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Inverse relationship between salinity and $^2\text{H}/^1\text{H}$ fractionation in leaf wax *n*-alkanes from Florida mangroves

Ding He^{a, b*}, S. Nemiah Ladd^d, Julian P. Sachs^e, Rudolf Jaffé^{b, c}

^a School of Earth Sciences, Zhejiang University, Hangzhou 310027, CHINA

^b Marine Science Program, Department of Chemistry & Biochemistry, Florida International University, 3000 NE 151st St., North Miami, FL 33181, USA

^c Southeast Environmental Research Center, Florida International University, Miami, FL 33199, USA

^d Department of Surface Waters Research and Management, Swiss Federal Institute of Aquatic Science and Technology (EAWAG), Seestrasse 79, 6047 Kastanienbaum, Switzerland

^e School of Oceanography, University of Washington, Box 355351, Seattle, WA 98195, USA

* Corresponding author. E-mail address: Ding He(dinghe@zju.edu.cn), School of Earth Sciences, Zhejiang University, Hangzhou 310027, CHINA.

ABSTRACT

The effect of salinity on hydrogen isotope fractionation during the production of leaf wax *n*-alkanes was assessed for *Laguncularia racemosa* (white mangrove), *Rhizophora mangle* (red mangrove), and *Avicennia germinans* (black mangrove) along a 31 ppt (parts per thousand) salinity gradient in the Shark River estuary, Florida, USA. Significant variation in hydrogen isotope ratios were observed among these three Atlantic-East Pacific (AEP) species, with increasing leaf wax *n*-alkane $^2\text{H}/^1\text{H}$ fractionation with increasing salinity. Net $^2\text{H}/^1\text{H}$ fractionation for hentriacontane ($n\text{-C}_{31}$) increased by 0.8, 1.4 and 1.8‰/ppt in *R. mangle*, *A. germinans* and *L. racemosa*, respectively. The observations are consistent with published $\delta^2\text{H}_{n\text{C}_{31}}$ data from 5 species of Indo-West Pacific (IWP) mangroves, which increased with salinity by 0.7 - 1.5‰/ppt. Although all measured species from both the AEP and IWP regions have more $^2\text{H}/^1\text{H}$ fractionation at high salinity, differences in slope and intercepts of these are relationships are observed among genera. These differences may result from variation in the composition of compatible solutes, reliance on storage carbohydrates, and/or physiological responses to salt. However, no statistically significant difference in the sensitivity of $\delta^2\text{H}_{n\text{C}_{31}}$ to salinity was observed in four *Rhizophora* species from both Indo-West Pacific and Americas-East Atlantic regions, which makes sedimentary *Rhizophora* lipids a promising target for paleohydroclimatic reconstruction.

Keywords: hydrogen isotope fractionation; mangroves; *n*-alkanes; paleoclimate; estuary.

1. Introduction

The stable hydrogen isotopic composition of leaf wax *n*-alkyl lipids, especially *n*-alkanes, and triterpenoids, can be used as paleoclimatological and paleohydrological proxies, especially when combined with other geochemical proxies, such as molecular biomarkers, bulk and compound specific carbon isotopes, etc. (Smith and Freeman, 2006; Polissar et al., 2009; Hren et al., 2010; Berke et al., 2012; Sachse et al., 2012 and references therein; Feakins et al., 2014; Aichner et al., 2015; Nelson and Sachs, 2016). Recently, a new application of lipid hydrogen isotope ratios (typically expressed as $\delta^2\text{H}$ values, where $\delta^2\text{H} = ({}^2\text{H}/{}^1\text{H})_{\text{Sample}}/({}^2\text{H}/{}^1\text{H})_{\text{VSMOW}} - 1$) as a paleosalinity proxy has garnered attention and various laboratory cultures and field studies have been performed to examine the relationship between $\delta^2\text{H}_{\text{lipid}}$ and salinity in phytoplankton (Schouten et al., 2006; Sachse and Sachs, 2008; Sachs and Schwab, 2011; Chivall et al., 2014; M'boule et al., 2014; Nelson and Sachs, 2014; Sachs et al., 2016; Maloney et al., 2016). Across multiple taxa and diverse settings these studies found that as salinity increased, ${}^2\text{H}/{}^1\text{H}$ fractionation decreased by ca. 0.5 - 2‰/ppt (Schouten et al., 2006; M'boule et al., 2014; Sachs et al., 2016). These results have prompted the application of algal $\delta^2\text{H}_{\text{lipid}}$ values to reconstruct salinity across a range of spatial and geological scales (van der Meer et al., 2007, 2008; Sachs et al., 2009; Smittenberg et al., 2011; Vasiliev et al., 2013; Kasper et al., 2014, 2015; Nelson and Sachs, 2016; Richey and Sachs, 2016).

While most research into the effect of salinity on hydrogen isotope fractionation in photosynthetic organisms has focused on phytoplankton, recent studies on halophytic vascular plants have found a similarly sensitive ${}^2\text{H}/{}^1\text{H}$ fractionation response, but surprisingly, of the opposite sign (Ladd and Sachs, 2012, 2015a, b, 2017). Mangroves – trees that inhabit the intertidal zone – display more net hydrogen isotope fractionation during the production of their leaf lipids as salinity increases (Ladd and Sachs, 2012, 2015a, b, 2017). This salinity response is in direct contrast to that of phytoplankton. Increased hydrogen isotope fractionation at higher salinity has been reported for both acetogenic and isoprenoidal lipids from six species of Indo-Pacific mangroves growing in estuaries and brackish lakes at tropical and subtropical sites

throughout the western Pacific (Ladd and Sachs, 2012, 2015a, b, 2017). The differing hydrogen isotope responses to salinity in phytoplankton and mangroves may result from, among other things, different biosynthetic responses to osmotic stress in higher plants and algae and/or to transpiration-related effects present only in higher plants (Ladd and Sachs, 2015b), as elaborated upon below.

The $^2\text{H}/^1\text{H}$ fractionation between salinity and leaf wax n -alkanes is defined as the hydrogen isotope fractionation factor $\alpha_{\text{alkane-sw}}$, where $\alpha_{\text{alkane-sw}} = (\delta^2\text{H}_{\text{alkane}} + 1)/(\delta^2\text{H}_{\text{surface water}} + 1)$. $^2\text{H}/^1\text{H}$ fractionation has been shown to increase by 0.7 - 1.7‰/ppt for leaf wax n -alkanes in Indo-West Pacific (IWP) mangroves (Ladd and Sachs, 2012, 2015a, b). However, it is unknown whether the inverse correlation between salinity and $\alpha_{\text{alkane-sw}}$ is characteristic of Americas-East Pacific (AEP) mangrove species and other mangrove genera such as *Laguncularia*. In addition, questions remain about the effect of different salt management strategies employed by mangroves on $\alpha_{\text{alkane-sw}}$. These salt management strategies include excluding salt from entering the roots by filtration during water uptake, secreting salt from their leaves, and accumulating salt in vacuoles (Hogarth, 2007). Not all mangroves are capable of using all three salt management strategies, which may affect the species-specific correlation between salinity and $\alpha_{\text{alkane-sw}}$. For example, if secreted salt on the leaf surface increases the relative humidity in the boundary layer around the leaf, this should result in less enrichment of leaf water and more net fractionation between surface water and leaf lipids (Ladd and Sachs, 2012). This effect would result in more pronounced hydrogen isotope fractionation at high salinity for taxa that secrete salt, such as *Avicennia*, relative to those that cannot secrete salt, such as *Rhizophora*.

Even if all mangrove species exhibit increased hydrogen isotope fractionation in their leaf lipids at high salinity, it is crucial to have a better understanding of interspecies differences in the slope and intercept of each calibration equation so that quantitative paleosalinity reconstruction can be performed where multiple mangrove taxa occur. In order to characterize the hydrogen isotope response of AEP mangrove lipids to salinity, we sampled the three most common species, *Rhizophora mangle* (red mangrove), *Laguncularia racemosa* (white mangrove) and *Avicennia germinans* (black mangrove), along a 31 ppt salinity gradient in the Shark River estuary in south Florida, USA. We present $\delta^2\text{H}_{n\text{-alkane}}$ data from 29 individual trees and compare it with published hydrogen isotope data from one additional genus and five additional species from the IWP mangroves (*Aegiceras corniculatum*, *Avicennia marina*, *Rhizophora stylosa*, *R. mucronata*, *R.*

apiculata) reported by Ladd and Sachs (2012, 2015a,b). *R. mangle* and *R. stylosa* are known as salt excluders and accumulators; *A. germinans* and *A. marina* are salt excluders, secretors and accumulators; *A. corniculatum* is a salt excluder and secretor and *L. racemosa* is a salt secretor only (Parida and Jha, 2010). With these data we aimed to evaluate whether increasing $^2\text{H}/^1\text{H}$ fractionation in leaf wax lipids is a response to increasing salinity shared by AEP mangroves, and if so, whether any inter-species differences in the response can be attributed to different salt management strategies. While it is difficult to prove interspecies variability in the H isotope response to salinity within a family or a genus with the limited taxa investigated to date, the results presented here add to a growing body of evidence supporting the use of mangrove lipid $\delta^2\text{H}$ in hydroclimate reconstructions.

2. Samples and methods

2.1. Site description and samples

The Shark River estuary is located in the southwest of the Florida Everglades, USA (Figure 1). It is a mangrove-dominated system, characterized by semi-diurnal tides with a mean amplitude of 1.1 m (Wanless et al., 1994). The dominant plant species in this oligohaline ecotone are mangroves (Rivera-Monroy et al., 2011), ranging in height between 5 and 13 m and decreasing in size along the estuary with distance inland from the mouth of the Shark River (i.e. decreasing average salinity) (Castañeda-Moya et al., 2013). Leaf lifetimes in the region are about 60, 111, and 160 days in *L. racemosa*, *R. mangle* and *A. germinans*, respectively (Suárez, 2003).

Mature leaves from these three mangrove species were collected by hand along the estuary in March 2013 (Fig. 1; Table 1). Multiple leaves (randomly selected) were collected from each mature mangrove tree and immediately placed in plastic Ziploc bags and stored on ice in an insulated box. At each site, multiple salinity measurements were made for surface water (ca. 10 cm below surface) adjacent to the sampled tree to calculate the average salinity (Table 1). In addition, the linear correlation equation between salinity and distance to the Shark River mouth was determined as: $\text{salinity} = -1.3949 \times (\text{km of distance}) + 31.745$; R^2 0.97, which is used to derive calculated salinity in order to minimize the bias due to limited salinity measurements during a tidal cycle (Table 1).

2.2. Lipid extraction

Intact whole leaves from each tree were used for extraction to avoid potential isotopic differences that occur from the base to the tip of a single leaf (Sessions, 2006; Gao and Huang, 2013; Gao et al., 2015). All leaf samples were freeze-dried at -50 °C and shredded. Freeze dried biomass (2 to 3 g) was then subjected 3 x to ultrasonic extraction (0.5 h each) with pure dichloromethane (Optima, Fisher, USA). Each extract was concentrated and fractionated via adsorption chromatography over 7.5 g silica gel to purify the *n*-alkanes using 20 ml *n*-hexane as the eluent.

2.3. Gas chromatography-mass spectrometry (GC-MS)

GC-MS was performed with a Hewlett-Packard 6890 GC instrument linked to a HP 5973 MS system, fitted with a Rtx-1 (30 m x 0.25 mm i.d., 0.25 µm df) column from Restek, USA. The GC oven temperature program was set from 60 (4 min) to 300 °C (held 20 min) at 6 °C/min. All the *n*-alkanes were quantified relative to squalane (internal standard), assuming a similar response factor, with an uncertainty of +/- 15% for replicates.

2.4. Compound specific hydrogen isotope analysis

A GC/pyrolysis/isotope ratio MS (IRMS) system consisting of a HP 6890 GC instrument connected to a Finnigan MAT delta Plus V mass spectrometer was used. An Rtx-1 fused silica GC column (30 m x 0.25 mm i.d., 0.25 µm df) was used. The pyrolysis temperature was 1440 °C in a micro volume ceramic tube. He was the carrier gas at 1.6 ml/min. The oven temperature program was as above (Section 2.3). An external standard (B3; Indiana University, Bloomington, USA) consisting of a mixture of *n*-alkanes from C₁₆ to C₃₀ (with different concentration and known δ²H value for each *n*-alkane) was analyzed between every four sample measurements and used for correction. Specifically, the slope and intercept of the relationship between measured and known δ²H values of each *n*-alkane in the B3 standard was used to construct a correlation line between measured and known δ²H values. This correlation line was used to correct *n*-alkane δ²H values for each mangrove leaf sample. A laboratory external standard containing methyl palmitate (-255‰) and squalane (-107‰) at three different concentrations (200 ng/µl, 1000 ng/µl and 1500 ng/µl each), and the internal co-injected squalane were used for checking instrument performance throughout the whole period of measurements. The H₃⁺ factor was measured daily prior to sample analysis using pulses of a reference gas of varying heights and averaged 5.3 +/-

0.1 during this study. All $\delta^2\text{H}$ values for the B3 standard (different concentrations), internal squalane and external mixture (methyl palmitate and squalane) replicated well, with standard deviation within $\pm 5\text{‰}$ ($n = 31$). Only odd n -alkanes present in sufficient quantity (intensity above 1500 mVs) for reliable $\delta^2\text{H}$ measurements are reported and discussed. All samples were analyzed 2x or 3x and standard deviation was generally within $\pm 5\text{‰}$ (Table 2).

2.5. Modeled surface water $\delta^2\text{H}$ values

Considering the large diurnal salinity changes in the Shark River estuary (He et al., 2014), measuring water $\delta^2\text{H}$ at time of sampling alone provides limited utility to represent the water pool mangroves used for leaf wax synthesis. As such, published rain and seawater $\delta^2\text{H}$ values were used as end-members to relate measured salinity values to estimated surface water $\delta^2\text{H}$ values. Henceforth the water $\delta^2\text{H}$ values estimated from measured salinity are referred to as “modeled surface water $\delta^2\text{H}$ values”. Positive correlations between salinity and surface water $\delta^2\text{H}$ values along estuaries are typical, and have been reported in multiple studies (Craig and Gordon, 1965; Gat, 1996; Ladd and Sachs, 2012, 2015b) (Figure 2).

The $\delta^2\text{H}$ composition of precipitation is variable in South Florida. It is known that the precipitation $\delta^2\text{H}$ values measured at Readland (within 30 km from our sampling sites at the Shark River) vary throughout the year from -50.6‰ to 2.4‰ during the wet season, and from -34‰ to 9.1‰ during the dry season (see table 5 in Price et al. 2008). The weighted average $\delta^2\text{H}$ values ($n = 43$) of precipitation at the Readland location is -12.3‰ (Price et al., 2008), which is assigned as the surface water $\delta^2\text{H}$ values for Everglades freshwater (0 ppt). Surface seawater in the Gulf of Mexico has an average $\delta^2\text{H}$ value of 15‰ (Sternberg and Swart, 1987). A modeled surface water $\delta^2\text{H}$ value was then calculated for each sampling site assuming conservative mixing between average freshwater and Gulf of Mexico surface water. This generates a mixing line as: modeled surface water $\delta^2\text{H}$ value (per mil) = $(15+12.2)/(32-0.7) \times \text{salinity} - 12.2$. Both measured and modeled average salinity (based on the linear correlation between distance from river mouth and measured salinity; Table 1) were used to calculate the uncertainty in modeled surface water $\delta^2\text{H}$ values and capture variation due to seasonality (Table 2 and indicated by error bars in Figs. 3 and 4). This resulted in modeled surface water $\delta^2\text{H}$ values of -12 to 13‰ along the 31 ppt salinity gradient of the Shark River estuary sampled (Fig. 2) and the uncertainty in modeled surface water $\delta^2\text{H}$ values equals $(15+12.2)/(32-0.7) \times (\text{measured salinity} - \text{calculated$

salinity). The range is within that reported for South Florida, where published fresh water and marine surface water values are from -14 to 15‰ (Sternberg and Swart, 1987; Price et al., 2008; Saha et al., 2009; Florea and McGee, 2010).

2.6. Calculation of concentration-weighted average leaf wax *n*-alkane δ^2H values (δ^2H_{C27-33})

Due to the inconsistent detection of *n*-C₃₃ (avg. $0.19 \pm 0.34\%$ of the total concentration of all *n*-alkanes) in *L. racemosa* and low concentration in *R. mangle* ($4.1 \pm 3.8\%$; Fig. 3), *n*-C₃₁ was used for comparison among the various mangrove species here and with previous studies (Ladd and Sachs, 2012, 2015a,b). Meanwhile, concentration-weighted average (CWA) of long chain leaf wax *n*-alkane δ^2H values (δ^2H_{C27-33}) was also calculated for each species using Eq. 1 (Table 1):

$$\delta^2H_{C27-33} = \sum_{k=27}^{33} \frac{\delta^2H_k \cdot \text{Conc.}_k}{\text{Con.}_{\text{tot}}} \quad (1)$$

where δ^2H_k is $\delta^2H_{27} - \delta^2H_{33}$ (odd homologues), conc._k the concentration of *n*-C₂₇ – *n*-C₃₃ alkanes (odd homologues) in $\mu\text{g/g}$ dry leaf material and $\text{conc.}_{\text{tot}}$ the total concentration of *n*-C₂₇ – *n*-C₃₃ alkanes (odd homologues).

We included *n*-C₂₇ for the CWA calculation because of the relatively large amount (27% to 33% of total) in *L. racemosa* (Fig. 3). In contrast, we excluded *n*-alkanes < C₂₇ since emergent and aquatic plants can contribute significant amounts of C₂₃ and C₂₅ *n*-alkanes to sediments (Ficken et al., 2000). The C₃₅ *n*-alkane was not included in this calculation due to its low concentration and inconsistent detection, which cannot generate accurate δ^2H values. The CWA δ^2H values can facilitate comparison among species with different *n*-alkane composition, as demonstrated in previous studies (e.g. Sachse et al., 2010; Kahmen et al., 2011).

2.7. Calculation of modeled apparent isotopic fractionation factor

The H isotopic ratio of *n*-alkanes relative to surface water, the modeled apparent or net fractionation factor α , was calculated according to Eq. 2:

$$\text{Modeled } \alpha_{\text{alkane-sw}} = \frac{\delta^2H_{\text{alkane}} + 1}{\text{modeled } \delta^2H_{\text{surface water}} + 1} \quad (2)$$

Uncertainties in the modeled $\alpha_{\text{alkane-sw}}$ ($\alpha_{\text{C}_{31}\text{-sw}}$ and $\alpha_{\text{C}_{27-33}\text{-sw}}$) values were propagated considering error from the modeled $\delta^2\text{H}_{\text{water}}$ values. This propagated error was calculated according to equations 3 and 4:

$$Z = \frac{X}{Y} \quad (3)$$

$$\frac{\Delta Z}{Z} = \frac{\Delta X}{X} + \frac{\Delta Y}{Y} \quad (4)$$

where Δx and Δy are the standard deviations of X ($\delta^2\text{H}_{\text{alkane}} + 1$) and Y (modeled $\delta^2\text{H}_{\text{surface water}} + 1$); Δz propagated error of Z (Modeled $\alpha_{\text{alkane-sw}}$).

2.8. Statistics

Statistical analysis was performed using SPSS 13.0 for Windows. Outliers were tested using the Grubbs test at $P = 0.05$ significance level (two-tailed). Linear regression lines fitted to data were tested at $P = 0.05$ significance level. The slope and y-intercept (with standard deviation for each) for each linear regression line were calculated using OriginPro 8. Unpaired t-test (two-tailed) was used to compare the slopes of various regressions at $P = 0.05$ significance level.

3. Results

3.1. Leaf wax *n*-alkane composition of *R. mangle*, *L. racemosa* and *A. germinans*

The leaf wax *n*-alkane concentrations for *R. mangle*, *L. racemosa* and *A. germinans* were 35-95 $\mu\text{g/g}$ d.w. (dry weight; $n = 13$), 60-154 $\mu\text{g/g}$ d.w. ($n = 9$) and 66-95 $\mu\text{g/g}$ d.w. ($n = 7$), respectively (Table S1). *L. racemosa* had significantly higher *n*-alkane concentrations than *R. mangle*, while no significant difference in concentration was observed between *R. mangle* and *A. germinans*. The two most abundant *n*-alkanes were C_{29} and C_{31} , C_{29} and C_{27} , C_{31} and C_{29} *n*-alkanes for *R. mangle*, *L. racemosa* and *A. germinans*, respectively (Fig. 3). Alkane concentration did not vary systematically with salinity in any of the three Shark River taxa (Fig. 4; Table S1). Average chain length (ACL) values ranged from 28.7 to 29.6, 28.0 to 28.7 and 29.4 to 29.8 for *R. mangle*, *L. racemosa* and *A. germinans*, respectively (Table S1). The averaged ACL of *L. racemosa* was significantly lower than that of *R. mangle* and *A. germinans* ($P < 0.05$). The carbon preference index (CPI) values were 4.8 to 7.3, 6.7 to 8.6 and 4.8 to 6.0 for *R. mangle*, *L. racemosa* and *A. germinans*, respectively (Table S1). *L. racemosa* had a significantly higher

CPI than *A. germinans* ($P < 0.05$). The proportion of longer chain odd n -alkanes (C_{27} - C_{33}) ranged from 68% to 83%, 80% to 85%, 65% to 73% for *R. mangle*, *L. racemosa* and *A. germinans*, respectively.

3.2. Variation in leaf wax n -alkane δ^2H values for *R. mangle*, *L. racemosa* and *A. germinans*

Generally, the CWA $\delta^2H_{C_{27-33}}$ values co-varied with n - C_{31} δ^2H ($\delta^2H_{C_{31}}$) values. The latter correlated significantly with $\delta^2H_{C_{31}}$ values across the full sample set ($n = 29$, $R^2 = 0.97$, $P < 0.001$; Fig. 5). On the basis of the high correlation, $\delta^2H_{C_{31}}$ values are discussed below.

The $\delta^2H_{C_{31}}$ values ranged from -131‰ to -147‰ (16‰ range), -135‰ to -170‰ (35‰ range) and -180‰ to -199‰ (19‰ range) in *R. mangle*, *L. racemosa* and *A. germinans*, respectively (Fig. 6; Table 2). The CWA $\delta^2H_{C_{27-33}}$ values ranged from -124‰ to -140‰ (16‰ range), -124‰ to -156‰ (32‰ range) and -167‰ to -183‰ (16‰ range), respectively (Table 2). The $\delta^2H_{C_{31}}$ values and CWA $\delta^2H_{C_{27-33}}$ values for *A. germinans* were significantly lower ($P < 0.01$) than those in *R. mangle* and *L. racemosa*. Meanwhile, the $\delta^2H_{C_{31}}$ values and CWA $\delta^2H_{C_{27-33}}$ values in *R. mangle* were significantly higher ($P < 0.05$) than those in *L. racemosa*.

3.3. Inverse correlation between modeled $\alpha_{alkane-sw}$ and salinity in *R. mangle*, *L. racemosa* and *A. germinans*

The apparent fractionation factor between n - C_{31} and modeled surface water, $\alpha_{C_{31-sw}}$, was negatively and significantly correlated ($P < 0.05$) with salinity for all 3 mangrove species (Fig. 7, Table 3). A significantly shallower slope for the linear relationship was observed for *R. mangle* relative to *L. racemosa* and *A. germinans*, while no significant difference in the slopes of the linear relationships existed between *L. racemosa* and *A. germinans*. A significantly lower y-intercept ($\alpha_{C_{31-sw}}$) of the linear relationship was observed in *A. germinans* than that of *R. mangle* and *L. racemosa*, while no such significant difference existed between *R. mangle* and *L. racemosa* (Fig. 8).

4. Discussion

4.1. Interspecies variability in leaf wax n -alkane concentration in Florida mangroves

Variation in leaf wax n -alkane concentration and composition is observed for the three species sampled in the Shark River estuary. Lack of correlation between leaf n -alkane

concentration and salinity may imply that salinity alone is not a dominant control on *n*-alkane concentration in mangroves from the Shark River estuary (Fig. 4). This contrasts with a laboratory culture study by Basyuni et al. (2012), in which a positive correlation was observed between salinity and triterpenoid content in leaves of mangroves grown in a glass house (for both salt secretor *A. marina* and non-salt secretor *R. stylosa*). The authors hypothesized that triterpenoids may fortify the plasma membrane structure to protect the mangrove plant from salt. Triterpenoids and *n*-alkanes are generally located in different parts of the leaf (Jetter et al., 2007; Jetter and Riederer, 2016), and different triterpenoids may be located in different parts of the leaf. For instance, in leaf cuticles of *Rhizophora mangle* from Florida, β -amyrin was largely confined to epicuticular wax, while taraxerol appeared to be a cutin component (Killpos and Frewin, 1994).

Compared to triterpenoids, *n*-alkanes commonly accumulate in highest abundance in the epicuticular wax, and consequently have a different physiological function (Jetter et al., 2007; van Maarseveen and Jetter, 2009; Buschhaus and Jetter, 2011; Jetter and Riederer, 2016). A recent study from eight plant species with wide chemical diversity showed that water transport resistances are associated with fatty acyl components such as *n*-alkanes rather than alicyclic components such as triterpenoids (Jetter and Riederer, 2016).

In addition, salinity gradients like those in the Shark River estuary do not exist in isolation from other environmental gradients (Mancera Pineda et al., 2009). Multiple factors such as seasonality, variable leaf lifetimes, leaf and soil nutrients (P and N concentration), sulfide toxicity (soil redox conditions), and the timing of leaf lipid synthesis also affect the growth of mangroves and thus may contribute to the variation in leaf wax *n*-alkane abundance (Wium-Anderson and Ghristensen, 1978; Leach and Burgin, 1985; Clough, 1992; Suárez, 2003). Moreover, mangroves produce new leaves throughout the year, so the leaf lipids may represent aggregate annual conditions (Clough, 1992; Coupland et al., 2005). The regeneration leaf lipids throughout the lifespan of a leaf could also influence the overall leaf wax composition (Sachse et al., 2009; Gao et al., 2012).

Along the salinity transect, a significant positive correlation ($P < 0.05$) was observed between salinity and ACL for *R. mangle* and *A. germinans* (Table S1). Leaf wax *n*-alkanes with higher ACL may better protect the leaf from water loss (Jetter et al., 2007), which may help *A.*

germinans resist salt stress, consistent with the presence of only *A. germinans* at higher salinity locations in the Shark River estuary.

4.2. Interspecies variability of $\alpha_{\text{alkane-sw}}$ among mangrove species

Average $\delta^2\text{H}_{\text{C}_{31}}$ values increased from *A. germinans* ($-186\text{‰} \pm 6\text{‰}$, $n = 7$) to *L. racemosa* ($-153\text{‰} \pm 12\text{‰}$, $n = 9$) to *R. mangle* ($-139\text{‰} \pm 5\text{‰}$, $n = 13$) (Table 2; Fig. 6). Modeled $\alpha_{\text{alkane-sw}}$ values increased from *A. germinans* (0.805 ± 0.008 , $n = 7$) to *L. racemosa* (0.845 ± 0.023 , $n = 9$) to *R. mangle* (0.860 ± 0.011 , $n = 13$) (Table 2). A similar observation was made by Ladd and Sachs (2015a), who observed that $\delta^2\text{H}_{\text{C}_{31}}$ values were consistently enriched by 25‰ to 30‰ in *R. stylosa* relative to *A. marina* and *A. corniculatum* during a 10 month study in Mobbs Bay, Australia, even though leaf-water $\delta^2\text{H}$ values were nearly identical in the three taxa (Ladd and Sachs, 2015a). While different H isotope responses to salinity in leaf wax *n*-alkanes are apparent among species, there are notable similarities in the sensitivity of the response. The similarities likely represent physiological responses to salinity and possibly other co-varying environmental parameters, and/or differences in isotopic fractionation during *n*-alkane biosynthesis. Interspecies variability is not surprising given reported differences in $\alpha_{\text{lipid-sw}}$ (isotopic fractionation between lipid and source water) of up to 100‰ within growth forms in several studies (e.g. Chikaraishi and Naraoka, 2007; Feakins and Sessions, 2010a, b; Eley et al., 2014).

A. marina ($\delta^2\text{H}_{\text{C}_{31}}$ averaged as -139‰ , $n = 17$; Ladd and Sachs, 2012), had $\alpha_{\text{C}_{31} \text{ } n\text{-alkane-sw}}$ values that differed by ca. 51‰ (Ladd and Sachs, 2012) compared to *A. germinans*, even they both belong to the same genus, *Avicennia*. This suggests the possibility of variation in $\alpha_{n\text{-alkane-sw}}$ within the same genus, especially when growing at settings with different environmental conditions. The significantly larger $^2\text{H}/^1\text{H}$ fractionation and thereby lower $\delta^2\text{H}_{\text{C}_{31}}$ values in *A. germinans* ($P < 0.01$) vs. *R. mangle* and *L. racemosa* is unlikely to be explained by the $\delta^2\text{H}$ values of both xylem and leaf waters because (i) no significant difference was observed among xylem or stem water $\delta^2\text{H}$ values in *R. mangle*, *L. racemosa* and *A. germinans* growing in south Florida across a salinity range from 4.0 to 30.6 ppt (Sternberg and Swart, 1987; Feakins et al., 2013); (ii) no significant difference in $\delta^{18}\text{O}_{\text{lw}}$ was observed between *L. racemosa* ($n = 5$) and *R. mangle* ($n = 5$) across salinity ranges of up to 30 ppt growing in south Florida (Ellsworth et al., 2013); and (iii) *A. marina*, *R. stylosa* and *A. corniculatum* growing at Mobbs Bay did not have significant differences in xylem water and leaf water $\delta^2\text{H}$ values throughout a 10 month period

(Ladd and Sachs, 2015a). Instead, the significant difference in $\alpha_{C_{31} \text{ } n\text{-alkane-sw}}$ values among mangrove species is more likely attributable to: (i) utilization of different compatible solutes, (ii) reliance on stored carbohydrates as lipid precursors, (iii) differing salt management strategies, (iv) seasonal timing of leaf wax synthesis and leaf wax turnover time and/or (v) opportunistic uptake of freshwater during rain events and foliar uptake of water, especially at high salinity (Ladd and Sachs, 2015a).

First, the amount and type of osmolyte (i.e. compatible solute) employed by each species could indirectly cause differences of $\alpha_{\text{alkane-sw}}$. Metabolic NADPH, such as that derived from the pentose phosphate cycle, is thought to be ^2H -enriched relative to photosynthetically-produced NADPH (from Photosystem I) by several hundred per mil (Schmidt et al, 2003; Zhang et al. 2009). *A. germinans* has been shown to invest more nitrogen in synthesis of compatible solutes, such as amino acids, than that of *L. racemosa* (Sobrado and Ewe, 2006). If oxidative pentose pathway derived NADPH, which is relatively ^2H -enriched and might ordinarily be used in *n*-alkane synthesis, gets funneled to amino acid synthesis, leaving photosystem I derived NADPH, which is relatively ^2H -depleted, for alkane synthesis, it may result in an enhanced incorporation of ^2H -depleted NADPH from photosystem I for *n*-alkane synthesis in *A. germinans* as compared with *L. racemosa*.

The second factor that could account for differences in $\alpha_{n\text{-alkane-sw}}$ values among species is differential use of stored carbohydrates (Ladd and Sachs, 2015a). Sobrado (2000) found higher photosynthetic rates and water-use efficiency in *A. germinans* than in *R. mangle* and *L. racemosa*. Lower photosynthetic rate in *R. mangle* and *L. racemosa* suggest they have less primary photosynthate available for leaf wax synthesis. In that case, *R. mangle* may use relatively more stored carbohydrates as a substrate for leaf wax production (e.g. Yakir, 1992; Roden et al., 2000). Stored carbohydrates are relatively enriched in ^2H because they undergo greater H exchange with intracellular water than the $\text{C}_3\text{-C}_6$ sugars emanating from the Calvin-Benson Cycle (e.g. Yakir, 1992; Roden et al., 2000; Hayes, 2001). The same mechanism was proposed by Ladd and Sachs (2015a) to explain the more ^2H -enriched alkanes in *R. stylosa* than in *A. marina*.

A third possible explanation for differences in $\alpha_{n\text{-alkane-sw}}$ values among species could be the different salt adaption mechanisms among species, including salt exclusion, salt secretion and salt accumulation. *A. germinans* can utilize all three mechanisms, *L. racemosa* can only secrete salt, and *R. mangle* can exclude and accumulate salt (Parida and Jha, 2010). The different salt

management mechanisms in mangroves could influence lipid $\delta^2\text{H}$ values, since the secreted salt on the leaf surfaces of *A. germinans* and *L. racemosa* should attract water from the air and maintain a constant relative humidity of 76% at the leaf surface (O'Brien, 1948). Additionally, this water of hydration for Na salts can be depleted in ^2H by up to -80‰ relative to the surrounding water vapor (Matsuo, et al., 1972). Both increased relative humidity at the leaf surface and lower $\delta^2\text{H}$ values of water vapor could result in less enrichment of leaf water for salt secreting mangroves relative to non-secreting plants. However, previous studies find no significant difference among leaf water $\delta^2\text{H}$ values and xylem or stem water $\delta^2\text{H}$ values in different mangrove species growing at the same site as each other (Sternberg and Swart, 1987; Feakins et al., 2013; Ladd and Sachs, 2015a), making this explanation less likely to account for interspecies differences in net hydrogen isotope fractionation. Since no direct xylem water and leaf water $\delta^2\text{H}$ values were measured in the Shark Bay estuary, we cannot rule out the possibility that these values differed among species, limiting our ability to determine how much different salt management strategies may have affected interspecies variability in $\alpha_{n\text{-alkane-sw}}$ at this location.

Other uncontrolled factors that could contribute to variation in $\alpha_{n\text{-alkane-sw}}$ include the timing of leaf wax synthesis, seasonal variation of the water $\delta^2\text{H}$ values, leaf anatomy and leaf longevity. For instance, leaf wax $\delta^2\text{H}_{n\text{-alkane}}$ values have been observed to be affected by the timing of leaf wax synthesis (Sachse et al., 2009, 2010, 2015; Kahmen et al., 2011; Gao et al., 2012; Tipple et al, 2013). Kahmen et al. (2011) suggested that leaf wax *n*-alkanes record only a brief period of the environmental variability that a leaf experiences throughout its life. Although mangroves are evergreens, leaf lifetimes are variable in the three Shark River species (60, 111, and 160 days in *L. racemosa*, *R. mangle* and *A. germinans*, respectively; Suárez, 2003). Since leaf wax *n*-alkanes will presumably have a shorter residence time than the leaf itself, and this may vary as a function of taxa and environmental conditions (Sachse et al., 2009, 2010, 2015; Kahmen et al., 2011; Gao et al., 2012; Tipple et al, 2013; Newberry et al., 2015; Oakes and Hren, 2016), this factor may contribute to the variation in $\alpha_{n\text{-alkane-sw}}$ values. In addition, opportunistic freshwater uptake during rain events and foliar uptake of water could also cause the variations in $\alpha_{n\text{-alkane-sw}}$ values, as suggested by Ladd and Sachs (2015a, b).

All in all, the factors affecting the difference in $\alpha_{n\text{-alkane-sw}}$ values among the three species are complex. Without a better understanding of the reasons for this interspecies variability in $\alpha_{n\text{-alkane-sw}}$ values, interpretation of sedimentary $\delta^2\text{H}_{n\text{-alkanes}}$ values in mixed mangrove areas is

complicated and taxon-specific lipid biomarkers, such as taraxerol for *Rhizophora* spp., should be used in paleo-hydroclimate studies when possible (e.g. Nelson and Sachs, 2016).

4.3. Different relationships between salinity and $\alpha_{\text{alkane-sw}}$ among mangrove species

The consistent inverse correlation between salinity and $\alpha_{\text{C31 } n\text{-alkane-sw}}$ in Shark River mangroves support the key findings from studies of Indo-West Pacific mangroves, and further demonstrates that the main factor controlling mangrove $\delta^2\text{H}_{n\text{-alkane}}$ values is not environmental water $\delta^2\text{H}$ values. As proposed by Ladd and Sachs (2015a, b), the robustly negative $\alpha_{\text{C31 } n\text{-alkane-sw}}$ – salinity relationship in mangroves can be best explained by varying biosynthetic fractionation (i.e. $\alpha_{n\text{-alkane-leaf water}}$) in response to increasing salinity, or by variable timing in lipid production. This is the first report of a similarly negative correlation in Americas-East Atlantic mangroves, as all the previous studies focused on the Indo-West Pacific mangroves (Ladd and Sachs, 2012, 2015a, b).

In order to explore the universality of the $\alpha_{\text{C31 } n\text{-alkane-sw}}$ – salinity among globally distributed mangrove taxa, the published results from five salinity transects and a single site monitored over time (at which salinity ranged from 1 to 36 ppt) (Ladd and Sachs, 2012, 2015a, b) were compared with the Shark River estuary results (Figs. 7 and 8). The linear regression results for $\alpha_{n\text{-alkane-sw}}$ vs. salinity are listed Table 3 and the slope and y-intercept values for each regression are indicated in Fig. 8. The significantly different and overall wide range of slope values from $-1.8 (\pm 0.2) \times 10^{-3}$ to $-0.7 (\pm 0.3) \times 10^{-3}$, suggests different correlations between $\alpha_{n\text{-alkane-sw}}$ and salinity among the mangrove species from the same spatial transect, irrespective of their salt management mechanism (Fig. 8). *R. mangle* and *R. stylosa* (salt excluders and accumulators) and *A. corniculatum* (a salt excluder and secretor) show no significant difference in the slope values of the linear regression for $\alpha_{n\text{-alkane-sw}}$ vs. salinity, and similar slope values are observed between *L. racemosa* (a salt secretor only) and *A. germinans* (a salt excluder, secretor and accumulator), suggesting salt management mechanism alone seems not a controlling factor affecting the slope values of the linear regression for $\alpha_{n\text{-alkane-sw}}$ vs. salinity among the mangrove species studied.

There is no significant difference among slopes of the four *Rhizophora* species, including *R. mangle* from Florida, and *R. apiculata*, *R. stylosa* and *R. mucronata* from Australia and Micronesia (Fig. 8a), implying similar mechanisms responsible for the $^2\text{H}/^1\text{H}$ fractionation

response to salinity within this genus. Similarly, no significant difference in slope values was observed for *A. marina* [$-1.5 (\pm 0.3) \times 10^{-3}$] in the Brisbane River estuary and *A. germinans* [$-1.4 (\pm 0.5) \times 10^{-3}$] in the Shark River estuary, although the slopes for *Rhizophora* species are significantly lower than for *L. racemosa* and *A. germinans*, suggesting variation among genera.

4.4. Implications for paleoclimate studies

Rhizophora seems to be a better candidate than *Avicennia* for paleo-hydroclimate reconstruction, considering the robust and similar negative linear correlation between $\alpha_{\text{alkane-sw}}$ and salinity in 4 species from Florida, Australia and Micronesia. With a high concentration of the lipid biomarker taraxerol produced by this genus (Versteegh et al., 2004; Koch et al., 2011), a future study ought to evaluate the $\alpha_{\text{taraxerol-sw}}$ -salinity relationship in Florida *R. mangle* leaves to evaluate whether it is similar to that observed in Australia and Micronesia (Ladd and Sachs, 2015a, b).

The opposing relationships between H isotope fractionation and salinity in lipids from mangroves and phytoplankton provide a potential new tool for quantitatively reconstructing water salinity and H isotope composition in locations that receive input from both organisms, as demonstrated by Nelson and Sachs (2016), who measured $\delta^2\text{H}$ values of taraxerol and a microalgal lipid in sediments from a brackish pond in the Galápagos to reconstruct salinity and water $\delta^2\text{H}$ values during the last 2 kyr (Nelson and Sachs, 2016). If the similar negative correlation between $\alpha_{\text{alkane-sw}}$ and salinity observed in this study is also applicable to $\alpha_{\text{taraxerol-sw}}$ -salinity relationship in *Rhizophora*, the uncertainty of reconstructed salinity and water $\delta^2\text{H}$ in the study by Nelson and Sachs (2016) will be reduced.

Future studies aimed at constraining the potential effects of the timing of leaf wax synthesis, tree height, leaf residence time, and tides, not evaluated here or in previous studies (Ladd and Sachs, 2012, 2015a, b), would further aid the application and interpretation of mangrove lipid $\delta^2\text{H}$ values.

5. Conclusions

This study confirms that an inverse correlation exists between $\alpha_{n\text{-alkane-sw}}$ and salinity in three AEP mangrove species, supporting the application of mangrove lipid $\delta^2\text{H}$ values as a paleosalinity and paleohydrologic proxy. This inverse relationship is characteristic of all

mangrove species surveyed (*A. marina*, *A. germinans*, *R. mangle*, *R. stylosa*, *R. apiculata*, *R. mucronata*, *L. racemosa* and *Aegiceras corniculatum*) from four major families with different salt management mechanisms and at different geographic locations (both AEP estuaries and IWP estuaries and lakes), covering a 53° latitudinal scale (28° S to 25° N).

Although our study finds that variation exists in the $\alpha_{n\text{-alkane-sw}}$ -salinity relationship among different taxa, with linear regression slopes between -0.7×10^{-3} to -1.8×10^{-3} (i.e. -0.7 to -1.8 ‰ ppt⁻¹) among eight mangrove species, the regression slopes for all *Rhizophora* species are the same within error. As such, coastal areas where this globally distributed genus is predominant could be ideal candidates for paleo-hydroclimate reconstruction using the approach discussed here. Our study highlights the need to better understand the biochemistry of water processing and metabolism, and of leaf wax synthesis, in different mangrove taxa with different salt adaption mechanisms. Laboratory cultivation experiments could be particularly fruitful in this regard.

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Figures

Figure 1. Map of the Shark River estuary. Sampling sites are marked with bold numbers from 1-11. Detailed sampling information is also shown in Table 1. Note: five-pointed star, triangle, circle denote samples from the Shark River estuary, Brisbane River estuary, Mobbs Bay, respectively (Ladd and Sachs, 2012, 2015a); diamonds denote samples from Pohnpei estuaries and Palau lakes (Ladd and Sachs, 2015b).

Figure 2. Surface water $\delta^2\text{H}$ values vs. salinity along various mangrove estuaries and lakes.

Figure 3. Leaf wax *n*-alkane compositions among mangrove species. Note: *A. marina*, *R. stylosa* and *A. corniculatum* were obtained from Mobbs Bay in Australia (Ladd and Sachs, 2015a).

Figure 4. Leaf wax *n*-alkane concentrations vs. salinity.

Figure 5. Correlation between $\delta^2\text{H}_{\text{C}_{31}}$ and $\delta^2\text{H}_{\text{C}_{27-33}}$ values across the entire sample set from the Shark River Estuary.

Figure 6. Leaf wax and surface water $\delta^2\text{H}$ values along Shark River Estuary vs. salinity. A, *n*-C₃₁ alkane (this study); B, *n*-C₂₇₋₃₃ alkanes (this study); C, *n*-C₃₁ alkane (reorganized from Ladd and Sachs, 2015a). Surface water $\delta^2\text{H}$ scale for all plots is on the right hand y-axis; lipid $\delta^2\text{H}$ scale for all plots is on the left hand y-axis. Note: *A. marina* C₃₁ *n*-alkane $\delta^2\text{H}$ values in Fig. 6 were obtained from a transect study in the Brisbane River Estuary, Australia (Ladd and Sachs, 2012); in some cases, the error bar is similar to or smaller than the size of the symbols and thus not visible. All C₃₁ *n*-alkane $\delta^2\text{H}$ values in Fig. 6 were obtained from a single site at Mobbs Bay, Australia, with time (Ladd and Sachs, 2015a).

Figure 7. Hydrogen isotope fractionation factor, α , of mangrove n -C₃₁ alkane (A; this study), n -C₂₇₋₃₃ alkanes (B; this study) and n -C₃₁ alkane (C; reorganized from Ladd and Sachs, 2015a) vs, salinity. Note: in some cases, the error bar is similar to or smaller than the size of the symbols. The detailed equation of each correlation line is shown in Table 3.

Figure 8. Slope and y-intercept values of each linear correlation between leaf wax n -C₃₁ alkane hydrogen isotope fractionation values (α_{C31-sw}) and surface water salinity among mangroves. Note: 1 to 5 denote samples from the Shark River estuary, Brisbane River estuary, Mobbs Bay, Pohnpei estuaries and Palau lakes, respectively (Ladd and Sachs, 2012; 2015a, b). The color denotes different species.

Fig. 1

Fig. 2

Note: the calibrations for Brisbane River estuary, Mobbs Bay, Pohnpei estuaries and Palau lakes were reorganized from previous studies (Ladd and Sachs, 2012; 2015a, b); dashed line shows calibration line used to calculate surface water $\delta^2\text{H}$ values for Shark River estuary assuming conservative mixing between freshwater and seawater end members.

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Note: different letters indicate a significant difference at $P < 0.05$.

Tables

Table 1

Locations of mangrove leaves sampled from Shark River estuary, southern Florida, USA.

Table 2

Mean salinity of surface water at sample sites, $\delta^2\text{H}$ of water (modeled), C_{31} *n*-alkane, CWA $\delta^2\text{H}$ values of C_{27} , C_{29} , C_{31} , C_{33} *n*-alkanes, and modeled hydrogen isotope fractionation factors, modeled $\alpha_{\text{alkane-sw}}$. Results for leaf samples from two different trees were determined at some locations and are indicated by subscript (i.e. a_1 , a_2 etc.).

Table 3

Linear correlations between salinity and $\alpha_{n\text{-alkane-sw}}$ for mangroves.

Site	Lat.	Long.	Distance (km) ^a	Measured mean salinity	Calculated salinity ^b	Uncertainty in salinity ^c
1	25°26'31.45"	80°54'21.21"	25.1	0.7	0.0	0.7
2	25°24'36.63"	80°57'49.98"	18.2	3.3	6.6	3.3
3	25°24'58.02"	80°59'51.60"	14.9	8.3	11.0	2.7
4	25°23'31.29"	80°59'50.90"	13.7	13.3	12.6	0.7
5	25°22'50.58"	81°1'31.68"	10.5	15.6	17.1	1.5
6	25°22'18.08"	81°2'31.04"	8.3	19.7	20.2	0.5
7	25°22'17.76"	81°3'17.34"	7.2	23.0	21.7	1.3
8	25°21'52.62"	81°4'40.50"	4.2	27.5	25.9	1.6
9	25°21'15.54"	81°5'48.40"	2.1	27.9	28.8	1.0
10	25°21'14.97"	81°6'18.24"	1.2	31.4	30.1	1.3
11	25°21'12.47"	81°6'73.14"	0.0	32.0	31.7	0.3

^a Distance from river mouth in Shark River; ^b salinity calculated via linear regression (Calculated salinity = $-1.3876 \times \text{distance} + 31.723$; R^2 0.97; $P < 0.0001$) of measured data vs. distance (km); ^c absolute difference between calculated and measured mean salinity values. The measured

salinity values were used to make figures 6 and 7 and the uncertainty in salinity values were used to make error bars in figures 6 and 7.

Florida species	Site	Salinity (ppt) ^a	Estimated $\delta^2\text{H}_{\text{water}}$ (‰) ^b	Uncertainty in $\delta^2\text{H}_{\text{water}}$ (‰) ^c	C_{31} $\delta^2\text{H}$ (‰)	1σ C_{31} (‰) ^d	Modeled $\alpha_{\text{C}_{31}\text{-sw}}$	1σ $\alpha_{\text{C}_{31}\text{-sw}}$ ^e	C_{27-33} $\delta^2\text{H}$ (‰) ^f	1σ C_{27-33} (‰)	Modeled $\alpha_{\text{C}_{27-33}\text{-sw}}$	1σ $\alpha_{\text{C}_{27-33}\text{-sw}}$ ^e
<i>R. mangle</i>	1a ₁	0.7	-12	1	-137	2	0.873	0.003	-126	2	0.885	0.003
<i>R. mangle</i>	1a ₂	0.7	-12	1	-140	3	0.870	0.004	-136	1	0.874	0.002
<i>R. mangle</i>	2a	3.3	-10	3	-131	1	0.878	0.004	-124	1	0.884	0.004
<i>R. mangle</i>	3a	8.3	-6	2	-140	1	0.865	0.003	-129	1	0.876	0.003
<i>R. mangle</i>	4a	13.3	-2	1	-133	4	0.868	0.005	-127	4	0.875	0.005
<i>R. mangle</i>	5a	15.6	0	1	-137	3	0.864	0.004	-132	3	0.868	0.004
<i>R. mangle</i>	6a	19.7	3	0.4	-146	2	0.851	0.002	-139	3	0.858	0.003
<i>R. mangle</i>	7a	23.0	6	1	-132	4	0.863	0.005	-126	3	0.869	0.004
<i>R. mangle</i>	8a	27.5	9	1	-136	6	0.856	0.007	-130	6	0.863	0.007
<i>R. mangle</i>	9a	27.9	9	1	-140	1	0.852	0.002	-133	1	0.859	0.002
<i>R. mangle</i>	10a	31.4	12	1	-142	1	0.848	0.002	-132	1	0.857	0.002
<i>R. mangle</i>	11a ₁	32.0	13	0.2	-140	2	0.849	0.002	-130	2	0.859	0.002
<i>R. mangle</i>	11a ₂	32.0	13	0.2	-147	4	0.842	0.004	-140	5	0.850	0.005
<i>L. racemosa</i>	2b ₁	3.3	-10	3	-135	2	0.873	0.005	-128	2	0.881	0.005
<i>L. racemosa</i>	2b ₂	3.3	-10	3	-143	4	0.865	0.007	-139	5	0.870	0.008
<i>L. racemosa</i>	3b	8.3	-6	2	-135	1	0.871	0.003	-124	2	0.882	0.004
<i>L. racemosa</i>	4b	15.6	0	1	-145	3	0.855	0.004	-135	3	0.865	0.004
<i>L. racemosa</i>	6b	19.7	3	0.4	-141	3	0.857	0.003	-135	3	0.862	0.003
<i>L. racemosa</i>	8b	27.5	9	1	-167	2	0.825	0.003	-148	1	0.844	0.002
<i>L. racemosa</i>	10b	31.4	12	1	-169	1	0.821	0.002	-153	1	0.837	0.002
<i>L. racemosa</i>	11b ₁	32.0	13	0.2	-170	3	0.819	0.003	-156	3	0.833	0.003
<i>L. racemosa</i>	11b ₂	32.0	13	0.2	-166	7	0.823	0.007	-154	7	0.835	0.007

<i>A. germinans</i>	6c	19.7	3	0.4	-180	2	0.817	0.002	-170	1	0.828	0.001
<i>A. germinans</i>	8c ₁	27.5	9	1	-186	3	0.807	0.004	-174	3	0.819	0.004
<i>A. germinans</i>	8c ₂	27.5	9	1	-185	2	0.808	0.003	-167	4	0.826	0.005
<i>A. germinans</i>	10c ₁	31.4	12	1	-183	2	0.807	0.003	-178	2	0.812	0.003
<i>A. germinans</i>	10c ₂	31.4	12	1	-185	3	0.805	0.004	-173	2	0.817	0.003
<i>A. germinans</i>	11c ₁	32.0	13	0.2	-199	5	0.791	0.005	-183	6	0.807	0.006
<i>A. germinans</i>	11c ₂	32.0	13	0.2	-187	4	0.802	0.004	-178	5	0.811	0.005

^a Measured average salinity; ^b Modeled $\delta^2\text{H}_{\text{water}}$ (surface water $\delta^2\text{H}$) values were calculated using two end member mixing equation for Shark River estuary assuming estuarine water well mixed; ^c uncertainty in estimated $\delta^2\text{H}_{\text{water}}$ values determined through propagating the uncertainty of salinity in Table 1; ^d the standard deviation based on two or three separate measurements of the same sample; ^e uncertainty in modeled $\alpha\text{C}_{31-\text{sw}}$ and $\alpha\text{C}_{27-33-\text{sw}}$ values propagated considering error from modeled $\delta^2\text{H}_{\text{water}}$ values; ^f uncertainty in C_{27-33} $\delta^2\text{H}$ values propagated considering error from $\delta^2\text{H}$ value of each individual *n*-alkane.

Species	α_{C31-sw} vs. salinity	R ²	P	N
<i>A. germinans</i> ¹	$\alpha_{C31-sw} = -1.4 (\pm 0.5) * 10^{-3} * \text{Salinity} + 0.845 (\pm 0.014)$	0.55	< 0.05	7
<i>A. marina</i> ²	$\alpha_{C31-sw} = -1.5 (\pm 0.3) * 10^{-3} * \text{Salinity} + 0.892 (\pm 0.006)$	0.68	< 0.05	18
<i>A. marina</i> ³	$\alpha_{C31-sw} = -0.8 (\pm 0.1) * 10^{-3} * \text{Salinity} + 0.868 (\pm 0.003)$	0.52	< 0.0001	38
<i>A. corniculatum</i> ³	$\alpha_{C31-sw} = -0.9 (\pm 0.7) * 10^{-3} * \text{Salinity} + 0.870 (\pm 0.02)$	0.17	0.21	11
<i>L. racemosa</i> ¹	$\alpha_{C31-sw} = -1.8 (\pm 0.2) * 10^{-3} * \text{Salinity} + 0.880 (\pm 0.004)$	0.92	< 0.01	9
<i>R. mangle</i> ¹	$\alpha_{C31-sw} = -0.8 (\pm 0.1) * 10^{-3} * \text{Salinity} + 0.875 (\pm 0.002)$	0.82	< 0.01	13
<i>R. stylosa</i> ³	$\alpha_{C31-sw} = -0.8 (\pm 0.2) * 10^{-3} * \text{Salinity} + 0.895 (\pm 0.004)$	0.50	0.0001	24
<i>Rhizophora</i> spp. ^{4, a}	$\alpha_{C31-sw} = -0.7 (\pm 0.2) * 10^{-3} * \text{Salinity} + 0.871 (\pm 0.005)$	0.42	0.004	18
<i>Rhizophora</i> spp. ^{5, b}	$\alpha_{C31-sw} = -0.7 (\pm 0.3) * 10^{-3} * \text{Salinity} + 0.858 (\pm 0.007)$	0.37	0.004	12

Table 3

¹⁻⁵ Samples from Shark River estuary, Brisbane River estuary, Mobbs Bay, Pohnpei estuaries and Palau lakes, respectively (Ladd and Sachs, 2012; 2015a, b); ^a *Rhizophora* spp. include *R. apiculata*, *R. stylosa* and *R. mucronata*; ^b *Rhizophora* spp. include *R. apiculata* and *R. mucronata*. The correlation between α_{C31} and surface water salinity is also shown in figure 7.

Highlights

• Leaf wax *n*-alkane hydrogen isotope fractionation factors (between *n*-alkane and surface water) correlated negatively with salinity in 3 Florida mangrove species. • Different sensitivity of lipid $^2\text{H}/^1\text{H}$ to salinity observed among mangrove species. • *A. germinans* and *L. racemosa* discriminated against ^2H more than *Rhizophora* during leaf wax *n*-alkane synthesis.