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Inverse relationship between salinity and *n*-alkane δD values in the mangrove *Avicennia marina*

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ABSTRACT

Hydrogen isotope ratios in lipids derived from mangroves have the potential to be used for paleohydrologic reconstructions and could serve as a much needed tool for establishing past climate variability in the tropics. We assessed the effect of salinity on the apparent fractionation factor, α_a, between mangrove derived *n*-alkanes and their source water for *Avicennia marina* (gray mangrove) specimens collected along a 28 PSU salinity gradient in the Brisbane River Estuary. Our results indicate that there is an inverse relationship between the apparent fractionation factor and salinity. This salinity effect is large enough to override variability in the isotopic composition of source water, which plays a dominant role in determining the hydrogen isotope ratio of leaf waxes in other vascular plants. We suggest that this relationship may be due to (i) increased discrimination against deuterium during water uptake at high salinity, (ii) increased production of compatible solutes from D enriched pyruvate at high salinity, resulting in more hydrogen from D depleted NADPH being incorporated in leaf waxes, and/or (iii) increased secretion of salty brine by leaves at high salinity, resulting in (iii.a) higher relative humidity at the leaf surface, and (iii.b) introducing the possibility that D depleted water of hydration is absorbed by the leaf. Our results indicate that hydrogen isotope ratios of mangrove lipid biomarkers can be developed as a paleosalinity indicator. They also imply that care must be taken when interpreting hydrogen isotopic variations in non-source specific higher plant lipids in sediments where both mangrove and non-mangrove plants contribute organic material.

1. Introduction

Hydrogen isotopes of lipid biomarkers have recently emerged as a promising paleoclimate proxy. Compound specific hydrogen isotope measurements were made possible by analytical developments during the late 1990s (Burgoyne and Hayes, 1998; Hilkert et al., 1999) and the technique rapidly garnered considerable interest because lipids can be source specific and well preserved in sediment. Thus measuring hydrogen isotope ratios of lipids in marine and lacustrine sediments allows for paleoclimate reconstructions in a myriad of archives for which no robust proxies previously existed. The basic premise of such reconstructions is that lipids produced by autotrophic organisms will reflect the hydrogen isotopic signature of their source water, a relationship that has been established by both laboratory cultures and field calibrations (Sessions et al., 1999; Sauer et al., 2001; Huang et al., 2002, 2004; Chikaraishi and Naraoka, 2003; Sachse et al., 2004; Englebrecht and Sachs, 2005; Zhang and Sachs, 2007; Sachs and Schwab, 2011; Schwab and Sachs, 2011). Water isotopes, in turn, are closely linked to the hydrologic cycle (Craig, 1961; Craig and Gordon, 1965; Gat, 1996) and by serving as a proxy for the isotopic composition of ancient water, lipids can provide valuable insights into paleohydrology.

During the past decade, hydrogen isotopes of lipid biomarkers have been employed around the world to reconstruct paleohydrologic changes on 10^1–10^7 year time scales (Huang et al., 2002; Schefuss et al., 2005; Pagani et al., 2006; Makou et al., 2007; Pahnke et al., 2007; Vandermeer et al., 2007, 2008; Sachs et al., 2009) and they have demonstrated the potential to significantly enhance our understanding of paleoclimate variability in the tropics. The tropics are a region of considerable interest in climate dynamics, as the flux of heat and moisture poleward from the tropics drives much of Earth's atmospheric circulation. Reorganizations of tropical climate thus have the potential to profoundly impact the rest of the planet (Chiang, 2009). However, our understanding of past variability in this important region has been limited by a dearth of high resolution paleoclimate records. Ice cores in this region are limited to a few high altitude locations. Likewise, tree rings are often difficult to interpret in the tropics and frequently do not exist in equatorial regions with no distinct dry season (Worbes, 1995; Jones et al., 2009). Marine sediments have been used to create long term records of climatic variability in the tropics using proxies such as alkenone $U^{k'}_{37}$ (Ohkouchi et al., 1994; Lawrence et al., 2006; Koutavas and Sachs, 2008; Herbert

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et al., 2010), and $\delta^{18}O$ and Mg/Ca of foraminifera (Koutavas et al., 2002; Stott et al., 2002; Lea et al., 2006). However, given the slow accumulation rates typical of pelagic sediments, temporal resolution is often limited to 10^2–10^3 years. Varved sediment sequences are found in a few locations (such as the Cariaco Basin and the Arabian Sea), but are quite uncommon. $\delta^{18}O$ of coralline $CaCO_3$ has been used to generate annually resolved records of climate variability in the tropics (Hendy et al., 2002; Cobb et al., 2003; Linsley et al., 2006), but are limited in duration to decades to a few centuries. Recently, high resolution records from hydrogen isotopes of lipid biomarkers have been produced from high accumulation rate sediments in brackish ponds in Palau and the Line Islands (Sachs et al., 2009; Smittenberg et al., 2011) and the coastal margin of Indonesia (Tierney et al., 2010).

1.1. Potential for mangrove based δD studies

A tropical sedimentary archive that has been unexploited for lipid D/H studies is peat in mangrove swamps. Mangrove peat has accumulated at rates on the order of 1 m/kyr in the tropical Pacific over the past two millennia (Ellison and Stoddart, 1991; Fujimoto, 1997) and is comprised in large part of decaying leaf matter from mangroves (Kristensen et al., 2008) which are woody trees and shrubs that have adapted to live in brackish or saline water along estuaries and intertidal flats.

The environment inhabited by mangroves is a harsh one, characterized by fluctuations in salinity and anoxic soils. As such, few higher plants are adapted to live in these waters, with only 20 genera existing worldwide (Hogarth, 2007). Nearly half of all mangrove species belong to either the Avicenniaceae or Rhizophoraceae families. This makes the mangrove forests in any given location relatively homogenous, with many prominent mangrove swamps dominated by 2 or 3 species (Polidoro et al., 2010). Because of the low species diversity in a mangrove swamp, there are very few potential sources of higher plant lipids to the peat that accumulates in the swamp, increasing the probability that a source specific biomarker for paleohydrologic reconstructions based on hydrogen isotopes can be exploited in the sediments.

1.2. Competing influences on leaf wax δD

It is beneficial to work with lipid biomarkers that can be attributed to a single species or to a small group of species when inferring past climate variability from hydrogen isotopes of leaf lipids. This is because a number of different studies have indicated that different groups of plants have different apparent fractionation factors between their source water and the leaf wax (Sachse et al., 2006; Smith and Freeman, 2006; Hou et al., 2007; Liu and Yang, 2008; Feakins and Sessions, 2010; Romero and Feakins, 2011). In this case, the apparent fractionation factor, hereafter referred to as α_a, is a measure of the isotopic difference between environmental water and lipids and is defined as $\alpha_a = (\delta D_{lipid} + 1000)/(\delta D_{water} + 1000)$, where $\delta D_{sample} = [(D/H)_{sample}/(D/H)_{VSMOW} - 1] * 1000$. It is important to note that α_a incorporates a number of steps during which fractionation might occur. In the case of terrigenous plants, these include enrichment of soil water due to evaporation (Riley et al., 2002; Eggemeyer et al., 2009), enrichment of leaf water due to transpiration (Dawson et al., 2002; Kahmen et al., 2008), and D depletion due to enzymatically mediated fractionation during biosynthesis of lipids (Sessions et al., 1999; Sachse et al., 2006; Sessions, 2006; Feakins and Sessions, 2010). Differences in water use efficiencies, leaf morphology and transpiration have been cited as causes of differences in α_a that have been observed between mosses and woody plants (Sachse et al., 2006), between grasses and woody plants (Liu et al., 2006; Hou et al., 2007), and between C_3 and C_4 grasses (Smith and Freeman, 2006; McInerney et al., 2011). Feakins and Sessions (2010) observed considerable variations in α_a among species of the same general plant type growing at the same site, although the average δD of leaf waxes from all trees at each site along their southern California transect was well correlated with local precipitation. The discrepancies among α_a for different plants suggest that changes in downcore δD of non-source specific lipids, such as *n*-alkanes, *n*-alcohols and *n*-fatty acids, may represent changes in plant community composition as well as hydrologic changes.

Other complications in interpreting δD of leaf waxes as an indicator of precipitation δD exist, for although the isotopic composition of precipitation plays a major role in determining that of lipids, a growing body of evidence suggests that other variables exert a significant influence on the δD values of lipid biomarkers. Results from a growth chamber study by Hou et al. (2008) suggested that relative humidity has a slight effect on α_a, presumably due to changes in evapotranspiration. However, no response to humidity was reported in a study of core tops along an aridity gradient in southwestern US, which Hou et al. (2008) attributed to changing contributions from grass and woody plants. More recently, Feakins and Sessions (2010), found little change in α_a along an aridity gradient in southern California. However, the magnitude of apparent enrichment values, ε_a, (where $\varepsilon_a = (\alpha_a - 1) * 1000$) reported by both Feakins and Sessions (2010) and Hou et al. (2008) are significantly smaller ($\sim -94‰$ ($\alpha_a = 0.906$) and $-99‰$ ($\alpha_a = 0.901$), respectively) compared to those reported in more humid regions such as the northeastern US (Hou et al., 2007) and Europe (Sachse et al., 2006), where ε_a is typically around $-120‰$ ($\alpha_a = 0.88$) and has been reported to be as low as $\sim -160‰$ ($\alpha_a = 0.84$) (Sachse et al., 2006). A recent review suggests that relative humidity >70% results in more depleted leaf wax δD values (Sachse et al., 2012).

Other variables that might affect leaf wax α_a are temperature and growth rate. Temperature has recently been proposed to affect D/H fractionation via evapotranspiration, although initial results do not suggest that it affects enzymatic fractionation (Zhou et al., 2011). The effect of growth rate on lipid α_a from plants has not been assessed, but cultures with algae suggest that it can have a significant effect on fractionation factors in algal lipids (Schouten et al., 2006; Zhang et al., 2009) and it is possible that similar effects may occur in higher plants.

Although the various controls on leaf wax δD described above complicate interpretation of this paleoclimate proxy, the majority of core-top studies show significant positive correlations between δD of precipitation and δD of leaf waxes (Sauer et al., 2001; Sachse et al., 2004; Hou et al., 2008; Polissar and Freeman, 2010). These empirical studies suggest that hydrogen isotopes of leaf waxes and other lipids are a viable paleoclimate proxy. Ideally, field calibrations will better constrain the factors influencing the leaf wax δD signal. If we wish to extend the use of leaf wax δD measurements to mangrove peat, it is important to assess the controls on δD values of leaf waxes from mangroves in the modern environment.

A major reason to evaluate mangrove derived lipids independently is that, unlike other higher plants, the salinity of a mangrove's source water is liable to change over time. Increasing salinity has been observed to increase δD values of lipids produced by phytoplankton and cyanobacteria by decreasing the apparent fractionation factor between the lipid and the source water (Schouten et al., 2006; Sachse and Sachs, 2008; Sachs and Schwab, 2011). The goal of this study is to establish what effect, if any, variations in salinity have on the apparent fractionation factor between mangrove lipids and their source water and to assess the potential of using the δD values of mangrove lipids as a paleosalinity indicator. If successful, this technique will be beneficial to our efforts to reconstruct paleoclimate variability at low latitudes,

since mangroves are ubiquitous to the tropics and often inhabit swamps with low species diversity.

2. Materials and methods

2.1. Sample collection

During a 1 week period in February 2010, whole *Avicennia marina* (gray mangrove) leaves were collected along the Brisbane River Estuary and from Moretown Bay in Queensland, Australia (Fig. 1) (Table 1). Multiple leaves were collected from each tree and were immediately placed on ice and subsequently kept frozen during storage. *A. marina* was chosen for this study because it is one of the most salt tolerant species of mangroves (Ball, 1988; Ye et al., 2005) and as such, it was present along the entire length of the Brisbane River Estuary from the coast to inland sites that had essentially fresh water at low tide.

At each site, surface water was collected adjacent to the sampled tree and the water temperature, conductivity and specific conductivity were measured using a YSI 85 conductivity probe (YSI Inc., Yellow Springs, OH, USA). When possible, multiple visits were made to the same site at different points in the tidal cycle in order assess the diurnal variation in salinity. For the six sites that were sampled near both high and low tide, the average tidal range of salinity was 4.2 ± 1.0 practical salinity units. Salinity in the Brisbane River Estuary is correlated (R^2 = 0.84) with distance from the mouth of the river (Fig. 2), and the line of best fit relating measured salinities to distance from the river mouth was used to infer the mean salinities used in Figs. 3, 4 and Table 1, which was deemed preferable to using the measured salinity at a single time point in the tidal cycle. The standard error of the regression in Fig. 2 is 2.9 PSU.

2.2. Analytical methods

2.2.1. Water δD

δD and $\delta^{18}O$ of water samples were measured in Brian Popp's laboratory at the University of Hawaii using a Picarro L1102-i Isotopic Liquid Water Analyzer in high precision mode and were normalized to VSMOW using lab standards. Each sample was analyzed six times and the first three analyses were discarded to avoid memory effects from the previous sample. The average precision of the water δD measurements was ±0.44‰.

2.2.2. Lipid extraction and purification

In order to avoid the significant isotopic differences that can occur from the base to the tip of a single leaf (Helliker and Ehleringer, 2000; Sessions, 2006), intact whole leaves from each tree were selected for analysis. In addition, in order to assess the isotopic variability among leaves from multiple locations on the same tree, five leaves each from two trees were prepared and treated as individual samples. Leaves were rinsed with DI water to remove debris and cut into small pieces using solvent cleaned scissors prior to freeze drying. Dry leaves were ground up with a solvent cleaned mortar and pestle and lipids were extracted using an Accelerated Solvent Extractor (ASE-200, Dionex Corp., Sunnyvale, CA, USA) with 9:1 dichloromethane:methanol (DCM:MeOH) at 100 °C and 1500 psi (10.3 MPa) for three five minute static cycles. The resulting total lipid extract (TLE) was evaporated to dryness under a stream of nitrogen gas on a Turbovap system (Caliper, Hopkinton, MA, USA).

A small aliquot of the TLE was dissolved in 20 μl of pyridine and silylated with 20 μl of bis(trimethylsilyl)trifluoroacetamide (BSTFA, Sigma–Aldrich, St. Louis, MO, USA) at 60 °C for 60 min. An initial screening of lipids present in the TLEs was conducted by gas chromatography–mass spectrometry (GC–MS) using an Agilent (Santa Clara, CA, USA) 6890 N gas chromatograph equipped with an Agilent 7683 autosampler, a split-splitless injector operated in splitless mode and an Agilent DB-5 ms capillary column (60 m × 0.32 mm × 0.25 μm) interfaced to an Agilent 5975 quadrupole mass selective detector. The oven temperature was increased from 60 °C to 150 °C at 15 °C/min, then at 6 °C/min to 320 °C, where it was held for 28 min. Lipids were identified based on published EI spectra and comparisons to the mass spectra of laboratory standards. The composition of the TLEs did not vary significantly as a function of salinity, and the most abundant lipids included normal alkanes (n-C_{31} and n-C_{33} alkanes), normal alcohols (n-C_{26}, n-C_{28} and n-C_{30} alcohols), sterols (stigmasterol

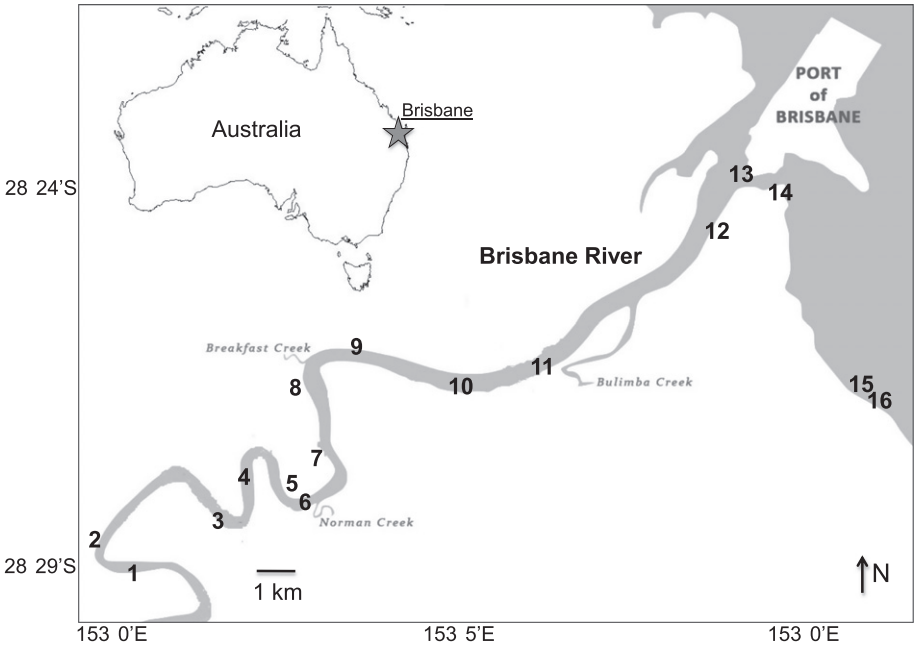

Fig. 1. Map of the Brisbane River Estuary. Sampling sites used for this study are marked with bold numbers, corresponding to data in Table 1.

Table 1
Locations of mangrove trees sampled for this study, mean salinity of the water at those sites, δD of water, C_{31} and C_{33} *n*-alkanes, and apparent fractionation factors, α, between water and each *n*-alkane.

Site	Latitude (S)	Longitude (E)	Inferred salinity (PSS)	δD_{water} (‰)	$\delta D_{C_{31}}$ (‰)	$1\sigma_{C_{31}}$ (‰)	$\alpha_{C_{31}}$	$\delta D_{C_{33}}$ (‰)	$1\sigma_{C_{33}}$ (‰)	$\alpha_{C_{33}}$
1a*	27°29.56′	153°0.12′	5.8	−10.15	−123.9	0.1	0.876	−121.1	0.8	0.879
1b*	27°29.56′	153°0.12′	5.8	−10.15	−127.8	1.1	0.881	−124.1	2.3	0.885
2	27°29.00′	152°59.78′	6.9	−9.78	−133.4	2.8	0.875	−122.9	2.9	0.886
3	27°28.58′	153°1.51′	10.0	−5.84	−124.7	0.9	0.880	−132.4	2.6	0.873
4	27°28.27′	153°2.05′	11.6	−6.17	−125.0	1.2	0.880	−117.1	2.4	0.888
5	27°28.52′	153°2.62′	13.8	−6.44	−131.9	5.7	0.874	−122.5	5.8	0.883
6	27°28.57′	153°2.98′	14.3	−4.01	−126.2	2.6	0.877	−118.9	2.0	0.885
7	27°28.29′	153°3.12′	14.7	−7.49	−132.9	2.1	0.874	−127.6	3.1	0.879
8	27°27.19′	153°2.98′	16.6	−7.36	−121.4	4.5	0.885	−113.6	4.4	0.893
9	27°26.40′	153°3.73′	18.7	−4.66	−157.9	3.2	0.846	−145.6	1.4	0.859
10	27°26.95′	153°5.03′	20.5	−3.27	−134.2	2.7	0.869	−125.2	1.7	0.878
11	27°26.87′	153°5.97′	21.6	−2.82	−136	2.6	0.866	−129.2	0.2	0.873
12	27°24.65′	153°8.95′	26.7	−2.45	−163.4	3.0	0.839	−156.8	0.4	0.845
13	27°23.97′	153°9.85′	28.4	2.21	−168.2	0.7	0.830	−164.1	0.6	0.834
14a**	27°24.09′	153°10.08′	32.5	4.26	−151.3	1.4	0.845	−158.5	2.6	0.838
14b**	27°24.09′	153°10.08′	32.5	4.26	−155.3	0.4	0.841	−148.3	0.8	0.848
15	27°28.08′	153°11.62′	34.8	3.16	−162.2	1.4	0.835	−155.7	1.0	0.842
16	27°28.10′	153°11.64′	34.8	2.99	−139.3	1.0	0.858	−128.1	2.4	0.869

* These two samples are procedural duplicates, where one total lipid extract was split in two and treated as two samples.
** These two samples are from two separate trees growing at the same site. 14a was a mature tree (height >5 m). 14b was a sapling (height <1 m).

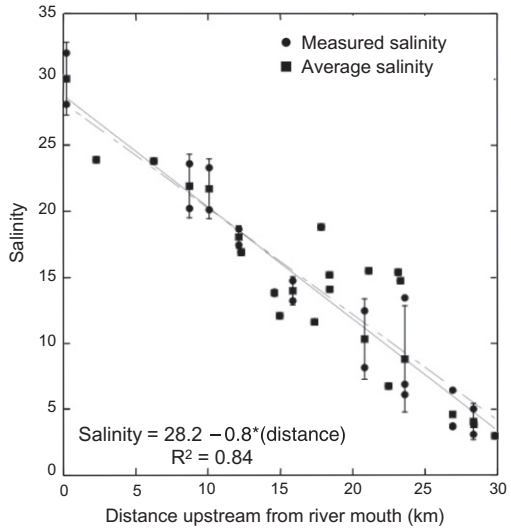

Fig. 2. Salinity vs. distance from river mouth in the Brisbane River. The linear regression of this field data was used to calculate the mean salinity values shown in Table 1.

and β-sitosterol) and triterpenols (α-amyrin, β-amyrin, and lupeol), with lupeol being particularly abundant (Fig. S1).

Another aliquot of the TLE (~12 mg) was purified using two step column chromatography. During the first step, with a solid phase of 500 mg of amino propyl silica gel (Supelco, Lot # 2511301, Part # 5-7205) the neutral fraction was eluted with 8 ml of 3:1 DCM:isopropyl alcohol, acids with 6 ml 4% acetic acid in ethyl ether and other polar compounds with 6 ml of MeOH. The neutral fraction was further purified with a solid phase of 1 g of silica gel (EMD, Lot # 45169543, Part # 11567-2) that was 5% deactivated with water. Hydrocarbons were eluted with 8 ml of hexane, sterols, alcohols and triterpenols with 6 ml of 4:1 hexane:ethyl acetate and remaining compounds with 4 ml of MeOH.

The hydrocarbon fraction was dominated by C_{31} and C_{33} *n*-alkanes, with relatively small amounts of *n*-C_{32} and other unidentified lipids (Fig. S2). Lipids in the hydrocarbon fraction were quantified with a gas chromatograph connected to a flame ionization detector (GC-FID). An Agilent 6890N gas chromatograph equipped with an Agilent 7683 autosampler, a programmable temperature vaporization inlet (PTV) operated in splitless mode and an Agilent DB-5 ms capillary column (60 m × 0.32 mm × 0.25 μm) was used with helium as the carrier gas (2.4 ml/min). The oven temperature was increased from 60 °C to 150 °C at 15 °C/min, then at 6 °C/min to 320 °C, where it was held for 28 min. Quantification of *n*-alkanes was performed by comparing their integrated peak areas to that of a known amount of *n*-C_{37}, which had been added as a quantification standard. The hydrocarbon fractions were then dried under a gentle stream of nitrogen and brought up in toluene such that the concentration of the less abundant target compound (C_{31} or C_{33} *n*-alkane) was ~200 ng/μl.

2.2.3. Lipid δD

δD values of the C_{31} and C_{33} *n*-alkanes were measured by GC–IRMS on a Thermo DELTA V PLUS system (Thermo Scientific, Waltham, MA, USA). The gas chromatograph (Trace Ultra, Thermo) was equipped with a split-splitless injector operated in splitless mode at 300 °C, a TRIPLUS autosampler (Thermo Scientific), and a VF-17 ms capillary column (60 m × 0.32 mm × 0.25 μm, Varian Inc., Middelburg, Nederlands). The GC was heated from 80 °C to 170 °C at 20 °C/min, then at 3 °C/min to 325 °C and then held at 325 °C for 20 min. Helium was used as the carrier gas at a constant flow of 1.3 ml/min. Compounds were pyrolyzed in a ceramic reactor at 1400 °C. A 1 μl sample was injected along with 0.5 μl of *n*-C_{38} of known isotopic composition (A. Schimmelmann, Indiana University, Bloomington, Indiana). Four samples were also injected with a mixture of four coinjection standards (C_{21}, C_{26}, C_{38} and C_{40} *n*-alkanes from A. Schimmelmann), in order to assess the effect of different isotopic corrections. Thermo ISODAT software V.2.5 was used to control instrumentation and calculate δD values. Correcting the raw δD values of the *A. marina* hydrocarbons with one or multiple coinjection standards did not result in any significant change in final δD values and it had no impact on the overall trends reported here. The *n*-alkane δD values reported below were corrected using only the C_{38} coinjection standard. Additionally, instrument performance was monitored using a mix of lab standards, whose δD values had previously been established using TC–EA–IRMS. This standard mix was run in triplicate at the beginning and end of each sequence of samples, as well as in duplicate after every six analyses. The H_3^+ factor was determined each day using pulses of a reference gas of varying heights and ranged between

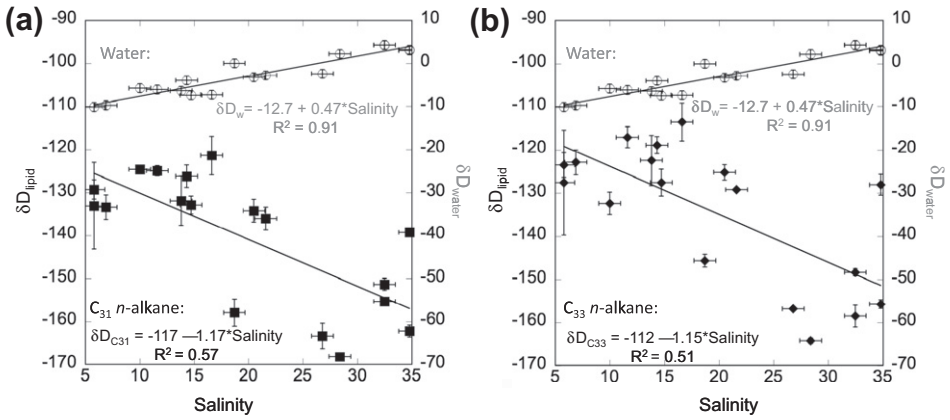

Fig. 3. Leaf wax and water δD values in the Brisbane River vs. salinity. A shows *n*-C_{31} alkanes; B shows *n*-C_{33} alkanes. Lipid δD scale for both plots is on the left-hand *y*-axis; the water δD scale for both plots is on the right-hand *y*-axis. The two δD axes have been scaled equivalently, but are offset by 100‰.

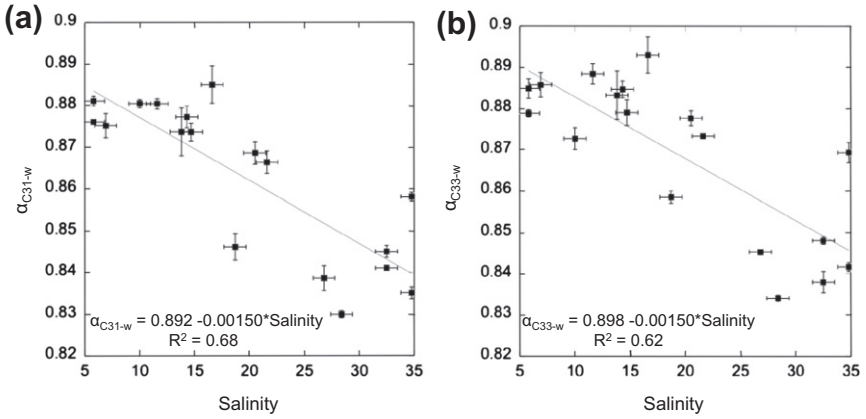

Fig. 4. Apparent fractionation factors, α_a, of mangrove *n*-C_{31} alkanes (A) and *n*-C_{33} alkanes (B) vs. salinity.

2.04 and 2.06. Each sample was measured in triplicate and the average standard deviation of triplicate analyses was 2.2‰.

3. Results

Water δD was positively correlated with salinity in the Brisbane River Estuary (R^2 = 0.88), reflecting the mixing of isotopically depleted freshwater and deuterium enriched seawater (Fig. 3). The overall range in water δD was 14.5‰ (values ranged from −10.2‰ to 4.3‰). The linear relationship between salinity and water δD implies that the fresh water end member of δD values (*y*-intercept) at the time of sampling was −12.7‰. From 1967–2002, mean February precipitation δD values in Brisbane averaged −15‰ ± 13.9‰, (1 s, IAEA, 2006), consistent with our estimate.

δD values were between −168‰ and −121‰ for *n*-C_{31}, a range of 47‰ and between −164‰ and −114‰ for *n*-C_{33}, a range of 50‰. δD values of *A. marina* *n*-alkanes were greatly depleted compared to that of their source water and were negatively correlated with salinity (Fig. 3). Linear regression analysis yielded correlation coefficients (R^2) of 0.52 for *n*-C_{31} vs. salinity (Fig. 3a) and 0.49 for *n*-C_{33} vs. salinity (Fig. 3b).

The apparent fractionation factor, α_a, was negatively correlated with salinity (R^2 = 0.63 for *n*-C_{31} and 0.59 for *n*-C_{33}) (Fig. 4). The apparent fractionation factors for the two lipids were highly correlated with each other (R^2 = 0.93), with *n*-C_{31} generally being 6‰ depleted relative to *n*-C_{33}.

Analysis of five leaves selected from a range of heights and sun exposure from the same tree generally yielded δD values within 4‰ of each other (Table 2). However, in one case, alkanes in one leaf were significantly enriched (>30‰) compared with the other four leaves from the same tree.

Table 2
δD values of replicate leaves from the same tree. 13a–e were five individual leaves from the mangrove growing at site 13 (Table 1); 10a–e were five individual leaves from the mangrove growing at site 10 (Table 1).

Sample	$\delta D_{C_{31}}$ (‰)	$1\sigma_{C_{31}}$ (‰)	$\delta D_{C_{33}}$ (‰)	$1\sigma_{C_{33}}$ (‰)
13a	−157.7	0.7	−157.5	0.7
13b	−154.7	1.2	−156.2	0.7
13c	−155.6	2	−157.4	0.6
13d	−127.9	1.7	−122.5	4.6
13e	−158.4	1.2	−158	0.3
10a	−117	5	−114.3	6.4
10b	−119.2	3	−115.7	2.4
10c	−118.3	0.2	−112.9	3.2
10d	−120.4	0.3	−113.7	1.7
10e	−119.4	2	−115.6	0.9

4. Discussion

4.1. Effect of salinity on mangroves and leaf wax δD values

4.1.1. Relationship between salinity and mangrove leaf wax δD values

The negative relationship between environmental water δD values and mangrove *n*-alkane δD values (Fig. 3) stands in sharp

contrast with the positive correlation seen in studies with terrigenous plants (Sachse et al., 2006; Hou et al., 2007). It implies that environmental water δD values are not the main controlling variable of *n*-alkane δD in mangroves and, by extension, that sedimentary mangrove leaf wax δD values would not provide a proxy for water δD values. Rather, for reasons discussed below, environmental water salinity likely exerts a stronger control in determining δD values of leaf waxes produced by mangroves than environmental δD values.

Noteworthy is the fundamentally different relationship between salinity and lipid δD values in mangroves as compared to aquatic phytoplankton and cyanobacteria, in which δD values of lipids are positively correlated with salinity (Fig. 5) (Schouten et al., 2006; Sachse and Sachs, 2008; Sachs and Schwab, 2011). In field and laboratory based studies of phytoplankton and cyanobacteria, the difference between δD_{water} and δD_{lipid} is smaller at higher salinities. This has been attributed to either (i) diminished growth rate at higher salinity, (ii) deuterium enrichment of intracellular water resulting from enhanced water recycling within the cell, or (iii) production of D depleted osmolites that counter the greater osmotic gradient at high salinity (Sachs and Schwab, 2011).

Perhaps it is not surprising that single celled aquatic organisms and vascular plants respond differently to the same environmental change. A similar discrepancy was observed for the effect of temperature on α_a. For phytoplankton, increasing temperature has been demonstrated to result in greater apparent fractionation (Zhang et al., 2009). However, increasing temperature had no effect on net fractionation in a study of C_3 and C_4 vascular plants (Zhou et al., 2011).

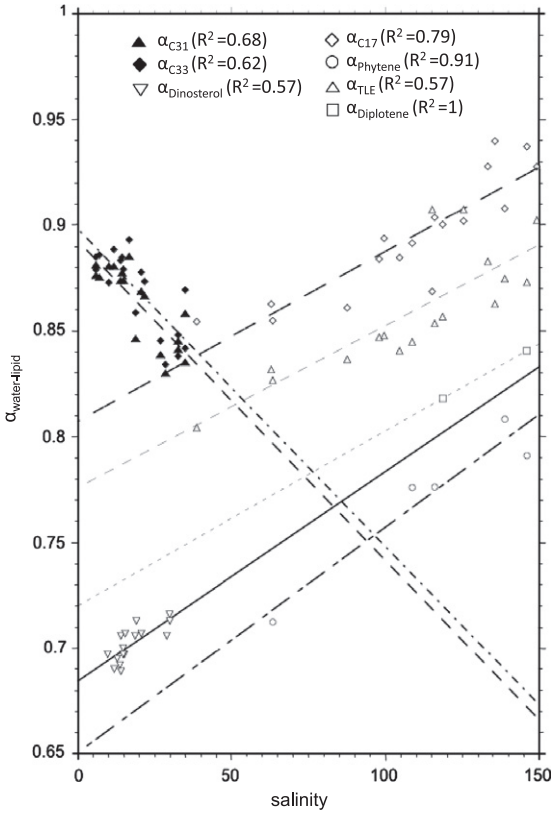

Fig. 5. Comparision of this study's results ($\alpha_{C_{31}}$ and $\alpha_{C_{33}}$ from Fig 4, solid symbols) with previously published comparisons of apparent fractionation in algal lipids with salinity (open symbols). Dinosterol data is from Sachs and Schwab (2011); other algal lipids are from Sachse and Sachs (2008). Linear regressions are continued beyond the salinity range of each study for illustrative purposes only.

Additionally, our results are not dissimilar to another study of leaf wax δD values from plants growing in a saline environment. Romero and Feakins (2011) measured xylem water, leaf water and leaf wax δD values for a variety of plants at four sites in a Californian salt marsh. They observed less apparent fractionation (α closer to 1) at the inland sites with more saline pore water, which seems to contrast with our results. However, by analyzing the xylem water of their plants, Romero and Feakins (2011) demonstrated that the plants at the more inland saline sites were using meteoric water, as the xylem water was depleted by 20‰ compared to the plants at the less saline sites, whose xylem water had δD values comparable to local seawater. Thus the plants with the greatest apparent fractionation were those that were obtaining most of their water from seawater, similarly to the mangroves we studied in the Brisbane River Estuary.

Since α_a represents the combined fractionation factors of several different processes, it is possible that the fractionation associated with one or more of those processes is negatively related to salinity. In general, evapotranspiration causes leaf water to become enriched in D relative to environmental water (Leaney et al., 1985; Walker et al., 1989; Dawson et al., 2002; Kahmen et al., 2008) and biosynthesis results in lipids becoming depleted in D relative to leaf water (Sessions et al., 1999; Sachse et al., 2006; Feakins and Sessions, 2010; Romero and Feakins, 2011). Biosynthetic D depletion (α_b) has a greater magnitude than the D enrichment associated with evapotranspiration (α_e), resulting in net fractionations on the order of 0.90–0.84 ($\varepsilon_a = -100‰$ to $-160‰$) (Chikaraishi and Naraoka, 2003; Sachse et al., 2006, 2009; Feakins and Sessions, 2010). At 0.88 ($\varepsilon_a = -120‰$), the net fractionation for *A. marina* trees at low salinity sites in our study is comparable to the net fractionation observed in other C_3 dicots (growing at zero salinity). The greater negative apparent fractionation observed in *A. marina* growing at high salinity ($\alpha_a = 0.835$; $\varepsilon_a = -165‰$) could be due to less enrichment of internal water, more D depletion during biosynthesis, or some combination of the two (Fig. 6).

Because salinity exerts a significant environmental challenge to mangroves, and because this is the primary difference between the trees in our study and those in other investigations of the controls on leaf wax δD values, it seems reasonable that our results can be explained in the context of the physiological responses of *A. marina* to increased salinity, and to the mechanisms *A. marina* has developed to manage salt contained in its water. In the following sections, we review the ways in which mangroves respond to increased salinity, including their strategies for dealing with salt, and assess the potential for these responses to impact α_e and α_b.

4.1.2. Physiological responses of mangroves to increased salinity

Salt imposes osmotic stress on plants and interferes with their enzymatic functions (Greenway and Osmond, 1972; Yeo, 1983; Morgan, 1984). Mangroves have evolved three strategies to manage salt. One strategy is to prevent salt from entering roots during water uptake (exclusion). Another strategy is to secrete salt from leaves (secretion). A final strategy is to accumulate salt in special vacuoles within the leaves (accumulation) (Hogarth, 2007; Parida and Jha, 2010). Although some species rely exclusively on one strategy, most mangroves employ two, or all three (Hogarth, 2007). The strategy employed by a mangrove can also vary as salinity changes. At low salinities, *A. marina* excludes 90% of the salt from its internal water (it both secretes and accumulates the remaining salt). As salinity increases, *A. marina* increases the fraction of salt its roots exclude, reaching a 97% exclusion rate when cultured in water with an NaCl concentration of 500 mol/m^3 (i.e., comparable to seawater; Ball, 1988).

Mangroves growing in saline water have also developed a number of adaptations to conserve water. These include smaller mature heights and lower growth rates (Ball, 1988; Lin and Sternberg,

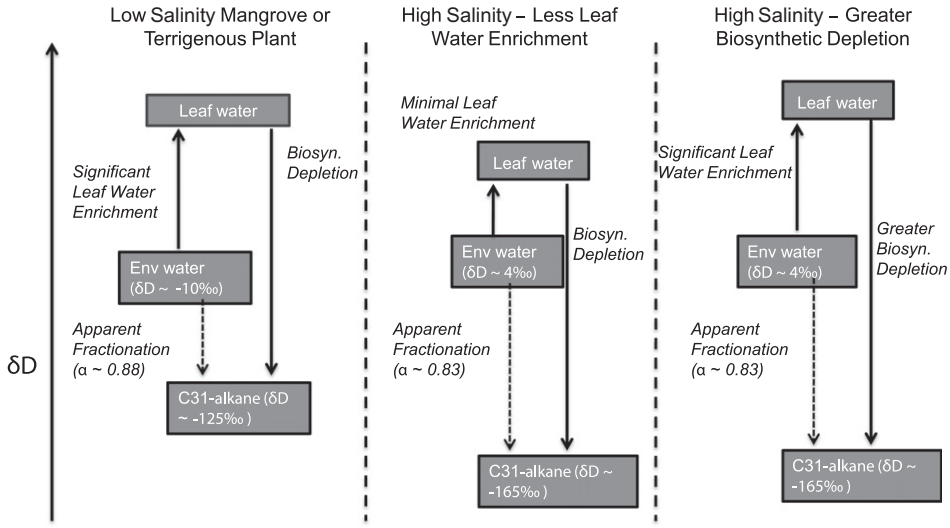

Fig. 6. Schematic representation of processes that contributed to the hydrogen isotope ratios of mangrove lipids. The left panel represents a mangrove growing in low salinity, and is comparable to a terrestrial tree. The right two panels represent two ways a mangrove growing in more saline water could display greater apparent fractionation. At present, we cannot determine which scenario is more appropriate.

1992; McKee et al., 2002; Naidoo, 2010), as well as lower concentrations of chlorophyll a and b (Naidoo, 2010), lower photosynthetic capabilities (Ball and Farquhar, 1984) and lower carbon assimilation rates (Ball and Farquhar, 1984; Lin and Sternberg, 1992). Increased salinity is also associated with lower stomatal conductance (Ball and Farquhar, 1984; Lin and Sternberg, 1992) and consequently diminished transpiration rates (Ball and Farquhar, 1984; Ye et al., 2005; Lopez-Hoffman et al., 2007).

4.1.3. Potential for reduced stomatal conductance to affect α_a at high salinity

Numerous studies of the relationship between leaf wax δD values and environmental water δD values have suggested that changes in α_a are due to changes in evapotranspiration, with higher evapotranspiration rates resulting in greater isotope fractionation of the leaf water and enrichment of D and ^{18}O in that water (e.g. Sachse et al., 2006; Feakins and Sessions, 2010; Romero and Feakins, 2011). At first glance the *A. marina* δD data would seem to be consistent with this scenario, whereby higher salinity causes reduced rates of transpiration and less isotopic enrichment of leaf water and the leaf waxes produced from that water. But this overlooks the cause of the reduced transpiration rates when mangroves encounter more saline water, which, as stated, is diminished stomatal conductance.

Stomatal conductance is the rate at which water vapor or CO_2 pass through the stomata, which in turn is controlled by the aperture of the stomata. A reduction in the size of the stomatal aperture is thought to increase the isotope fractionation of water and cause isotopic enrichment of leaf water (Leaney et al., 1985; Walker et al., 1989; Barbour and Farquhar, 2000; Farquhar et al., 2007). Hence, since higher salinity causes mangroves to decrease their stomatal conductance (Ball and Farquhar, 1984; Lin and Sternberg, 1992), it ought to cause leaf water δD values to increase. This is opposite the dependence observed in *n*-alkanes from *A. marina* (Fig. 3), implying that lower transpiration rates brought on by diminished stomatal conductance in response to saltier water cannot explain the observed decrease in δD values.

4.1.4. Potential for mangrove salt tolerance strategies to affect α_a at high salinity

We have developed four hypotheses related to the salt tolerance mechanisms of *A. marina* that could explain some or all of the increased magnitude of α_a at high salinity: (i) increased exclusion of salt at the roots could lead to more discrimination against deuterium during water uptake, (ii) salt secretion could increase the local relative humidity on the leaf surface, resulting in less enrichment of leaf water, (iii) depleted water of hydration could enter the leaves and result in less net enrichment of leaf water, or (iv) enhanced production of compatible solutes could use up pyruvate and cause more of the hydrogen in leaf waxes to come from relatively depleted NADPH. We will explore each of these possibilities below.

Although most plants do not fractionate water isotopes during uptake by the roots (White et al., 1985; Dawson and Ehleringer, 1991; Walker and Richardson, 1991), mangroves and other halophytic plants have been shown to discriminate against HDO (but not $H_2{}^{18}O$) during water uptake (Lin and Sternberg, 1993; Ellsworth and Williams, 2007). This fractionation has been attributed to the well developed Casparian band that is found on the roots of halophytic plants such as *A. marina* (Moon et al., 1986), which limits apoplastic transport (a bulk transfer process) of water into the roots, in favor of symplastic transport (which is a molecular transfer process). It is energetically favorable to disassociate H_2O rather than HDO from water aggregates, but there is little difference in the energy needed to disassociate $H_2{}^{16}O$ and $H_2{}^{18}O$ (Chacko et al., 2001). Therefore, increased symplastic transport could explain the fractionation of water isotopes that has been observed during water uptake by mangroves and other halophytes (Lin and Sternberg, 1993; Ellsworth and Williams, 2007). *Avicennia germinans*, a salt secreting mangrove that is closely related to *A. marina*, displayed less D discrimination when grown in freshwater than in saline water (Lin and Sternberg, 1993). The magnitude of the effect observed by Lin and Sternberg was <10‰, so at most, this mechanism accounts for less than 20% of the 50‰ implied change in α_a we observed for *A. marina* in the Brisbane River.

It is also possible that increased salt secretion at high salinity could partially explain the negative relationship between α_a and salinity. One way this could occur is by increasing the local relative humidity on the leaf surface. Saturated NaCl solutions maintain a constant relative humidity of 76% above them (O'Brien, 1948), greater than the mean annual humidity in Brisbane, which is 53% (http://www.bom.gov.au). Elevating relative humidity reduces transpiration rates due to a decreased gradient in vapor pressure across the leaf surface and reducing transpiration by this mechanism also results in reduced isotopic enrichment of leaf water

(Helliker and Ehleringer, 2002a, 2002b; Farquhar and Cernusak, 2005). Helliker and Ehleringer (2002b) observed that leaf water in grass blades grown at a relative humidity of 76% had a $\delta^{18}O$ value ~7.5‰ less than grass growing at a relative humidity of 53%. Since the enrichment associated with evaporation is typically five times greater for hydrogen than oxygen, it is plausible that this increase in relative humidity could account for a ~35‰ decrease in δD_{leaf} water.

However, it is unclear whether changes in leaf water δD are captured in lipid δD, and some studies suggest that relative humidity may not have a large role in determining leaf wax δD (Hou et al., 2008; McInerney et al., 2011). A recent review (Sachse et al., 2012) concluded that relative humidity likely influences leaf wax δD, but the relationship is not straightforward and needs to be investigated further. It would be necessary to grow *A. marina* at different relative humidities (and constant salinity) in order to determine its impact on *n*-alkane δD values.

Another way that increased salt secretion at high salinity could result in more apparent fractionation is the potential for D depleted hydration water from exchange with atmospheric water vapor in secreted salts to mix with leaf water, lowering its δD value. Water of hydration in Na salts can be depleted in deuterium by 80‰ relative to source water at 25 °C (Matsuo et al., 1972). If hydration water derived from the atmosphere in secreted salts exchanges with internal leaf water the latter would undergo isotopic depletion. In order to account for the entire ~50‰ variation we observed in *A. marina* leaf wax δD values, hydration water would need to account for more than 50% of the water in the leaf, which seems improbable.

Finally, it is possible that the increased production of compatible solutes at high salinity is responsible for some of the increased fractionation observed at high salinity. *A. marina* produces compatible solutes (predominantly asparagine, but also stachyose, glycine betaine, alanine, pinitol and proline) at high salinity, which reverse the osmotic gradient within the leaf and help the plant maintain isolation of salt ions in vacuoles (Ashihara et al., 1997; Hibino et al., 2001; Kathiresan and Bingham, 2001; Datta and Ghose, 2003). Amino acids, organic acids and soluble carbohydrates, such as the compatible solutes listed above, are enriched in D relative to pyruvate, a biosynthetic precursor of *n*-alkanes (Schmidt et al., 2003). The hydrogen in pyruvate is derived from two sources: water via soluble carbohydrates and NADPH via the pentose phosphate cycle (Schmidt et al., 2003). Of these, NADPH-derived hydrogen is significantly more depleted in D ($\delta D \sim -250‰$) than that from carbohydrates ($\delta D \sim -70‰$) (Luo et al., 1991; Hayes, 2001; Schmidt et al., 2003). Therefore, at high salinity it may be that a greater proportion of the H in pyruvate would, by necessity, be derived from NADPH due to the increased demand for compatible solutes to reverse the osmotic gradient. This would result in D depletion of the *n*-alkanes and other lipids synthesized from pyruvate (Schmidt et al., 2003; Zhou et al., 2010). Since the majority of hydrogen in lipids is derived from water in all cases, it seems unlikely that this mechanism could, in and of itself, account for the entire ~50‰ signal we observed in *A. marina* *n*-alkane δD values along the Brisbane River Estuary.

Of the four mechanisms we propose that could contribute to the increased D/H fractionation in *A. marina* *n*-alkanes as salinity increases, none seems able to account for the entire signal independently. However, a combination of some or all of these mechanisms could be large enough to cause the overall observed reduction in δD values of *A. marina* *n*-alkanes along the salinity gradient.

4.1.5. Deviations from the linear relationship between salinity and α_a

There are a few outlying *n*-alkane δD values at the higher end of the salinity spectrum. One of those outliers is from a tree on the coast of Moreton Bay, outside the Brisbane River Estuary (site 16). The *n*-alkanes from this tree are enriched in D compared to the trend defined by the linear regression of *n*-alkane δD vs. salinity. The mangrove sampled at this site was growing on the edge of a public park on a sandy intertidal flat. At a similar site further north on the coast, groundwater seepage was noted to be contributing to the water around a mangrove we sampled, which caused us to ultimately exclude that sample from this study, since groundwater seeps are often not constant through time. It is possible that a similar source of freshwater intermittently impacted the Moreton Bay site, causing the leaf *n*-alkanes to have higher δD values than predicted by the trend line in Fig. 3. Consistent with this notion, *A. germanins* trees growing on sand bars amidst hypersaline water in South America have been observed to utilize groundwater percolating through the sand as opposed to the more prevalent saline surface water (Lambs et al., 2008) and *R. mangle* trees from the Florida Everglades have been observed to switch their source water to ground water during the dry season (Ewe et al., 2007). It is possible that the tree sampled here was employing a similar strategy and thus was consuming less saline water than the surface water we measured.

It is also probable that much of the scatter observed in the relationship between salinity and α_a in *A. marina* shown in Fig. 4 is due to the wide range of maturities and microenvironments of the mangroves in the sample set. Plants in the sample set ranged from large mature trees to small saplings. Our results give some indication of how much this might have contributed to the scatter in Fig. 4. At the mouth of the river (site 14), we sampled a mature tree with a height of ~5 m as well as a small sapling that was <1 m tall. The δD and α_a values for *n*-alkanes in these two trees are plotted as separate data points in Fig. 3 and Fig. 4 and fall within 4‰ of one another for the *n*-C_{31} alkane. For the C_{33} *n*-alkane, the offset was 10‰.

Additionally, the amount of sunlight incident on sampled leaves was not controlled, contrary to many other studies (e.g. Sachse et al., 2009; Feakins and Sessions, 2010). It is possible that this could affect either the amount of transpiration in a leaf and the D/H fractionation during *n*-alkane biosynthesis (i.e., α_b). Low level continuous light (such as that during summer at polar latitudes) has been observed to result in a 40‰ enrichment of leaf waxes relative to leaf waxes in plants grown with diurnal light cycles, which could be due to more evapotranspiration under continuous light conditions (Yang et al., 2009). However, no systematic study of the effect of varying light intensity within a single tree has been published to date.

Finally, we did not control for the age of the leaf sampled. Seasonal variations in leaf wax δD have been observed in sea grasses and some trees (Sessions, 2006; Sachse et al., 2009), but not in other species of trees and some grasses (Sachse et al., 2010; Kahmen et al., 2011). It is unclear whether leaf waxes are continually produced in leaves, or whether they are predominantly produced when the leaf is young (Jetter et al., 2007; see discussion in McInerney et al., 2011). In either case, leaf wax *n*-alkanes from older leaves may have a different isotopic signature than those from younger leaves if they formed during different times of year.

At most sites we measured *n*-alkane δD values in a single leaf. Multiple analyses of leaves from the same tree generally justified this approach, but there was one leaf that had *n*-alkanes that were greatly enriched (by >30‰) compared with the mean of the other 4 leaves from the same tree (which had δD values within 4‰ of one another) (Table 2). Future studies should seek to minimize the range of these variables in an effort to better isolate the effect of salinity on D/H fractionation. Despite the limitations of this particular sample set, there remains a significant negative correlation between salinity and the apparent fractionation factor between source water and mangrove *n*-alkanes, suggesting that salinity plays a major role in controlling leaf wax δD values in mangrove leaves.

We might expect that the relationship between salinity and α_a would not be strictly linear, because it is likely that the environmental challenge presented by salinity is non-linear and diminishes at lower salinity. Indeed, the relationship between salinity and growth rate is not strictly linear. Cultivation of *Avicennia* and *Rhizophora* in different salinity treatments has consistently shown that these genera of mangroves obtain their highest growth rates in brackish water with salinity between 5 and 15 PSU (Gordon et al., 1992; Ye et al., 2005; Patel et al., 2010).

4.2. Implications for paleoclimate reconstructions

If the relationship between salinity and *A. marina* *n*-alkane δD values observed here is characteristic of other lipids, in other mangrove species, and at other locations, it suggests that mangrove lipid δD may be used as a paleosalinity proxy. This would be useful in developing records of past hydrologic change, especially in settings where mangrove biomarkers are found alongside biomarkers of other organisms that are more strongly influenced by source water δD. For example, the δD value of dinosterol, a sterol produced primarily by dinoflagellates, is controlled by both source water δD and salinity in roughly equal proportions (Sachs and Schwab, 2011). An independent measure of salinity variability could constrain dinosterol δD values and provide for a more robust measure of past changes in water δD values.

A paleosalinity proxy based on mangrove lipids could be applied in a variety of settings, including mangrove swamps. Advantages of working in mangrove swamps include their prevalence along tropical coastlines, their relatively high accumulation rates, and the fact that are dominated by relatively few higher plants. However, material within a swamp could be subject to lateral advection by currents, or vertical movement by roots or animals. The organic material in mangrove swamps comes both from the mangroves and from marine sources, with some swamps being influenced more by one source than the other (Bouillon et al., 2003). Contributions of lipids from inland terrestrial plants are less common in mangrove areas, even where a major river is present (Volkman et al., 2007).

Bioturbation within a mangrove swamp is most commonly due to mangrove crabs, who may disturb the upper portion of the sediment when burrowing (Hogarth, 2007). Additional vertical mixing may be caused by mangrove roots, which are shallow (typically <1 m deep) compared to other trees (Lin and Sternberg, 1994; Hogarth, 2007). The roots themselves contribute younger organic material to the surrounding sediment, but often contain different lipids than the leaves (Wannigama et al., 1981; Basyuni et al., 2007), making their avoidance possible. Complications from vertical and horizontal mixing need to be assessed individually for specific mangrove swamps, as, indeed, they do for any sedimentary archive.

Our results are pertinent not only to mangrove swamps, but also to brackish lakes and ponds surrounded by mangroves and to sediments along continental margins in the tropics and subtropics, with the caveat that a mangrove-specific lipid biomarker would be necessary for the paleosalinity proxy to work in such an environment. Leaf waxes, which include the *n*-alkanes measured in this study, are produced by a wide range of plants. In a depositional setting with mixed contributions of mangrove and non-mangrove leaf waxes, the δD values of *n*-alkanes and other common lipids would be difficult to interpret, since different variables would influence the δD value of similar lipids to varying degrees. Given the negative correlation between salinity and α_a of mangrove leaf wax *n*-alkanes, drier periods should result in lower δD values in mangrove lipids, a consequence of increased water salinity caused by higher evaporation relative to precipitation and less freshwater runoff. The same processes would be expected to result in D enrichment of the source water used by non-mangrove trees, thereby causing D enrichment of their leaf waxes. A sedimentary mixture of the leaf waxes from both types of trees, characterized by opposing slopes of α_a vs. salinity, would produce a muted signal at best, while down-core changes in leaf wax δD would be influenced to an unknown extent by changes in the relative amounts of mangrove vs. non-mangrove leaf waxes.

A mix of mangrove and non-mangrove contributions to sediment leaf waxes could explain the small amplitude of variation in the hydrogen isotope ratios of leaf waxes in some published studies. For example, Smittenberg et al. (2011) measured δD of dinosterol, palmitic acid and long chain *n*-alkanes from Spooky Lake and OTM in Palau. These lakes are surrounded by mangroves, but in catchments characterized by a wide range of tropical non-mangrove trees. The dinosterol δD values during the last 500 years, produced exclusively by dinoflagellates within the lake, had a wide range of variability (~50‰) that correlated with the instrumental record of precipitation from the region. Palmitic acid δD values showed variability of ~35‰ over the past century and generally agreed with the trends observed for the dinosterol δD values. However, the long chain *n*-alkane δD values, presumably produced by a mix of mangrove and non-mangrove trees, had a much smaller range (~10‰) of values.

Likewise, Tierney et al. (2010) produced a record of hydrologic variability from leaf wax fatty acids preserved in marine sediments off the coast of Sulawesi, Indonesia. δD values of the leaf waxes were well correlated with the instrumental record of precipitation δD. However, the range of δD in precipitation (~40‰) was much larger than the corresponding range in leaf wax δD (~8‰). Tierney et al. (2010) proposed that the high sediment accumulation rates at their site implied large amounts of terrigenous runoff. Coastal Indonesia has abundant mangrove swamps and it is probable that both mangrove and non-mangrove leaf waxes contributed to the sedimentary lipid pool. A contribution of mangrove leaf waxes could partly explain why the range of sedimentary leaf wax δD variability during the last century is just 20% of the precipitation δD variability.

5. Conclusions

We collected leaves from *A. marina* along a salinity gradient in the Brisbane River Estuary and measured δD values of their *n*-alkanes, as well as of their source water. These measurements allowed us to calculate the apparent fractionation factor, α_a, for leaf wax *n*-alkanes produced by *A. marina*. The *n*-alkane δD values had a range greater than three times that of the water δD values and the two were inversely correlated with salinity such that the deuterium depletion in *n*-alkanes compared to water was only about 15‰ at 5 PSU and 70‰ at 35 PSU. There was a significant negative correlation between α_a and salinity for the mangrove leaf wax *n*-alkanes, the opposite of the trend observed for algal lipids. Additionally, δD values of mangrove leaf waxes differ from those of other vascular plants, in that they do not seem to be primarily controlled by source water δD. Rather, the stress of added salinity appears to result in increased discrimination against deuterium. We have suggested that this trend could be caused by increased fractionation against D in the roots during water uptake, increased relative humidity at the leaf surface due the secretion of salty brines, water of hydration of leaf salts being incorporated in leaf water, or increased contributions of isotopically depleted hydrogen from NADPH at a high salinity. Of these, only one of the mechanisms involving secreted salts appears large enough to independently cause the hydrogen isotope response we observe in *n*-alkanes from *A. marina*. But a combination of two or more of them seems ample.

Regardless of the mechanism, the empirical relationship between salinity and δD_{leaf} wax in mangroves suggests that hydrogen isotope measurements of source specific mangrove lipids may prove to be a valuable paleosalinity indicator. Likewise, our results indicate that caution must be applied in interpreting δD values of non-source specific higher plant leaf waxes, such as the *n*-alkanes measured here, in areas where both mangroves and non-mangrove trees contribute plant matter to the sediments.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orggeochem.2012.04.009.

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