with increasing chain length (Fig. 5B), while those same fatty acids in sediments exhibit a nearly opposite pattern (Fig. 5C). The pattern in surface POM appears most similar to that in sediments, but with 18:0 as an outlier. Third, unsaturated fatty acids are uniformly enriched in D relative to their saturated counterparts in deep POM samples, whereas they are nearly identical in sedi-

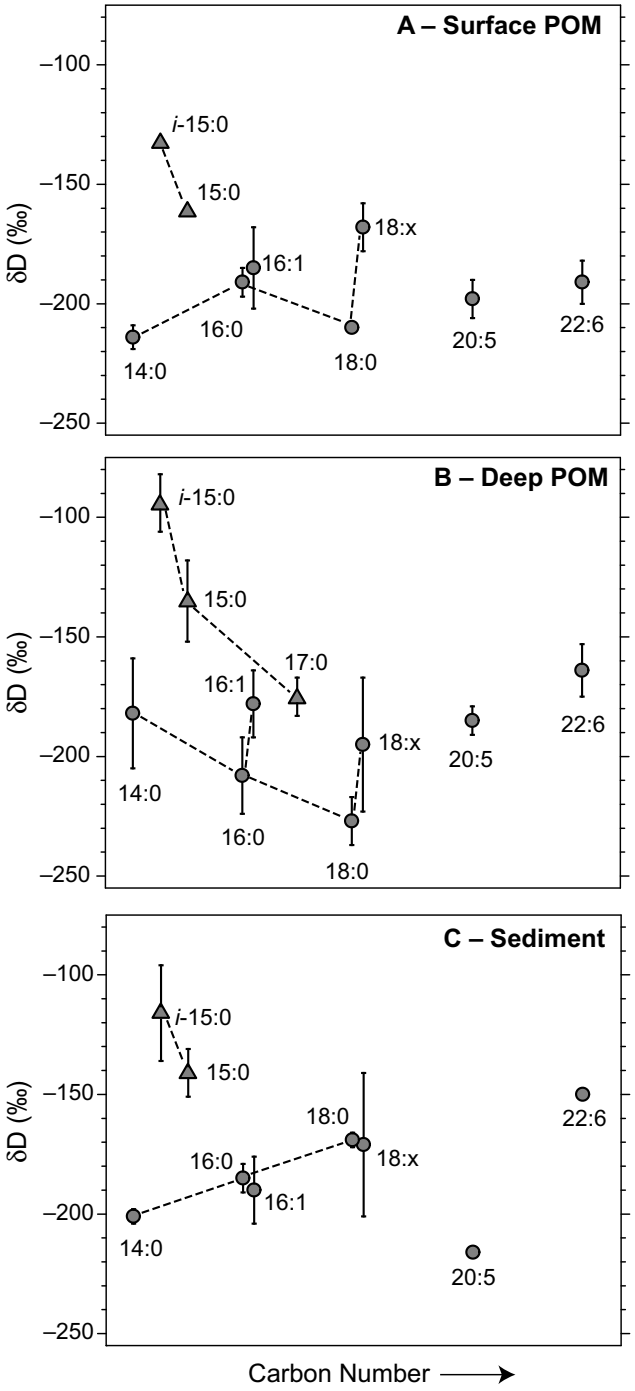

Fig. 5. Average δD values for fatty acids from surface POM, deep POM, and surface sediment samples. Error bars indicate the total range of observed values. Dashed lines indicate typical biosynthetic relationships between compounds, but do not necessarily imply common origins for the fatty acids.

ment samples. These patterns are examined in detail below.

3.4.1. Source mixing

How should we interpret the large variability in D/H ratios demonstrated in Fig. 5? While it might be interpreted to reflect highly variable biosynthetic fractionations between similar organisms (i.e., phytoplankton), it might also reflect simple mixing of two distinct sources with different D/H ratios, such as marine versus terrestrial, or algal versus bacterial sources. We address this latter possibility first. At all three sampling locations, there is a moderate, inverse correlation between sediment fatty acid δD values and concentration (Fig. 6A). Similar correlations are present in deep POM from SBB1 and GSC1 but not SMB1 (Fig. 6B). No correlation is observed in surface POM from any station (data not shown). These trends are strongly defined by the low-concentration fatty acids, which are mostly those of presumed bacterial origin with more D enriched isotopic compositions. The absence of such a correlation in deep POM from SMB1 can be readily attributed to the fact that bacterial fatty acid concentrations are quite low at SMB1, and as a result there are no corresponding δD values in our dataset. Likewise, there are only two data points for bacterial fatty acids in all three surface samples.

The inverse correlation of δD and concentration is entirely consistent with mixing of fatty acids from two isotopically distinct sources (Hayes, 1983). Based on molecular structures, one appears to be algal with a δD value near −200‰, and the other bacterial with a δD value near −100‰. Correlation coefficients (Fig. 6) indicate that such a 2-member mixing model could explain roughly half the variance in deep POM data, and nearly three quarters of the variance in sediment data. On the other hand, there are clearly some problems with this model, for example that it cannot plausibly explain the D/H variations among fatty acids with solely bacterial sources. We conclude that while mixing of sources with different characteristic D/H ratios is clearly an important process in our dataset, it cannot explain all of the variability we observe.

3.4.2. Bacterial fatty acids

The observation that two of three bacterial fatty acids are strongly D enriched is particularly puzzling, and has two possible explanations: either there is a significant terrestrial source for the most enriched compounds, or select groups of marine bacteria have unusually small lipid/water fractionations, or both. The southern California coastal landscape is generally arid, and significant evaporative enrichment of D in soil bacteria living there is

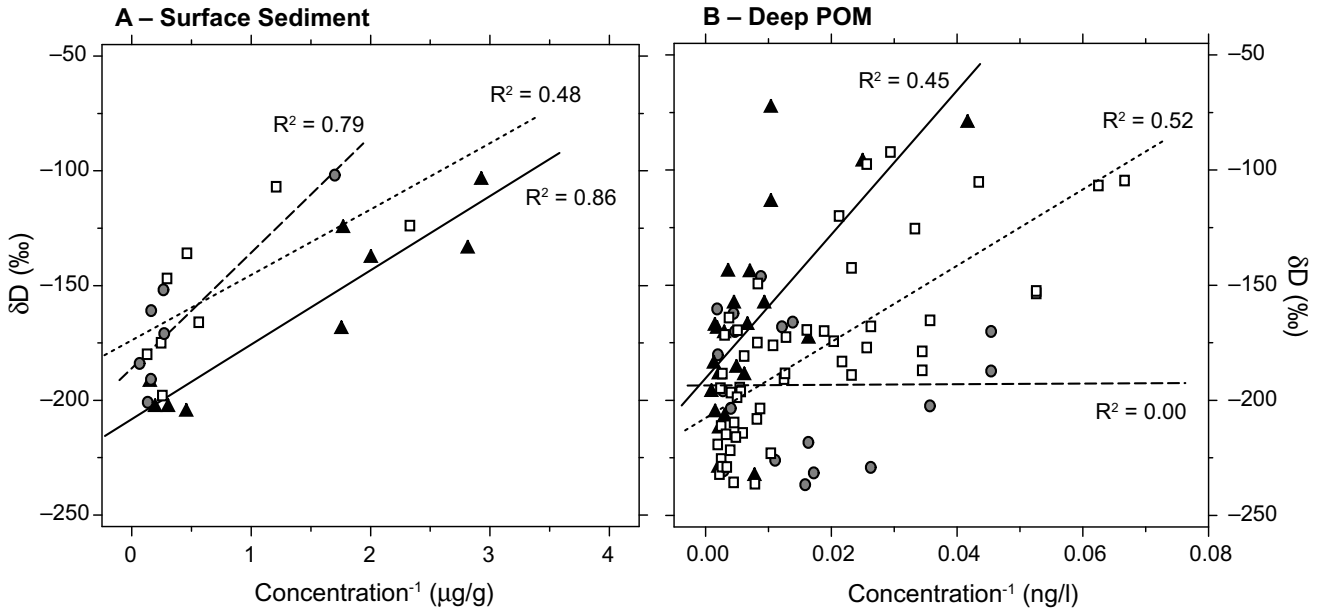

Fig. 6. Fatty acid δD values plotted against inverse concentration for sediments (panel A) and deep POM (panel B). Correlation coefficients pertain to the linear regression of data from each sample location: SBB1 (black triangles, solid line), SMB1 (gray circles, dashed line), and GSC1 (white squares, dotted line).

plausible even though no relevant D/H data for terrestrial soil bacteria are currently available. The pattern of decreasing δD with chain length among bacterial fatty acids can also be explained by varying mixtures of terrestrial and marine sources, with longer-chain acids becoming more dominantly marine. This would be consistent, for example, with the increase in absolute concentration of *cyc*-17:0 at depth but not of *i*-15:0 (Table S-1). It is also consistent with the ^{14}C data of Pearson et al. (2001) from Santa Monica Basin. In surface sediments, $\Delta^{14}C$ values for *i*-15:0 and 15:0 were +33 and +44‰, whereas for 16:0 and 18:0 they were +86 and +83‰, respectively. The two bacterial fatty acids are thus systematically older than the putative algal fatty acids, consistent with a terrestrial origin. $\Delta^{14}C$ values for *i*-15:0 were also virtually identical to those of the *n*-C_{29} alkane (+30‰), interpreted by Pearson and Eglinton (2000) as having an almost entirely terrestrial origin. Arguing against this interpretation, we see no evidence in our samples of a significant terrestrial plant contribution (long chain fatty acids are absent, for example).

The alternative explanation is no less surprising. A systematic D enrichment of bacterial fatty acids relative to those of eukaryotes has not previously been reported. There are, however, very few published studies of D/H fractionations by marine bacteria for comparison. Cultures of *Escherichia coli* (the only aerobic heterotroph studied thus far) grown on glucose exhibit fatty acid – water fractionations ranging from −117‰ to −146‰ for all fatty acids (A. Sessions, unpublished data). Assuming a seawater δD value of ~0‰ for our sampling locations, these fractionations are similar to those for *i*-15:0 and 15:0 in POM and sediments. For *E. coli*, there is no significant change in δD values with fatty acid chain length, or between odd- and even carbon number fatty acids. Unsaturated fatty acids are moderately depleted in D relative to their saturated counterparts, opposite the pattern observed for deep POM.

In four species of anaerobic, sulfate-reducing bacteria grown both autotrophically on H_2 and heterotrophically on acetate or lactate, fractionations between fatty acids and water ranged from −99‰ to −298‰ (A. Sessions, unpublished data). The average fractionation for all heterotrophic experiments was −179‰ ($n = 39$), larger than that observed for bacterial fatty acids in the current study. The pattern of intermolecular isotopic ordering was one of increasing D enrichment with chain length. Odd- numbered fatty acids had δD values similar to those of even-numbered compounds.

Several other H isotopic studies of bacteria are also available for comparison. These include the homoacetogen *Sporomusa* sp. strain DMG 58 (Valentine et al., 2004), the aerobic H_2-oxidizer *Cupriavidus necator* (Campbell, 2007), the sulfate-reducer *Desulfobacterium autotrophicum* grown both on $H_2 + CO_2$ and on formate (Campbell, 2007), and the aerobic methanotroph *Methylococcus capsulatus* (Sessions et al., 2002). In general, organisms utilizing H_2 are found to exhibit fatty acid-water fractionations larger than those observed in POM, while *M. capsulatus* exhibited smaller fractionations. Because such organisms likely contribute very little to total fatty acids in POM, these results serve only to demonstrate that significant variability in the biosynthetic fractionation of hydrogen isotopes does occur amongst different bacteria. Thus a systematic enrichment of D in *i*-15:0 compared to *cyc*-17:0 does not negate the possibility that both are products of marine heterotrophic bacteria.

3.4.3. D/H fractionations in deep POM

One of the most striking features of deep POM data is the systematic ordering of fatty acid δD values according to chain length and degree of unsaturation (Fig. 5B). Particularly interesting is the fact that the chain length pattern is reversed in sediments relative to deep POM (compare Fig. 5B and C), and the offset in saturated versus unsaturated counterparts seen in deep POM disappears in sediments. We first consider whether these patterns of isotopic ordering might reflect fractionations associated with biosynthesis. The most detailed study to date of intermolecular H isotopic ordering of fatty acids is for macrophyte brown and red algae (Chikaraishi et al., 2004c). These species produce fatty acids with δD values that increase with chain length and decrease with degree of unsaturation. More generally, a broad survey of the published literature reveals that in every organism studied to date, δD values of acetogenic lipids remain roughly constant or increase with chain length. Relevant data are available for C3 and C4 terrestrial plants, aquatic plants, macroalgae, diatoms, dinoflagellates, coccolithophores, green algae, and bacteria (Sessions et al., 1999; Chikaraishi et al., 2004a; Chikaraishi et al., 2004b; Chikaraishi et al., 2004c; Chikaraishi and Naraoka, 2005; Chikaraishi and Naraoka, 2006; Schouten et al., 2006; Sessions, 2006; Smith

and Freeman, 2006; Zhang and Sachs, 2007). Thus both surface POM and sediment samples from our study display patterns of intermolecular fractionation that are typical of those observed in living organisms. However, the pattern observed in deep POM (decreasing δD with chain length) has no known counterpart in living organisms, and is unlikely to reflect biosynthetic processes.

While searching for alternative explanations, we observed that δD values for deep POM fatty acids are qualitatively related to their aqueous solubility. Fatty acid solubility increases regularly with shorter chain length (Robb, 1966) and with the addition of carbon–carbon double bonds. Similarly, fatty acids from deep POM tend to become D enriched with shorter chain length and increasing unsaturation (Fig. 5B). Accurate data for the solubility of fatty acids in seawater are not available, so as a proxy we use the partition coefficients (K_p) measured by Anel et al. (1993) for the partitioning of fatty acids between live bacterial cells and water. While the measured values of K_p are obviously not directly applicable to natural marine systems, the relative order of solubility should be similar. Values of δD for deep POM fatty acids are strongly correlated with $1/K_p$ ($K_p = [FA]_{solution}/[FA]_{membrane}$), indicating a systematic enrichment of D with increasing aqueous solubility (Fig. 7). The 20:5 PUFA is an obvious outlier in this trend.

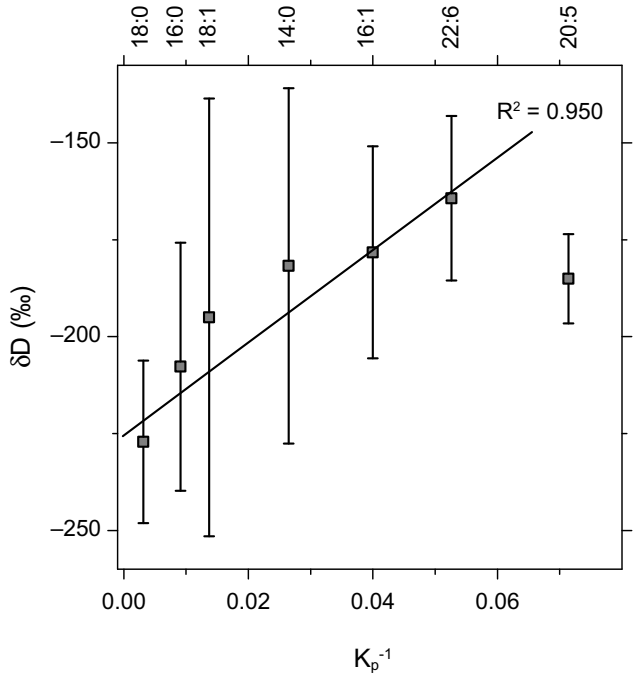

Fig. 7. Average fatty acid δD values versus the inverse membrane/water partition coefficient (K_p) measured by Anel et al. (1993). K_p is directly proportional to the aqueous solubility of a fatty acid. Error bars denote the 2σ range across the mean values.

The mechanism by which solubility (or some other correlated physical property) might affect deep POM remains uncertain. Kinetic fractionations accompanying dissolution and/or degradation are possible, and a 'normal' kinetic isotope effect would lead to preferential degradation of D depleted compounds (Hayes, 1993). Pond et al. (2002) studied D/H fractionation during the laboratory biodegradation of *n*-alkanes, and observed that the residual alkanes became D enriched by up to 25‰. Degradation was more rapid for shorter chain compounds, and the magnitude of D/H fractionation was greater for shorter chain compounds. These observations provide a plausible mechanistic link between marine fatty acid δD values and solubility, namely that more soluble compounds are degraded more rapidly and with a larger isotopic fractionation. Data for PUFA – thought to be of entirely marine origin, and among the most labile compounds – are also consistent with this hypothesis. On the other hand, there is no evidence for increasing δD values with depth in the water column, in spite of the fact that concentrations decrease dramatically. Moreover, fatty acid concentrations and K_p are uncorrelated ($R^2 = 0.04$) in all samples. If solubility is indeed a proxy for rates of microbial degradation, then additional processes must exert significant control over particulate fatty acid concentrations.

Partitioning of fatty acids between different types of particles (e.g., mineral versus organic, suspended versus sinking), or between adsorbed and dissolved phases, is another possible mechanism linking δD values with solubility. Although direct experimental data for relevant fractionation factors are not available, chromatographic separation of deuterated fatty acids is often observed in reverse phase HPLC (reviewed by Filer, 1999). In this case, the deuterated compound always leads the undeuterated compound, indicating that the addition of D increases aqueous solubility. This in turn should cause dissolved phase fatty acids to be D enriched relative to the adsorbed phase, thus decreasing δD values of particulate fatty acids with increasing solubility. The opposite trend is observed in our deep POM samples, in contradiction to this hypothesis. Partitioning between other reservoirs remains a possibility.

4. Conclusions

We have measured δD values for 12 different fatty acids extracted from POM and surface sediments at three coastal marine locations. Comparison of data between locations, across depth transects, and between differing molecular structures leads to the following conclusions.

1. Significant D/H variability exists between different fatty acids (~170‰ total range). These isotopic contrasts should prove useful for studying marine biogeochemical cycling of organic matter, including the distinction of marine versus terrestrial, and algal versus bacterial sources.
2. There are no systematic D/H variations associated with seasonally anoxic bottom waters. There are few obvious variations with depth, but those that are statistically significant indicate a slight D enrichment of compounds through the thermocline, and a slight D depletion with depth below the thermocline.
3. Certain bacterial fatty acids (*i*-15:0 and 15:0) are strongly D enriched by up to 130‰ relative to even carbon numbered fatty acids in all samples. This observation could be explained by a systematically smaller D/H fractionation in certain marine bacteria living in the mixed layer, or by a significant terrestrial source for these compounds, or both. Regardless, it is clear that even carbon numbered fatty acids are not derived from the same organisms producing *i*-15:0 and 15:0, either in POM or in sediments.
4. In deep POM samples, fatty acid δD values decrease systematically with chain length and increase with unsaturation. This pattern of isotopic variation is opposite that observed in all living organisms, and so is unlikely to be biosynthetic in origin. The D enrichment is correlated with the aqueous solubility of individual fatty acids, but the mechanism(s) responsible are not yet identified.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.orggeochem.2007.11.001.

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