Inverse relationship between D/H fractionation in cyanobacterial lipids and salinity in Christmas Island saline ponds

Dirk Sachse *, Julian P. Sachs

School of Oceanography, University of Washington, Seattle, WA, USA

Received 30 August 2007; accepted in revised form 15 November 2007

Abstract

Sediments from 28 saline and hypersaline (salinity 13.6–149.2) ponds on Christmas Island (Kiritimati), in the Central tropical Pacific Ocean, were investigated for the effect of salinity on the D/H ratios of lipid biomarkers. Hydrogen isotope ratios (expressed as δD values) of total lipid extracts, and individual hydrocarbons heptadecane, heptadecene, octadecane, octadecene, diploptene, and phytene from cyanobacteria, became increasingly enriched in deuterium as salinity increased, spanning a range of 100 ‰, while lake water δD values spanned a range of just 12 ‰. Net D/H fractionation between lipids and source water thus decreased as salinity increased. Isotope fractionation factors (\(a_{lipid-water}\)) were strongly correlated with salinity, and increased in all compound classes studied by up to 0.0967 over a salinity range of 136. Differences in the hydrogen isotopic composition of lipids derived from three biosynthetic pathways (acetogenic, mevalonate, and non-mevalonate) remained similar irrespective of the salinity. This suggests that the mechanism responsible for the observed \(a_{lipid-water–salinity}\) relationship originates prior to the last common biosynthetic branching point, the Calvin Cycle. We propose that a decrease in the exchange of intra- and extra-cellular (ambient) water resulting from down-regulation or closure of water channels (aquapores) within cyanobacterial cell walls, and subsequent isotopic enrichment of the intracellular water, likely resulting from metabolic reactions. These findings imply that caution must be exercised when attempting to reconstruct source water δD values using lipid δD values from environments that may have experienced salinity variations. On the other, hand our results can be used to establish a paleo-salinity proxy based on lipid δD, if additional constraints on source water δD values can be made.

© 2007 Elsevier Ltd. All rights reserved.

1. INTRODUCTION

It has been shown recently, that the stable hydrogen isotopic composition (expressed as δD value, where δD = \(\frac{R_{sample}}{R_{standard}}\) × 1000; with R being the ratio between the abundances of deuterium and protium, \(R = \frac{D}{H}\)) of a variety of lipid biomarkers can be used to infer the hydrogen isotopic composition of the water used by photosynthetic organisms (Sessions et al., 1999; Sauer et al., 2001; Chikaraishi and Naraoka, 2003; Huang et al., 2004; Sachse et al., 2004; Englebrecht and Sachs, 2005; Zhang and Sachs, 2007). It has been postulated that the net amount of hydrogen isotopic fractionation between source water and lipids is primarily a function of the biosynthetic pathway, with an as yet unquantified role for environmental factors (Sessions et al., 1999). In terrestrial vascular plants an additional influence on D/H ratios in lipids is the amount of evaporation of leaf water, largely a function of relative humidity and plant physiology (Sachse et al., 2006; Smith and Freeman, 2006). These results have been used to reconstruct changes in the hydrological cycle over various geological timescales (Andersen et al., 2001; Schefuss et al., 2005; Pagani et al., 2006; Pahnke et al., 2007). However, the biological mechanisms and possible responses to environmental conditions that influence the hydrogen isotopic composition of lipid biomarkers remain poorly understood.

* Corresponding author. Present address. Universität Potsdam, Institut für Geowissenschaften, Leibniz Center for Earth Surface and Climate Studies, Karl-Liebknecht-Str. 24–25, 14476 Potsdam, Germany.
E-mail address: dsachse@geo.uni-potsdam.de (D. Sachse).

0016-7037/S - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.gca.2007.11.022

Recent laboratory studies on algal lipids have found significant differences in net isotopic fractionation between fatty acids from different species of freshwater algae (Zhang and Sachs, 2007). Another study showed that salinity and growth rate, but not temperature, influenced D/H fractionation between source water and alkenones produced by two species of coccolithophorids in batch cultures (Schouten et al., 2006). While the use of species-specific lipid biomarkers can circumvent the problem of variable net D/H fractionation in different species, the influence of salinity on D/H fractionation in lipid biomarkers in the natural environment is unstudied. Further work is also needed on the effects of salinity changes outside the salinity range of 25 to 35% used by Schouten et al. (2006), and in lipids and species other than alkenones from the coccolithophorids Emiliania huxleyi and Gephyrocapsa oceanica. For these reasons, we targeted the hypersaline lakes on Christmas Island to study the influence of salinity on D/H fractionation in cyanobacteria.

Cyanobacteria are responsible for a substantial fraction of primary production in the ocean today (Lochte and Turkey, 1988) and complex microbial communities, organized in microbial mats, are found today in the most extreme environments, such as hypersaline lakes. It is hypothesized that such environments may be analogs for the origin of life on Earth and elsewhere (Simonett et al., 1998). The widespread occurrence of cyanobacteria makes lipid biomarkers, derived from these photosynthetic organisms, an ideal target for a variety of biogeochemical investigations.

Here, we present an investigation of the hydrogen isotopic fractionation between cyanobacterial lipids and environmental water for the three major lipid biosynthetic pathways along a natural salinity gradient of 13.6 to 149.2‰ in surface sediments and microbial mats from 28 lakes on Christmas Island in the central equatorial Pacific Ocean to evaluate the possibility of using lipid δD values as a proxy for salinity.

2. MATERIAL AND METHODS

2.1. Sampling sites and methods

Christmas Island is part of the Northern Line Islands in the Republic of Kiribati and lies at 15°52′N and 157°20′W (Fig. 1). It is the world’s largest coral atoll with a surface area of 360 km² (Valencia, 1977). The island’s climate is uniformly dry with a long-term net rainfall minus potential evaporation value of ~2 mm/year (Saenger et al., 2006 and references therein). A quarter of the island is covered by hypersaline ponds of various salinities, ranging from the local seawater value (38) up to 150, or higher depending on the year and season. Some ponds are fed by fresh groundwater within the carbonate bedrock and there have lower salinities (e.g., lake 168, see Table 1). Temperatures of the lake waters varied between 25.7 and 34.5 °C in June–July 2005. Lake water pH values were between 6.80 and 8.88 (Table 1). A complete description of the physical properties of the lakes sampled on Christmas Island in June–July 2005 is given in Saenger et al. (2006), locations are shown in Fig. 1.

Surface sediments of most of the lakes consisted of gelatinous bacterial mats from a few mm to half a meter in thickness (Fig. 2). The depths of ponds sampled were between 0.19 and 1.71 m (Table 1). For the purpose of this study, surface sediments were divided into bacterial mats (containing several layers of living microbial communities with at least 5 cm total thickness), and surface sediments (containing abundant mineral matter, mostly carbonate debris). The overwhelming sources of organic matter in both types of sediment were cyanobacteria (Trichet et al., 2001), as also revealed by light microscopy (Fig. 2). The upper layers of microbial mats (between 1 and 5 cm) and surface sediments were recovered using an interface coring apparatus that maintained the integrity of the sediment–water interface and left the mat or sediment surface intact (c.f., Fig. 9 in Saenger et al., 2006), and the upper 1 cm of each core was carefully extruded in the field, bagged, stored frozen prior to freeze-drying and extracted in the laboratory.

2.2. Microscopy

Microscopy was performed with Zeiss Axiosimager A1 microscope and Zeiss Axioscam MRc Imaging system and software (Carl Zeiss GmbH, Germany). Identification of microbial morphotypes was done in accordance with traditional phycological determination manuals (Geitler, 1932; Komárek and Anagnostidis, 1999; Castenholz, 2001; Komárek and Anagnostidis, 2005).

2.3. Analysis of water δD values

Water samples were analyzed for their hydrogen isotopic composition at Dartmouth College using an H-Device (ThermoFisher, Bremen, Germany) connected to a Delta plusXL Isotope Ratio Mass Spectrometer (IRMS, ThermoFisher, Bremen, Germany). Values were corrected to the VSMOW scale, using two laboratory standards with values of −69.4‰ and −234.4‰ (previously calibrated to VSMOW and SLAP) and the VSMOW standard (0‰). Precision of water δD measurement was ±0.5‰. A detailed description of the water isotopes measurement procedure can be found in Zhang and Sachs (2007).

2.4. Sample preparation and biomarker identification and quantification

Between 0.5 and 5 g of dried sediment or mat material was extracted with an Accelerated Solvent Extractor (ASE 200, Dionex Corp., Sunnyvale, CA, USA) with dichloromethane at 100 °C and 103 bar (1500 psi) for 5 min in each of 3 cycles. Total lipid extracts were blown dry under a stream of N₂. A subset of 17 samples was extracted separately using the same conditions in order to conduct compound-specific isotopic analyses. Silica gel chromatography was used to separate total lipid extracts into hydrocarbons (hexane) and more polar materials (dichloromethane and methanol). The hydrocarbon fraction was analyzed on a GC-MSD (Agilent GC 6890N...
connected to an Agilent MSD5975 detector, Agilent, Santa Clara, CA, USA) to identify lipids and assess their purity, then quantified on a GC-FID (Agilent GC 6890N with an FID detector) using an external standard mixture of \( n \)-alkanes (\( n-C_{14} \) to \( n-C_{36} \)).

### 2.5. Analysis of \( \delta D \) values of total lipid extracts

About 100 \( \mu g \) of each total lipid extract (TLE) was transferred into silver capsules (Costech, Valencia, CA, USA) using \( \text{CCl}_4 \)—to avoid contamination by solvent-derived hydrogen. Folded capsules were loaded into a Costech Zero Blank Auto-sampler connected to a Thermo/Finnigan thermochemical elemental analyzer (TC/EA) (ThermoFisher, Bremen, Germany), where they were pyrolyzed at 1400 °C to \( \text{H}_2 \) gas. \( \text{H}_2 \) gas was introduced into a Thermo Electron Corp. Delta V Plus isotope-ratio mass spectrometer (irMS) via a Conflo III combustion interface (ThermoFisher, Bremen, Germany). After each sample was analyzed in triplicate, \( \delta D \) values were normalized to the VSMOW scale using two laboratory standards (UWB-1, benzoic acid, \( \delta D = -64.9\% \) and a \( n-C_{28} \) alkane, \( \delta D = -232.5\% \), that had previously been calibrated to organic reference materials obtained from A. Schimmelmann, University of Indiana) and measured after every six samples. A scale stretch and drift correction (Werner and Brand, 2001) was applied for each sequence (typically 16 samples, including a blank). The average standard deviation (\( \sigma \)) of all measured samples was 3.8‰.

### 2.6. Analysis of \( \delta D \) values on specific lipids

One microliter of the hydrocarbon fraction dissolved in hexane was injected into a TraceGC II gas chromatograph (ThermoFisher, Rodano, Italy) equipped with a DB5ms column (60 m; ID: 0.32 mm; film thickness: 0.25 \( \mu m \), Agilent). The split/spillless injector was operated in spillless mode at 280 °C. The oven was programmed from 80 to 170 °C at 20 °C/min and then to 325 °C at 3 °C/min, where it was held for 20 min. The effluent from the GC entered a high-temperature conversion oven at 1400 °C to pyrolyze the sample quantitatively to \( \text{H}_2 \) gas.
The hydrogen isotopic composition of water in the saline ponds of Christmas Island varied by 11.8‰, from +4.0‰ to +15.8‰ (Fig. 3 and Table 3), over the salinity range 38.5 to 149.2 (practical salinity units). Lake water with a salinity of 13.6 in lake 168, received groundwater from a freshwater lens, which had a δD value of −7.6‰. The lowest δD values for waters with salinities greater than seawater (∼38), +5.4‰ and +4.0‰, were found in the least and most saline lakes respectively (Fig. 3). δD values reached a maximum at a salinity of approximately 60 and declined above that value.

In low humidity environments, an increase in salinity resulting from an excess of evaporation over precipitation is usually accompanied by an increase in water δD values, according to the Rayleigh distillation model (Craig and Gordon, 1965). However, under high humidity conditions, which commonly exists on oceanic islands, (deuterium-depleted) atmospheric moisture exchanges with the evaporating liquid in the air–water boundary layer. Depending on...
the relative humidity, there is an upper limit to deuterium enrichment in the water. At very high salinities, the effect of solutes contributed to D-depletion in water, as the thermodynamic activity of water decreased, effectively reducing the humidity contrast and the rate of evaporation (Gonfiantini, 1986). In addition, the hydration sheath for polyvalent ions became enriched in deuterium relative to the free water such that, in the last stages of evaporation, as salts precipitate, this D-enriched water was removed from free water and preserved in the hydration water of salts (Gonfiantini, 1986). The observed decrease in δD values, at higher salinities in the Christmas Island ponds was consistent with the combined action of these processes. From the standpoint of paleoclimate and paleoenvironmental reconstructions, the polynomial shape of the salinity vs. δDwater curve limits the estimation of salinity from water δD values (to the extent that the latter can be determined) in humid environments when salinities are ~60 or greater.

### 3.2. Lipid identification, concentration, and biological origin

Samples from 17 lakes on Christmas Island were analyzed for their lipid composition to determine the dominant organic matter sources (Table 2). In most samples, the concentration of all lipids in the hydrocarbon fraction was higher in microbial mats than in surface sediments.
Table 2
Concentration of the specific lipids in the analysed samples (in μg/g dry sediment)

<table>
<thead>
<tr>
<th>Lake</th>
<th>Salinity</th>
<th>n-C17</th>
<th>n-C17.1</th>
<th>n-C18</th>
<th>n-C18.1</th>
<th>Phytene</th>
<th>Diploptene</th>
</tr>
</thead>
<tbody>
<tr>
<td>151</td>
<td>38.5</td>
<td>0.06</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>62.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>165</td>
<td>63.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>87.6</td>
<td>12.94</td>
<td>5.79</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>123</td>
<td>98.0</td>
<td>3.45</td>
<td>0.37</td>
<td>0.94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K6</td>
<td>104.6</td>
<td>3.79</td>
<td>0.22</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>173</td>
<td>108.7</td>
<td>3.06</td>
<td>0.25</td>
<td>0.17</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>116</td>
<td>115.2</td>
<td>5.38</td>
<td>0.56</td>
<td>0.25</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A</td>
<td>116.0</td>
<td>1.16</td>
<td>0.52</td>
<td>0.22</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K10</td>
<td>118.6</td>
<td>2.28</td>
<td>0.33</td>
<td>0.29</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>178</td>
<td>125.4</td>
<td>4.99</td>
<td>0.25</td>
<td>0.43</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>128</td>
<td>133.8</td>
<td>0.31</td>
<td>0.25</td>
<td>0.25</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>137</td>
<td>133.3</td>
<td>0.35</td>
<td>0.25</td>
<td>0.25</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>135</td>
<td>138.8</td>
<td>1.14</td>
<td>1.36</td>
<td>1.15</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>124</td>
<td>146.0</td>
<td>1.52</td>
<td>0.56</td>
<td>0.25</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>122</td>
<td>149.2</td>
<td>0.18</td>
<td>0.52</td>
<td>0.25</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Due to the similarity of δD values in lipids produced by a single biosynthetic pathway (e.g., acetogenic, DOXP/MEP, and MVA), we discuss our results by grouping lipids according to their biosynthetic origin. Also, because there were no apparent differences in δD values obtained from sediments or microbial mat materials we have combined the discussion.

3.3.1. Acetogenic lipids (hydrocarbons)

δD values of acetogenic hydrocarbons in ponds (n-C17, n-C17.1, n-C18, n-C18.1) varied by more than 100‰, from −173‰ to −57‰ (Table 3). δD values of n-C17, n-C17.1, and n-C18.1 were very similar within a single sample. The n-C18 alkane, which was only found in sufficient concentrations for isotope measurements in four samples, was always depleted in deuterium relative to other hydrocarbons (by 27‰ to 66‰). δD values for each compound were positively correlated with salinity. δD values for the n-C17 alkane, the major hydrocarbon in all samples, increased by 80‰ over a salinity range of 110. The most negative δD value for n-C17 (−141‰) was measured in the least saline sample (Lake 151) with a salinity of 38.5, while the highest δD value for n-C17, −57‰, was measured in Lake 124 (salinity of 146).

Table 3
δD values for the analyzed lipids from the Christmas Island saline lakes

<table>
<thead>
<tr>
<th>Lake</th>
<th>Salinity</th>
<th>δD water (‰)</th>
<th>n-C17 Stdev</th>
<th>n-C17:1 Stdev</th>
<th>n-C18 Stdev</th>
<th>n-C18:1 Stdev</th>
<th>Phytene Stdev</th>
<th>Diploptene Stdev</th>
<th>TLE Stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td>151</td>
<td>38.5</td>
<td>5.4</td>
<td>-141 1.5*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-192 3.3</td>
</tr>
<tr>
<td>F3</td>
<td>62.9</td>
<td>13.0</td>
<td>-126 3.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-158 4.1</td>
</tr>
<tr>
<td>165</td>
<td>63.4</td>
<td>15.8</td>
<td>-132 1.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-160 2.2</td>
</tr>
<tr>
<td>101</td>
<td>87.6</td>
<td>11.4</td>
<td>-129 1.0</td>
<td>-123.5 1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-154 3.5</td>
</tr>
<tr>
<td>123</td>
<td>98.0</td>
<td>10.0</td>
<td>-107 0.4</td>
<td></td>
<td>-156 5.1</td>
<td></td>
<td></td>
<td></td>
<td>-145 0.4</td>
</tr>
<tr>
<td>125</td>
<td>99.6</td>
<td>10.4</td>
<td>-97 2.9*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-144 0.9</td>
</tr>
<tr>
<td>K6</td>
<td>104.6</td>
<td>10.0</td>
<td>-107 0.7</td>
<td></td>
<td>-166 4.9</td>
<td></td>
<td></td>
<td></td>
<td>-151 0.4</td>
</tr>
<tr>
<td>173</td>
<td>108.7</td>
<td>10.0</td>
<td>-100 0.7</td>
<td></td>
<td>-124 9.7</td>
<td></td>
<td></td>
<td></td>
<td>-147 1.4</td>
</tr>
<tr>
<td>116 (mat)</td>
<td>115.2</td>
<td>9.3</td>
<td>-123 3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-84 16.6</td>
</tr>
<tr>
<td>2A</td>
<td>116.0</td>
<td>10.2</td>
<td>-87 3.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-138 2.1</td>
</tr>
<tr>
<td>K10</td>
<td>118.6</td>
<td>11.3</td>
<td>-90 2.5</td>
<td>-98.4 3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-172 7.6</td>
</tr>
<tr>
<td>178</td>
<td>125.4</td>
<td>8.8</td>
<td>-90 5.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-85 2.3</td>
</tr>
<tr>
<td>128</td>
<td>135.6</td>
<td>4.0</td>
<td>-56 0.6</td>
<td></td>
<td>-90 16.7</td>
<td></td>
<td></td>
<td></td>
<td>-134 3.4</td>
</tr>
<tr>
<td>137</td>
<td>133.3</td>
<td>9.8</td>
<td>-63 13.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-109 4.4</td>
</tr>
<tr>
<td>135</td>
<td>138.8</td>
<td>5.8</td>
<td>-87 1.3</td>
<td>-67.0 n/a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-120 0.7</td>
</tr>
<tr>
<td>124</td>
<td>146.0</td>
<td>5.8</td>
<td>-57 2.9</td>
<td></td>
<td>-76 5.2</td>
<td>-204 3.3</td>
<td>-154.5</td>
<td></td>
<td>-122 1.7</td>
</tr>
<tr>
<td>122</td>
<td>149.2</td>
<td>6.5</td>
<td>-66 9.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-92 4.0</td>
</tr>
</tbody>
</table>

Q1 Standard deviation is given for three replicate measurements.

* The n-C17 alkane and n-C17:1 alkene were not baseline separated in these samples, therefore peaks were integrated as one.

Fig. 4. Linear relationship between δD values of total lipid extracts (TLE) and salinity. Lake water δD values are shown for comparison.

Fig. 5. Relationship between δD values of hydrocarbons and salinity. Water δD values are shown for comparison. 1σ error bars shown for triplicate analysis of lipid δD values, if not visible they are smaller than the symbol. Regression line is for n-C17.

There was a strong linear relationship between the n-C17 δD values and salinity ($r^2 = 0.78$, $n = 17$) with a slope of 0.77 (Fig. 5), with a similar relationship for n-C18:1 ($r^2 = 0.76$, $n = 6$). Overall, δD values of acetogenic hydrocarbons showed a strong positive linear relationship with salinity, even though water δD values changed parabolically with salinity (Fig. 5).

The n-C18 alkane, which was depleted in deuterium relative to n-C17 on average by 50‰ (ranging from 27‰ to 66‰), indicated a different source for this lipid than the other hydrocarbons. Whether n-C18 was produced by a different species or was the product of a degradation process involving hydrogen exchange, cannot be determined from our data. However, n-C18 was only identified in salinities up to 100, supporting the notion that it might have been derived from a different species preferring moderate salinities.

There was no other statistically significant relationship between the lipid δD values and other physico-chemical parameters, that were independent of variations in salinity (such as lake temperature or pH).

3.3.2. Lipids produced via DOXP-MEP (phytene) and MVA (diploptene) pathways

Due to lower abundances of phytene and diploptene, relative to acetogenic lipids only five samples of the former and two of the latter could be analyzed isotopically. The δD

values of phytene were between $-276\%e$ and $-187\%e$. There was a significant positive linear relationship between salinity and phytene $\delta D$ values ($r^2 = 0.9$, $n = 5$), with a slightly steeper slope than for the acetogenic lipids (0.98) (Fig. 6). $\delta D$ values of phytene averaged $140\%e$ for the lower and higher salinity samples. Diploptene $\delta D$ values followed the trend of all other lipids analyzed with increasing values at higher salinities, but with a slope slightly less than $n-C_{17}$ at 0.66. With only two diploptene $\delta D$ analyses little significance can be attributed to a different slope.

Diploptene was depleted in deuterium relative to $n-C_{17}$ by $97\%e$ on average ($91\%e$ and $103\%e$), within the range reported for the differences between sterols (which are produced via the same MVA biosynthetic pathway as diploptene) and hydrocarbons (Sessions et al., 1999; Sauer et al., 2001). There was no systematic variation in these differences with salinity. The difference between the $n-C_{17}$ alkane and phytene showed a negative correlation with temperature ($r^2 = 0.63$; $n = 5$), but with only five data points a causal relationship remains speculative.

### 3.3.3. Total lipid $\delta D$ values

Total lipid extract (TLE) $\delta D$ values were between $-192\%e$ and $-84\%e$, over a salinity range of 136 (Table 4). Sediment and mat samples from Lake 116 were extracted separately and showed no substantial isotopic differences ($-91\%e$ and $-84\%e$, respectively). Furthermore, the upper two layers of microbial mats from Lakes 101 and F4, identified by color and microbial species composition (Stal, 1995), were analyzed separately and found to have no substantial isotopic differences (Lake 101, layer 1: $-154\%e$; layer 2: $-148\%e$; Lake F4, layer 1: $-140\%e$; layer 2: $-131\%e$). Conversely, a sample taken under a halite crust on the bottom of Lake 128, was enriched by $14\%e$ compared to a sample from above the salt crust, likely resulting from more saline conditions.

### 3.4. Lipid–water D/H fractionation and its relationship to salinity

There was an increase of cyanobacterial lipid $\delta D$ values by more than $100\%e$, over a salinity range of 136. Conversely, water $\delta D$ values varied by just $12\%e$ and were not correlated with salinity, which strongly implied a decrease in net D/H fractionation between source water and lipids (or increase in fractionation factors $\alpha_{\text{lipid-water}}$ or $\epsilon_{\text{lipid-water}}$, with $\alpha = (1000 + \delta_{\text{lipid}})/(1000 + \delta_{\text{water}})$; $\epsilon = 1000 \times (\delta_{\text{lipid}} + 1000)/(\delta_{\text{water}} + 1000) - 1$). When plotting $\delta D$ values of water vs. $\delta D$ values of lipids, relationships with negative slopes were obtained for all lipids (significant only for $n-C_{17}$; $r^2 = 0.81$, $n = 4$; and phytene $r^2 = 0.91$, $n = 5$).

#### Table 4

<table>
<thead>
<tr>
<th>Lake</th>
<th>Salinity</th>
<th>$\delta D$ water (%e)</th>
<th>$\delta D$ TLE (%e)</th>
<th>Stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>13.6</td>
<td>-7.6</td>
<td>-190</td>
<td>3.9</td>
</tr>
<tr>
<td>160</td>
<td>38.8</td>
<td>6.1</td>
<td>-189</td>
<td>6.0</td>
</tr>
<tr>
<td>152</td>
<td>39.4</td>
<td>6.9</td>
<td>-183</td>
<td>9.7</td>
</tr>
<tr>
<td>F1b</td>
<td>41.2</td>
<td>7.7</td>
<td>-183</td>
<td>0.3</td>
</tr>
<tr>
<td>F2</td>
<td>41.6</td>
<td>8.4</td>
<td>-185</td>
<td>1.6</td>
</tr>
<tr>
<td>F4</td>
<td>80.3</td>
<td>14.2</td>
<td>-131</td>
<td>2.2</td>
</tr>
<tr>
<td>F5</td>
<td>86.9</td>
<td>13.6</td>
<td>-126</td>
<td>1.5</td>
</tr>
<tr>
<td>175</td>
<td>102.6</td>
<td>11.3</td>
<td>-147</td>
<td>nd</td>
</tr>
<tr>
<td>K2</td>
<td>103.8</td>
<td>9.9</td>
<td>-101</td>
<td>1.4</td>
</tr>
<tr>
<td>F7</td>
<td>114.9</td>
<td>13.3</td>
<td>-130</td>
<td>2.5</td>
</tr>
<tr>
<td>116 (sediment)</td>
<td>115.2</td>
<td>9.3</td>
<td>-91</td>
<td>2.3</td>
</tr>
<tr>
<td>F6</td>
<td>116.5</td>
<td>13.3</td>
<td>-122</td>
<td>2.5</td>
</tr>
<tr>
<td>101 (layer 2)</td>
<td>87.6</td>
<td>11.4</td>
<td>-148</td>
<td>0.7</td>
</tr>
<tr>
<td>F4 Mat (layer 1)</td>
<td>80.3</td>
<td>14.2</td>
<td>-140</td>
<td>3.2</td>
</tr>
<tr>
<td>128 (underneath salt crust)</td>
<td>135.6</td>
<td>4.0</td>
<td>-120</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Standard deviation is given for three replicate measurements.

Fig. 6. Relationship between the $\delta D$ values of phytene and diploptene vs. salinity. Water $\delta D$ values shown for comparison. Regression line is shown for phytene.

However, seemingly large and negative $\alpha_{\text{lipid-water}}$ values ($-2.9$ to $-8.1$) were obtained from the slopes of these regressions. $\alpha_{\text{lipid-water}}$ values derived from the intercepts of these regressions were 0.980 for $n$-C$_{17}$ and 0.854 for phytene. In a simple one-component (i.e., single hydrogen source) system, the intercept of a $\delta$D$_{\text{water}}$ vs. $\delta$D$_{\text{lipid}}$ relationship represented $\alpha_{\text{lipid-water}}$ and the slope $\beta_{\text{lipid-water}}$ (i.e., $\delta$D$_{\text{lipid}} = \alpha \delta$D$_{\text{water}} + \beta$), whereby both fractionation factors should have yielded the same value ($\beta = \alpha - 1$) (Sessions and Hayes, 2005). On the contrary, calibration studies on batch cultures and natural systems have demonstrated that such a simple model was inadequate for hydrogen isotopes in algae (Zhang and Sachs, 2007).

The stark disagreement between slope- and intercept-derived fractionation factors in Christmas Island microbial mat lipids implied that more than one fractionation process or hydrogen source contributed to the observed net D/H fractionation (Sessions and Hayes, 2005) and that the isotopic composition of the source water was not the parameter determining the $\delta$D value of the lipids investigated here, as its isotopic composition did not change substantially. Nevertheless, net isotopic fractionation between source water and lipids decreased (fractionation factor $\alpha$ increased) in all three compound classes with increasing salinity and showed strong linear correlations (Fig. 7). Differences in $\alpha_{\text{lipid-water}}$ for lipids derived from the three biosynthetic pathways remained almost constant over the entire salinity range as indicated by the similar slopes of the regressions (0.00080 to 0.00107).

Isotopic differences between the three compound classes were within the ranges reported in the literature; acetogenic lipids are least depleted in deuterium (Sessions et al., 1999; Sauer et al., 2001). Acetogenic biosynthesis starts with the production of pyruvate, (a product of the Calvin Cycle) from which acetyl-CoA is derived, the building block for aliphatic lipids (Kolattukudy, 1967; Kolattukudy, 1981; Chikaraishi et al., 2004). Lipids produced via the MVA pathway, such as diploptene, are derived from pyruvate, which is converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), reduced to mevalonic acid (MVA) by HGM-CoA reductase, transformed into farnesyldiphosphate (FPP), and finally to squalene (Lange et al., 2000; Chikaraishi et al., 2004). Squalene is the precursor to all sterols and hopanoids, including diploptene.

The most deuterium-depleted lipids, such as phytol, are produced via the DOXP/MEP pathway (Rohmer et al., 1993; Schwender et al., 1996; Lichtenthaler, 2004). DOXP is produced by pyruvate and glyceraldehydes-3-phosphate (GA-3P) from the Calvin Cycle. DOXP is converted into MEP, and then transformed into isopentyl diphosphate (IPP). Phytol is ultimately formed from geranylgeranyldiphosphate (GGPP) through the elimination of three carbon–carbon double bonds (see discussion in Zhang and Sachs (2007), and references therein). It is currently unknown at which biosynthetic step and by which mechanism hydrogen isotope fractionations arise that deplete isoprenoids in deuterium (compared to acetogenic lipids). Isotopically different pools of NADPH have been suggested to exist, as well as additional, yet unknown fractionation steps in the DOXP/MEP pathway, see discussion in Sessions et al. (1999).

Fig. 7. Relationship between the isotopic fractionation (given as $\alpha$ and $\varepsilon$ values) of three lipid biomarkers (n-C$_{17}$, phytene, diploptene) and the TLEs from which those lipids were purified, plotted as a function of salinity.
lower, or at the lower end of reported values, for all compound classes analyzed. A possible explanation for this observation could be that different species exhibit different D/H fractionation during lipid synthesis, as demonstrated for different species of cultured green algae (Zhang and Sachs, 2007), and/or a shifting cyanobacterial population in the ponds and microbial mats as salinity increased. An alternative explanation is that the relationship between salinity and $\Delta$lipid-water might cease to be linear at lower salinities. In fact, when applying a polynomial (2nd order) regression to the relationship between $\Delta_{n-C_{17}}$-water and salinity, a higher $R^2$ of 0.84 is obtained with an intercept of 0.860, within the range of previously reported values (Sessions et al., 1999; Sachse et al., 2004). Also, the $\Delta_{lipid-water}$ values for total lipid extracts for the six low-salinity ponds (salinities from 13.6 to 41.6) were very similar (0.806 to 0.816). The small number of samples analyzed from other compound classes did not permit such an evaluation, nor did our calibration have sufficient data points at low salinities to confirm a possible polynomial relationship.

The importance of species-specific differences was further evident when comparing our calibration curve to data from two haptophyte marine algae, E. huxleyi and G. Oceanica (Schouten et al., 2006). Schouten et al. (2006) analyzed the isotopic fractionation between source water and alkenones, biomarkers specific to these algae, over a salinity range from 24.8 to 35.1. Although this was a much narrower salinity range than our salinity range of 0 to 1.4 $d^{-1}$ (for $\Delta_{lipid-water}$) that also correlated with $\Delta_{water}$. In the microbial mat ecosystems of Christmas Island one can assume a comparable distribution of growth rates, given similar light, temperature, and nutrient levels. However, it has been shown that photosynthetic activity in cyanobacterial mats can decrease as salinity increases (Garcia-Pichel et al., 1999). Yet, studies on halotolerant cyanobacteria, such as members of the Aphanothece genus, which were identified in our Christmas Island mats by light microscopy (data not shown), have shown that growth rates were low and constant throughout a salinity range of 30–210 (Garcia-Pichel et al., 1998). The tendency to form gelatinous coverings also supports a slow but steady growth. The maximal range of growth rates for halotolerant cyanobacteria varied only by 0.4 $d^{-1}$ for a salinity range of 0 to 250 ppt (Garcia-Pichel et al., 1998; Nübel et al., 2000). Assuming the (steepest) calibration curve presented by Schouten et al. (2006) for growth rate dependency of $\Delta_{lipid-water}$, a decrease of 0.4 $d^{-1}$ in growth rate would result in an increase of only about 0.028 $d^{-1}$ (i.e., an $\Delta_{lipid-water}$ increase of 28%). Although species-specific differences were to be expected, it is unlikely that such small changes in growth rate, as observed in halotolerant cyanobacteria, can account for the changes in $\Delta_{water}$ observed in the Christmas Island mats. The majority of the observed 0.083 unit increase in $\Delta_{water}$ (or increase in $\Delta_{lipid-water}$, of 83%), over a salinity range of 136 in Christmas Island ponds, must have been causally related to the salinity increase itself.

3.5. Mechanisms for decreasing net fractionation during increasing salinity

When salinity (the total mass of ions per unit mass of water) in a closed water body increased, a number of chemical and physical parameters were also affected (Javor, 1989). For example, vapor pressure and activity of water (defined as vapor pressure of water divided by vapor pressure of pure water) decreased. Water activity has been shown to be an important controlling factor of the ecophysiology of microorganisms in hypersaline brines (Javor, 1989). Diffusivity, solubility of gases, and the specific heat all decreased with increasing salinity, whereas viscosity increased. As these physical properties did change with salinity, cells were subjected to osmotic gradients. It has been shown that green algae did alter their membrane fatty acid composition in response to increasing osmotic pressure, whether ionic (e.g., salinity) or molecular (e.g., sorbitol) in origin (Xu et al., 1998). Additionally, pH and alkalinity can be affected, depending on the nature of the ions present. Thus, it is likely that the combined action of these processes, across steep salinity gradients affected the metabolism of the microorganisms of Christmas Island lakes.

While our experiment did not allow us to identify or link these effects to the observed changes in D/H fractionation between water and lipids, the similarity of the slopes and consistent isotopic differences between lipids from the three biosynthetic pathways suggested that biological control over the $\Delta_{lipid-water}$-salinity relationship, was exerted before the last common biosynthetic branching point. The last common precursor molecule to all three biosynthetic pathways was pyruvate, which was produced from 3-phosphoglyceric acid (3-PGA), and originated in the Calvin cycle. Hydrogen was added from NADPH, when 3-PGA was converted to GA-3-P. This and the variety of chemical and physical properties that change with salinity lead us to hypothesize that the cause for the $\Delta_{water}$ dependency on salinity is not in the lipid biosynthesis itself, but rather in the transport of water into the cell.

There are at least two modes of water transport into cells. The primary mode of water transport into cells was believed to be diffusion through cell walls (Haines, 1994). However, in the 1990s it was discovered that a number of organisms, including plants and cyanobacteria, possess water channels within their cell walls, called aquaporins (Agre et al., 1993; Chrispeels and Agre, 1994; Tanghe et al., 2006), where water enters and leaves the cell through facilitated diffusion (e.g., along concentration gradients). Proton and ion channels constitute an additional means of (active) transport for hydrogen into and out of cells (so-called Na+/H+ antiporters) (Waditee et al., 2001).

It has been shown that aquaporins play an essential role in regulating water transport into the cell, especially during periods of (environmental) stress. Saltwater outside the cell generated an osmotic pressure gradient, drawing water out of the cell. Aquaporins were downregulated or closed to prevent water loss from cells, which has been demonstrated in plants and cyanobacteria.
in plants exposed to saltwater (Boursiac et al., 2005). Thus, ambient water is not necessarily the same as intracellular water from which hydrogen is derived for biosynthetic reactions. In fact, a recent study on the heterotrophic bacterium Escherichia coli showed that up to 53 ± 12% of the hydrogen from intracellular water, can be isotopically distinct from ambient water (Kreuzer-Martin et al., 2006)—due to internal water produced from metabolic reactions. A decreasing net D/H isotopic fractionation requires a deuterium-enriched intracellular water.

To explain how intracellular water becomes more deuterium-enriched intracellular water than ambient water two mechanisms are conceivable:

1. It may be there is less fractionation against HDO during water transport into the cell at higher salinities. However, even if such a process takes place, it is unlikely that the heavier molecules (HDO instead of H₂O) of water, or D⁺ instead of H⁺ ions, are preferentially transported into the cell. Indeed, certain proton channels (e.g., voltage gated channels) have been shown to fractionate against deuterium (DeCoursey, 2003), resulting in the opposite isotope effect.

2. A more likely process is that under high salinities less water is exchanged through the cell walls and the metabolic water inside the cell is continuously “recycled”. Studies on cyanobacteria have shown that under salt stress the volume of water in the cytosol of cyanobacteria decreases by as much as 25% (Allakhverdiev et al., 2000a,b). Aquaporins and Na⁺ channels are restricted after exposure to high salinities to maintain non-toxic levels of Na⁺ inside the cell (Pomati et al., 2004; Boursiac et al., 2005). Thus, any isotopic enrichment of the remaining intracellular water is due to continuous recycling of this water, as a variety of metabolic reactions produce water. A continuous removal of deuterium-depleted hydrogen from intracellular water during NADPH production must enrich the residual water with deuterium, if the rate of hydrogen extraction from that pool is large relative to the rate of hydrogen replenishment (from ambient water).

Biosynthetic fractionation during lipid biosynthesis itself might not be affected directly by salinity at all, as evidenced by the constant isotopic differences of products from three biosynthetic pathways. However, net D/H fractionation between all lipids and water decreased. Therefore, deuterium enrichment in the intracellular water would explain the observed D-enrichment of cyanobacterial lipids as salinity increased. Experimental studies comparing the isotopic composition of intracellular and extracellular water and its relationship to salinity are needed to confirm the operation of such a mechanism.

An additional reason for the observed increase in lipid δD values with increasing salinity may lie in the operation of “dark metabolism” in cyanobacteria when no light is present or under nutrient-limited conditions (Stal, 1995). Some cyanobacteria are able to accumulate large amounts of storage products, such as poly-glucose, which are used to produce energy at times when no photosynthesis is possible (e.g., anoxic or dark conditions). Glucose is converted to lactate, acetate, ethanol, CO₂ and H₂ via mixed acid fermentation (Moezelaar and Stal, 1997). Since protium should be preferentially released as H₂ gas, the remaining intracellular pool of hydrogen should become enriched in deuterium. We have no evidence to suggest this mechanism is operating in the Christmas Island mats, but if the dark metabolism became increasingly important as salinity increased this mechanism could enrich the intracellular hydrogen pool in deuterium.

Although, we cannot rule out possible differences in δlipid-water for different cyanobacterial species, we note that the strong correlation between δlipid-water and salinity for all lipids analyzed, as well as the constant isotopic differences between lipids produced from the three biosynthetic pathways, points to a similar behavior of δlipid-water for all cyanobacteria in the Christmas Island mats. Although a variety of cyanobacterial species have been observed in these microbial mats, the extremely halotolerant filamentous (Phormidium-Leptolyngbia genus) and coccoid (Aphanothecae genus) cyanobacteria seem to dominate the microbial community in the Christmas Island ponds (Fig. 2). Additionally, the composition of lipids in the mats is similar to previously reported distributions for Phormidium-type microbial mats (Grimalt et al., 1992; Simonet et al., 1998). Due to the high salinities the diversity of organisms capable of surviving in these environments is limited and the mats might be expected to consist of a relatively narrow range of species.

In order to establish δlipid-water as a proxy for salinity in paleoclimate applications, future studies should focus on other groups of organisms (such as marine algae). Schouten et al. (2006) confirmed the operation of a similar mechanism in two marine algae in batch cultures, although with a steeper slope of δlipid-water vs. salinity linear regression, perhaps resulting from variable growth rates in the cultures, and/or species-specific differences in water uptake. In fact, the species-specific differences observed in δlipid-water for freshwater algae, as observed by Zhang and Sachs (2007), might also be related to different water uptake strategies by these algae. These results stress the importance of independent constraints on the origin of the lipids in sediments, using biomarkers with a known source.

### 4. CONCLUSIONS

We have demonstrated for the first time that cyanobacterial lipids (hydrocarbons, dioloptene and phytyene as well as total lipids) from naturally occurring microbial mats became increasingly enriched in deuterium (by almost 100‰ for n-C₁₇ and more than 100‰ for TLEs) with rising salinity (38 to 149.2) in the absence of any substantial increase in water δD values. There was a strong dependency of D/H fractionation between lipids and water decreased by about 0.8‰ to 1.1‰ for every 1 unit increase in salinity (i.e., δlipid-water increased by 0.0008 to 0.001 per unit increase in salinity). These findings show that care must be exercised when using lipid δD values to reconstruct source water δD values in systems where changes in salinity might have.
occurred. On the other hand, changes in lipid δD values could become a useful indicator of paleosalinity if additional constraints on the source water isotopic composition (e.g., using δ18O values from foraminifera or ostracods) can be made.

Since the isotopic differences between compounds produced via the three major biosynthetic pathways for lipids remain unaffected by changes in salinity, we argue the biological control over this mechanism is exerted before the last common biosynthetic branching point for these three pathways, the Calvin Cycle. As a likely mechanism for the observed decrease in net D/H fractionation at higher salinities we propose a reduced exchange with the extracellular source water owing to down-regulation of water channels (aquaporins) and consequent increased incorporation of recycled metabolic water in biosynthetic products. Metabolic or intracellular water ought to become enriched in deuterium as isotopically-depleted hydrogen is continuously removed for NADPH production (Schmidt et al., 2003), leaving D-enriched water for biosynthesis.

These results and subsequent studies could lead to the establishment of a paleo-salinity proxy based on lipid δD values. However, the mechanism responsible for changes in the net isotopic fractionation that accompanies salinity changes must be confirmed. Additional factors, such as the nature of the solute (NaCl vs. NaSO4 for instance, which influences a number of physico-chemical parameters in the brine), species-specific differences, and the nature of xLipid/water at very low and very high salinities must be investigated to fully understand variations in lipid δD values from saline and hypersaline systems, as it is likely that D/H fractionation between source water and lipids under extremely hypersaline conditions will approach a minimum value, which might well be species-specific.

ACKNOWLEDGMENTS

This material is based upon work supported by the National Science Foundation under Grant No. 0639640 and by the Gary Comer Science and Education Foundation to J.P.S. The Alexander von Humboldt foundation is acknowledged for providing a Feodor-Lynen Research fellowship to Dirk Sachse. We thank Rienk H. Smittenberg, Casey Saenger, and Mike Miller for assistance during microscopy and with the identification of cyanobacteria. Special thanks to Mr. Rudi Rottenfusser, Carl Zeiss Inc. at Marine Biological Laboratory, Woods Hole, MA for providing support and advice in microscopy. The logistical support of Alla Skorokhod, Kim Anderson, John Bryden, Sue Fukada, and Chuck Corbett was also greatly appreciated. Thanks to the Kitimat Indian Ministers of the Environment and Fisheries for permission to conduct research on Christmas Island. The services of Bill Paupe, South Seas Air and the staff of the Captain Cook Hotel were also instrumental in the success of this research. We thank Anthony Faia for the water δD analysis at Dartmouth College. We are grateful to Orest Kawa and Valerie Schwab at the University of Washington for technical assistance and discussions. We would like to thank Alex L. Sessions and an anonymous reviewer as well as the Associate Editor Thomas S. Bianchi for their constructive comments on the original manuscript.

REFERENCES


Lipid–water D/H fractionation and salinity


Academische Verlagsgesellschaft.


