



## Journal Article

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## Note

## Hydrogen isotopic ratios of long-chain diols reflect salinity

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## ABSTRACT

Long-chain diols (LCDs) are ubiquitous lipids produced by freshwater and marine algae. A combination of semi-preparative high performance liquid chromatography and gas chromatography isotope ratio monitoring mass spectrometry, allowed the measurement of  $\delta^2\text{H}$  of individual LCDs from cultures, which indicated a correlation with the hydrogen isotope composition of the growth water and a species-specific effect. Results from environmental samples along a salinity gradient indicated the potential of  $\delta^2\text{H}$  ratios of LCDs to trace the hydrogen isotopic composition of water and sea surface salinity.

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## 1. Introduction

Hydrogen isotopes ratios of lipids from photosynthetic organisms have been shown to trace the hydrogen isotopic composition of the water ( $\delta^2\text{H}_{\text{H}_2\text{O}}$ ) as well as salinity in cultures (e.g., Schouten et al., 2006; Zhang and Sachs, 2007; Zhang et al., 2009; Sachs et al., 2016; Weiss et al., 2017). This principle has been used in particular with  $\delta^2\text{H}$  ratios of long-chain alkenones as a proxy for  $\delta^2\text{H}_{\text{H}_2\text{O}}$  and sea surface salinity (van der Meer et al., 2007; Weiss et al., 2019 and references therein). However, there are few other marine biomarkers used for this purpose.

Long-chain diols (LCDs) are ubiquitous marine compounds possibly produced by eustigmatophytes (Volkman et al., 1992, 1999; Villanueva et al., 2014). The  $\text{C}_{28}$  and  $\text{C}_{30}$  1,13- and the  $\text{C}_{30}$  1,15-diols are the major marine LCDs and have been used in a sea surface temperature proxy, the Long-chain Diol Index (Rampen et al., 2012). Furthermore, the fractional abundance of the  $\text{C}_{32:0}$  1,15-diol has been used to trace riverine input in marginal seas as it is mainly produced in river systems by freshwater eustigmatophytes (Lattaud et al., 2017), and is in low abundance in open marine environments (Rampen et al., 2012). Thus,  $\delta^2\text{H}$  ratios of

LCDs might be potential tools to reconstruct the  $\delta^2\text{H}_{\text{H}_2\text{O}}$  of sea water as well as sea surface salinity and  $\delta^2\text{H}_{\text{H}_2\text{O}}$  of river water, respectively. However, LCD isomers are not separated by traditional gas chromatography (GC) (cf. Versteegh et al., 1997) so semi-preparative liquid chromatography (prep-LC) is necessary to obtain  $\delta^2\text{H}$  ratios of individual LCDs (De Bar et al., 2016).

Here we used a prep-LC method adapted from de Bar et al. (2016) to isolate individual LCDs and measured their  $\delta^2\text{H}_{\text{LCDs}}$  in cultures and marine surface sediments from an environmental salinity gradient.

## 2. Materials and methods

## 2.1. Cultures and surface sediments

Three strains of *Nannochloropsis* (*Nannochloropsis gaditana* strain CCMP526, *Nannochloropsis oceanica* strain CCMP1779, and *Nannochloropsis oculata* strain CCMP2195) were grown in triplicate with a light intensity of  $280 \mu\text{mol m}^{-2} \text{s}^{-1}$  and salinity of  $37.5 \text{ g kg}^{-1}$  following methods described in Balzano et al. (2017). *N. oculata* was also grown at five salinities (22.5, 27.8, 30.1, 32.7 and  $34.5 \text{ g kg}^{-1}$ ) at  $15 \text{ }^\circ\text{C}$  and a light intensity of  $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Cell counts were obtained by flow cytometry (BD Accuri™ C6). The cells were harvested in exponential or, for the salinity experiment, stationary growth phase by filtration through  $0.7 \mu\text{m}$  GF/F filters (Whatman, Maidstone, UK).

Six surface sediments (Fig. 1) were collected in the northern Gulf of Mexico in March 2018 using a multi-corer (R/V *Pelagia*

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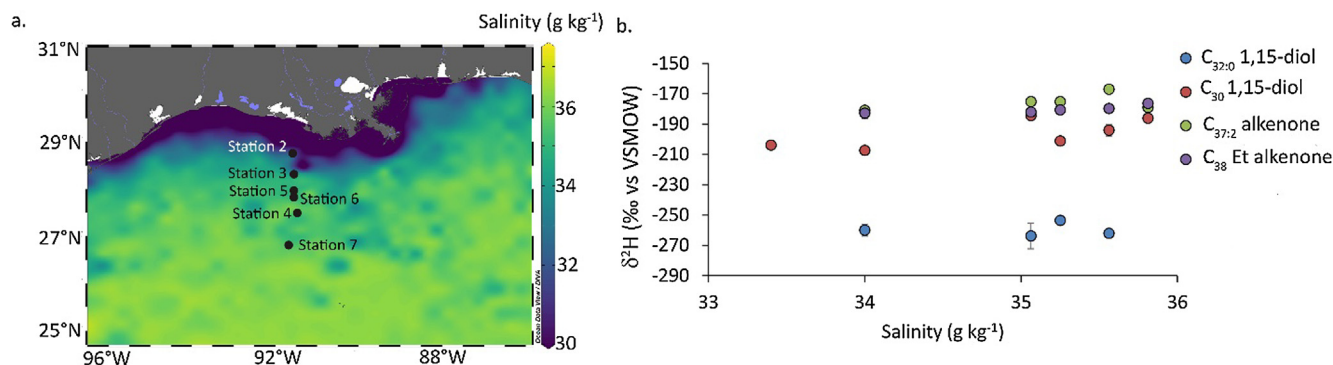


Fig. 1. (a) Location of the surface sediments and sea surface salinity (Antonov et al., 2010) and (b)  $\delta^2\text{H}$  ratios of the lipids.

cruise 64PE434). The annual mean salinity and annual mean temperature varies from 33.4 to 35.2  $\text{g kg}^{-1}$  and from 24.5 to 25.4  $^{\circ}\text{C}$ , respectively (Locarnini et al., 2009; Antonov et al., 2010).

$$\delta^2\text{H}_{\text{LCD}} = \frac{(\text{nH}_{\text{LCD}} + 2 \times \text{nH}_{\text{derivative}}) \times \delta^2\text{H}_{\text{LCD}}^{\text{measured}} - 2 \times \text{nH}_{\text{derivative}} \times \delta^2\text{H}_{\text{derivative}}}{\text{nH}_{\text{LCD}}} \quad (1)$$

## 2.2. Lipid extraction and separation

All surface sediments were freeze-dried and extracted using Accelerated Solvent Extraction following Lattaud et al. (2017). The filters from *Nannochloropsis* cultures were extracted following Lattaud et al. (2018) using base and acid hydrolysis. An internal standard ( $\text{C}_{22}$  7,16-diol) was added to all total lipid extracts and all extracts were subsequently separated into three fractions (apolar, ketone and polar) on an  $\text{Al}_2\text{O}_3$  column as described in Lattaud et al. (2018). For the quantification of the LCDs the polar fractions were analyzed by GC-MS as described in Lattaud et al. (2018). For the salinity experiment, saturated LCDs were isolated from the polar fraction by elution through an  $\text{Ag}^+$  impregnated Si column. The polar fractions were then acetylated with acetic anhydride ( $\delta^2\text{H} = -125.8 \pm 3.3\text{‰}$ ) following Das and Chakraborty (2011).

## 2.3. Isolation of long-chain diol isomers

Prior to stable isotope analysis, the LCDs were isolated from the polar fraction following a method from De Bar et al. (2016) using semi-preparative normal phase High Performance Liquid Chromatography (HPLC). An Agilent 1260 infinity HPLC equipped with a thermostatted autoinjector, and a Foxy R1 fraction collector with a Sunfire silica column (Waters, 250 mm  $\times$  10 mm; 5  $\mu\text{m}$ ) was used and 20 s fraction were collected. Fractions were combined in three pools: with pool 1 (33.4–34.4 min) containing 100% of  $\text{C}_{32:0}$  1,15-diol, pool 2 (35–35.2 min) containing 95% of  $\text{C}_{30}$  1,15-diol and pool 3 (35.4–37.4 min) containing 100% of  $\text{C}_{28}$  1,13 and <90% of  $\text{C}_{28}$  1,14-diols. Hydrogen isotope fractionation during HPLC isolation was tested using a pure LCD standard with known hydrogen isotope composition ( $\text{C}_{28}$  1,13-diol;  $\delta^2\text{H} = -142.6 \pm 0.6\text{‰}$ ). Prior to isotopic measurements, the purified LCDs were silylated with BSTFA with a known hydrogen isotopic composition ( $\delta^2\text{H} = -212 \pm 1\text{‰}$ ).

## 2.4. $\delta^2\text{H}$ measurements

Isolated LCDs from the polar fraction and long-chain alkenones from the ketone fraction were analyzed using gas chromatography isotope ratio monitoring mass spectrometry (GC-irMS) using a CPSil5 GC column (Agilent, 25 m  $\times$  0.32 mm  $\times$  0.4  $\mu\text{m}$ ). The sam-

ples were analyzed in triplicate when possible. The  $\delta^2\text{H}$  ratios of the LCDs were corrected for BSTFA (surface sediments) or acetylation (for culture experiments) as followed:

With  $\text{nH}_{\text{LCD}}$  = number of hydrogen atoms of LCD, and the derivative being either BSTFA or acetyl anhydride.  $\text{nH}_{\text{BSTFA}} = 9$  and  $\text{nH}_{\text{acetyl}} = 3$ ,  $\delta^2\text{H}_{\text{BSTFA}} = -212\text{‰}$  and  $\delta^2\text{H}_{\text{acetyl}} = -125.8\text{‰}$ .  $\delta^2\text{H}_{\text{BSTFA}}$  has been determined by measuring the  $\delta^2\text{H}$  of a BSTFA-derivatized phthalic acid with known isotopic ratio ( $-81.9 \pm 1.2\text{‰}$ ).

## 3. Results and discussion

### 3.1. Hydrogen isotopic composition of LCDs in cultures

A  $\text{C}_{28}$  1,13-diol standard was collected over three separate fractions using prep-LC containing 17, 59 or 21% of the original compound, respectively. A large isotope effect was noted ( $\delta^2\text{H} = -190.5 \pm 1.9\text{‰}$ ,  $-152.1 \pm 0.9\text{‰}$ ,  $-101.7 \pm 1.0\text{‰}$ , respectively) as observed previously for other compounds (Atwood and Sachs, 2012). Thus, it is necessary to ensure that 95% of the targeted peak is recombined to avoid any chromatographic fractionation effect ( $\leq 5\text{‰}$ ).

We analyzed three different *Nannochloropsis* species to assess whether there are differences in  $\delta^2\text{H}_{\text{LCD}}$  between species. The cultures contained mainly the  $\text{C}_{32:1}$  1,15- and  $\text{C}_{32:0}$  1,15-diols as reported previously (Volkman et al., 1992; Balzano et al., 2017), and no  $\text{C}_{30}$  1,15-diol. In *N. oculata* and *N. gaditana*, the  $\text{C}_{32:0}$  1,15-diol was more abundant than the  $\text{C}_{32:1}$  1,15-diol, whereas the latter was the major LCD in *N. oceanica*. The  $\delta^2\text{H}$  ratios of the  $\text{C}_{32:1}$  1,15- and the  $\text{C}_{32:0}$  1,15-diols did not differ significantly between *N. oceanica* and *N. oculata* (Table 1). In contrast, the  $\delta^2\text{H}$  of the  $\text{C}_{32:1}$  1,15-diol was more depleted ( $-135 \pm 6\text{‰}$ ) than the  $\text{C}_{32:0}$  1,15-diol ( $-129 \pm 2\text{‰}$ , Table 1) in *N. gaditana*, and both values were depleted considerably relative to the corresponding values in the other two species, suggesting that there is a species effect on the hydrogen isotopic fractionation of LCDs. Salinity experiments with *N. oculata* showed that the  $\delta^2\text{H}$  ratio of the  $\text{C}_{32:0}$  1,15-diol is more enriched at higher salinity, but that fractionation is relatively constant with slightly lower values at the highest salinity (Table 2). These observations indicate that  $\delta^2\text{H}_{\text{LCD}}$  might reflect the  $^2\text{H}$  of growth medium, and potentially salinity, in which LCD-producers grow. The  $\delta^2\text{H}$  ratios of *N. oculata* LCDs in the salinity experiment are enriched compared to the initial culture grown at 37.5  $\text{g kg}^{-1}$  (Table 1), suggesting other factor such as light and growth phase

**Table 1**

$\delta^2\text{H}$  ratios of *Nannochloropsis* spp. cultures with standard deviation, and fractionation values between the lipids and the medium indicated as  $\alpha$ .

Names	CCMP 526 ( <i>N. gaditana</i> )	CCMP 1779 ( <i>N. oceanica</i> )	CCMP 2195 ( <i>N. oculata</i> )
$\delta^2\text{H}_{\text{C}_{32:0} 1,15}$ (‰)	$-129 \pm 2$	$-118 \pm 10$	$-116 \pm 4$
$\delta^2\text{H}_{\text{C}_{32:1} 1,15}$ (‰)	$-135 \pm 6$	$-114 \pm 2$	$-116 \pm 3$
$\delta^2\text{H}_{\text{medium}}$ (‰)	8.9	8.7	8.9
$\alpha$	0.863	0.874	0.876

**Table 2**

$\delta^2\text{H}$  ratios of *N. oculata* cultures with standard deviation (samples without a value were only measured once due to low concentration), and fractionation values between the lipids and the medium indicated as  $\alpha$ . Salinity and  $\delta^2\text{H}_{\text{medium}}$  are the mean of measurements made at the start of the culture and at harvesting.

Salinity (g kg <sup>-1</sup> )	22.5	27.8	30.1	32.7	34.5
$\delta^2\text{H}_{\text{C}_{32:0} 1,15}$ (‰)	$-118 \pm 11$	$-116 \pm 3$	-105	$-105 \pm 6$	-95
$\delta^2\text{H}_{\text{medium}}$ (‰)	$-13.7 \pm 5.4$	$-8.1 \pm 6.8$	$-1.8 \pm 3.5$	$+0.8 \pm 6.7$	$+4.0 \pm 3.4$
$\alpha$	0.894	0.891	0.897	0.894	0.901

likely impact  $\delta^2\text{H}_{\text{LCD}}$  (Wolhowe et al., 2009; Zhang et al., 2009; Chivall et al., 2014; van der Meer et al., 2015).

### 3.2. Hydrogen isotopic composition of LCDs in marine surface sediments

The  $\delta^2\text{H}$  ratio of the  $\text{C}_{32:0}$  1,15-diol in surface sediments from the Gulf of Mexico were similar to each other ( $-260 \pm 5\text{‰}$ ,  $n = 4$ , Fig. 1) whereas the  $\delta^2\text{H}$  ratios of the  $\text{C}_{30}$  1,15 diol was more enriched and increased with salinity (from  $-207\text{‰}$  to  $-184\text{‰}$ , Fig. 1). The large isotopic difference (up to  $70\text{‰}$ ) between the  $\text{C}_{32:0}$  1,15-diol and  $\text{C}_{30}$  1,15-diol supports the idea that the former originates mainly from river input (Lattaud et al., 2017). Indeed, the  $\text{C}_{30}$  1,15-diol concentrations were lower close to the river mouth ( $0.2$  vs  $1.0 \mu\text{g g}^{-1}$ ), in contrast to the  $\text{C}_{32:0}$  1,15-diol ( $0.1$  vs  $0.05 \mu\text{g g}^{-1}$ ). The  $^2\text{H}$ -depletion of the  $\text{C}_{32:0}$  1,15-diol likely originates from the lower  $\delta^2\text{H}_{\text{H}_2\text{O}}$  values of the Mississippi or Atchafalaya rivers ( $-31 \pm 8\text{‰}$  for the Atchafalaya River in Melville and  $-39 \pm 3\text{‰}$  for the Mississippi River in St Francisville, Lee and Veizer, 2003) rather than the marine  $\delta^2\text{H}_{\text{H}_2\text{O}} \approx 15\text{‰}$  (calculated from  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  ratios, LeGrande and Schmidt, 2006 and the global meteoritic water line, Craig, 1961). The  $\delta^2\text{H}_{\text{C}_{30} 1,15}$  weakly correlated with annual mean salinity ( $r = 0.7$ ,  $p = 0.1$ ,  $n = 6$ ) and autumn mean salinity ( $r = 0.7$ ,  $p = 0.08$ ,  $n = 6$ ) in contrast to the  $\delta^2\text{H}_{\text{C}_{37}}$  ( $r = 0.49$ ,  $p = 0.4$ ,  $n = 5$ ).

## 4. Conclusions

We report, for the first time, the  $\delta^2\text{H}$  ratios of LCDs obtained from cultures of three strains of marine eustigmatophytes and environmental samples along a salinity gradient in the Gulf of Mexico. The  $\text{C}_{32:0}$  1,15-diol seems to reflect the  $\delta^2\text{H}$  ratio of the rivers/lakes where this LCD was produced while the  $\text{C}_{30}$  1,15 reflected the  $\delta^2\text{H}$  ratio and salinity of marine surface waters. The use of LCDs in conjunction with long-chain alkenones could be useful for understanding hydrological changes in the geologic record.

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