Biosynthetic hydrogen isotopic fractionation factors during lipid synthesis in submerged aquatic macrophytes: Effect of groundwater discharge and salinity

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Biosynthetic hydrogen isotopic fractionation factors during lipid synthesis in submerged aquatic macrophytes: Effect of groundwater discharge and salinity

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Highlights

- Hydrogen isotopic fractionation for *Potamogeton* grown in a lake and lab measured.
- δD values for plants collected from a lake unaffected by groundwater.
- δD values of plants grown in the lab became lower at higher salinity.
- Average ε value −136 ± 9‰ for freshwater samples

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Abstract

Sedimentary lipid biomarkers have become widely used tools for reconstructing past climatic and ecological changes due to their ubiquitous occurrence in lake sediments. In particular, the hydrogen isotopic composition (expressed as δD values) of leaf wax lipids derived from terrestrial plants has been a focus of research during the last two decades and the understanding of competing environmental and plant physiological factors influencing the δD values has greatly improved. Comparatively less attention has been paid to lipid biomarkers derived from aquatic plants, although these compounds are abundant in many lacustrine sediments.

We conducted a field and laboratory experiment to study the effect of salinity and groundwater discharge on the isotopic composition of aquatic plant biomarkers. We analyzed samples of the common submerged plant species, *Potamogeton pectinatus* (sago pondweed), which has a wide geographic distribution and can tolerate high salinity. We tested the effect of groundwater discharge (characterized by more negative δD values relative to lake water) and salinity on the δD values of *n*-alkanes from *P. pectinatus* by comparing plants (i) collected from the oligotrophic freshwater Lake Stechlin (Germany) at shallow littoral depth from locations with and without groundwater discharge, and (ii) plants grown from tubers collected from the eutrophic Lake Müggelsee in nutrient solution at four salinity levels. Isotopically depleted groundwater did not have a significant influence on the δD values of *n*-alkanes in Lake Stechlin *P. pectinatus* and calculated isotopic fractionation factors $\varepsilon_{l/w}$ between lake water and *n*-alkanes averaged -137 ± 9‰ (*n*-C_{23}), -136 ± 7‰ (*n*-C_{25}) and -131 ± 6‰ (*n*-C_{27}), respectively. Similar ε values were calculated for plants from Lake Müggelsee grown in freshwater nutrient solution (-134 ± 11‰ for *n*-C_{23}), while greater fractionation was observed at increased salinity values of 10 (163 ± 12‰) and 15 (-172 ± 15‰).

We therefore suggest an average ε value of -136 ± 9‰ between source water and the major *n*-alkanes in *P. pectinatus* grown under freshwater conditions. Our results demonstrate that isotopic fractionation can increase by 30-40‰ at salinities 10 and 15. These results could be explained either by inhibited plant growth at higher salinities, or by metabolic adaptions to salt stress that remain to be elucidated. A potential salinity effect on δD values of aquatic lipids requires further examination, since this would impact the interpretation of downcore isotopic data in paleohydrologic studies.

1. Introduction

The hydrogen isotopic composition (expressed as δD values) of leaf wax lipids extracted from sediments is increasingly being used by paleoclimatologists to reconstruct past hydrological conditions (e.g. reviewed by Castaneda and Schouten, 2011; Sachse et al., 2012). These compounds track the hydrogen isotopic signature of ambient water, which is lake water for submerged aquatic plants and soil water for terrestrial vegetation (Sauer et al., 2001; Huang et al., 2004; Sachse et al., 2006), the latter with additional influence from transpiration (Kahmen et al., 2013a, b). Due to deuterium (D) discrimination during lipid synthesis, these compounds are usually D depleted (more negative δD value) relative to the source water (Sessions et al., 1999; Chikaraishi and Naraoka, 2003; Zhang and Sachs, 2007; Zhang et al., 2009). To reconstruct the isotopic signature of precipitation or lake water it is therefore important to estimate the magnitude of the fractionation factor between leaf wax lipids and source water ($\varepsilon_{l/w}$ or sometimes referred to as the apparent fractionation ε_{app}). $\varepsilon_{l/w}$ integrates several potentially influencing factors on isotopic fractionation, such as relative humidity, plant physiological differences and biochemical reactions (ε_{bio}) (Sachse et al., 2012). Previous studies have demonstrated that $\varepsilon_{l/w}$ has significant inter and intra species variation (reviewed by Sachse et al., 2012). Culturing experiments have further shown that salinity, growth rate, temperature, and irradiance may have an effect upon $\varepsilon_{l/w}$ of lipids derived from algae (reviewed by Sachs, 2014; e.g. Schouten et al., 2006; Sachs and Schwab, 2011;

M´Boule et al., 2014; Chivall et al., 2014; Nelson and Sachs, 2014; Heinzelmann et al., 2015; van der Meer et al., 2015; Maloney et al., 2016).

Aquatic plant lipids are useful for paleohydrological reconstructions because submerged aquatic macrophytes do not use leaf water for lipid biosynthesis as terrestrial plants do. Instead they utilize lake water as a hydrogen source hence directly record the lake water isotopic composition. Despite their ubiquitous occurrence in lake sediments (Ficken et al., 2000; Aichner et al., 2010a, b), the amount of $\varepsilon_{l/w}$ data for submerged aquatic plant lipids, such as *n*-C_{23} and *n*-C_{25} alkanes is low (Chikaraishi and Naraoka, 2003; Mügler et al., 2008; Duan and Xu, 2012; Duan et al., 2014). Some other studies have attempted to estimate $\varepsilon_{l/w}$ of aquatic plants from δD measurements of sedimentary lipids (e.g. Sachse et al., 2006; Günther et al., 2013). However, sedimentary mid-chain *n*-alkanes, which are commonly attributed to aquatic organisms, are also produced by terrestrial plants, albeit in lower amounts (Aichner et al., 2010a; Gao et al., 2011; Liu et al., 2015). Thus, sedimentary accumulations often constitute mixtures which could bias the estimation of $\varepsilon_{l/w}$-values.

Further, the potential impact of environmental factors such as groundwater discharge and salinity on the isotopic signatures of aquatic plant lipids has not been examined. δD values of groundwater could be distinct from lake water δD. As such, groundwater discharge into littoral areas potentially affects δD of aquatic plants (Périllon and Hilt, 2016). Aquatic plant lipids are also found in saline lakes, but it is unclear whether or not salinity might exert a control on $\varepsilon_{l/w}$, as has been shown for

cyanobacteria (Sachse and Sachs, 2008), algae (Schouten et al., 2006; Sachs and Schwab, 2011), and mangroves (Ladd and Sachs, 2012 and 2017). This lack of understanding results in uncertainty when interpreting paleoclimate records, particularly when the aim is to quantitatively reconstruct δD values of source water.

In this study, we analyzed *n*-alkanes from the submerged aquatic angiosperm sago pondweed, *Potamogeton pectinatus* (also named *Stuckenia pectinata*) to test if salinity and groundwater discharge influence their δD values. *P. pectinatus* occurs nearly worldwide from sea level to 5,000 m above sea level. It can grow on a variety of bottom substrates and is able to tolerate a wide range of chemical environments (Van Wijk, 1988; Van Wijk et al., 1988; Casey, 2010). To test for the effect of groundwater discharge, we analyzed plants collected from a groundwater-fed freshwater lake at locations with and without groundwater discharge. To examine the effect of salinity, we grew *P. pectinatus* from tubers collected from a eutrophic lake under controlled laboratory conditions in four different salinity treatments.

2. Material and methods

2.1. Samples

Above ground parts of submerged *P. pectinatus* from the oligo-mesotrophic Lake Stechlin were collected in July 2014 with at least two plants per treatment (Fig. 1; for lake characteristics see Supplementary Table S1). Samples were taken at 50 cm water depth, at one site without the influence of groundwater discharge (C1), and

two different sites proximal to groundwater discharge (GW1 and GW2) (Fig. 1). Detailed analysis of groundwater discharge in Lake Stechlin was performed in a previous study (Périllon et al., 2017). Plants were gently freed from periphyton, dried at 60 °C, and homogenized using a Mini Mill (Pulverisette 23; Fritsch, Germany). Lake water was collected at the same location as the plants and interstitial water was sampled 70 cm below the sediment using a piezometer.

For the laboratory experiment, *P. pectinatus* tubers were collected from sediment at 30-60 cm water depth at the northern shore of Lake Müggelsee (Germany) on 21st March 2016. The tubers were instantly planted into 50 ml glass beakers filled with acid-rinsed sea sand (Roth, Germany) containing a phosphorous source (0.3 mg P/g). The beakers were placed into 2 l glass cylinders filled with artificial lake water (MIIIKS-nutrient solution; Nicklisch, 1999). Different salinity values were achieved by adding 0, 5, 10, 15, 20 and 30 g NaCl/l to the cylinders, with four replicates for each salinity treatment. This resulted in 24 beakers labelled 0 a - d (salinity 0) to 30 a – d (salinity 30). Each beaker was equipped with two tubers ("1" and "2"). Glass cylinders were covered with lids and placed under 12:12h of light (60 to 180 µmol photon/m²/s) delivered from the top with four fluorescent lamps (Biolux ® L 18W/965; OSRAM) in a temperature controlled room (20 ± 2 °C). On 20th April, plants were harvested for lipid biomarker analysis, dried and weighed and cut into small pieces. Dry weights of plant biomass are listed in the Supplementary Table S6. To monitor potential isotopic drift of the source water, water samples were collected from the glass beakers on days 1, 17 and 31 of the experiment (22nd March,

6th April and 20th April). We also analyzed water samples taken from Lake Müggelsee in January and July 2016 (n = 20) from different locations (Supplementary Tables S2 and S3) to assess a potential "storage effect" i.e. an isotopic signal stored in the tubers from the last vegetation period.

2.2. Organic geochemical and water isotope analysis

Plant samples were extracted into glass vials for 2 h in an ultrasonic bath filled with dichloromethane (DCM) / methanol (MeOH) (9:1). The *n*-alkane containing fractions of the extracts were purified by using columns containing a silica gel stationary phase which were flushed with hexane as eluent. Compounds were quantified using gas chromatography (GC; Agilent 7890) equipped with flame ionization detection (FID; Agilent 7683B) and a 30 m Restek DB-5 column (30 m, inner diameter 0.25 mm, film thickness 0.25 µm). 5α-androstane was added to the samples as internal standard prior to column chromatography. For compound specific hydrogen isotopic analysis we used a GC (Thermo Scientific® Trace) with a Restek RTX-5 column (30 m, inner diameter 0.25 mm, film thickness 0.25 µm) and a split-splitless (SSL) injector operated in splitless mode at an evaporation temperature of 60 °C. The GC instrument (temperature program: 2 minutes static at 70°C; 15°C/min to 150°C; 5°C/min to 320°C; 10 min static time) was connected via an Isolink with a high temperature conversion (HTC) reactor (1420 °C) and a Conflo IV interface to a DeltaVPlus isotope ratio mass spectrometer. Data derived were normalized to the Vienna Standard Mean Ocean Water (VSMOW) hydrogen isotopic scale by

calibrating values with an external standard containing 15 *n*-alkanes (A5-mix with C_{16} to C_{30} of known isotopic composition; A. Schimmelmann, Indiana University, Bloomington). The average root mean square error of replicate measurements of the compounds in the standard-mix across the measurement period was 3.96‰. To monitor the stability of the H_3^+ factor (Sessions et al., 2001) this correction parameter was determined before and after each sequence and accounted for 1.84 ± 0.05 ppm/mv over the three days measuring period.

In most cases the two lab grown plants from one beaker were combined to one sample for isotope ratio mass spectrometry (IRMS) analysis. To achieve a sufficient signal (i.e. > 2000 mV), single measurement was conducted for five samples by way of manually injecting the complete purified sample extract into the GC-IRMS-system. Duplicate measurements were conducted for all the other samples. Concerning plants from Lake Stechlin, all samples except for one were measured in triplicate.

Water isotopic values were measured using cavity ring down spectroscopy with a Picarro L2140-*i* and a Picarro L2130-*i*. Pore water and lake water were filtered (45µm) before the analysis, and contaminations were screened by using the ChemCorrect software. Each sample was measured ten times, but the first three measurements were discarded when calculating mean values to avoid memory effects. The average standard deviation of those seven replicates was <0.2‰. For calibration of data a VSMOW and a SLAP (Standard Light Antarctic Precipitation) standard (both from International Atomic Energy Agency IAEA, Vienna, Austria;

each measured 13x) were used. Additionally, 10 measurements of tap water served as a control to monitor drift.

The isotopic fractionation (or apparent enrichment factor) between δD values of lipid compounds and those of water were calculated as follows: $\varepsilon_{l/w}$ = 1000 x [(δD_{lipid} + 1000)/(δD_{water} + 1000) - 1]. To estimate the average uncertainty of these fractionation factors the following values were included into an error calculation: the average root mean square error of the A5-standard mix (3.96‰), the average standard deviation of duplicate δD-analysis of *n*-C_{23}-alkanes (5.20‰), and the standard deviation of measured δD-values of water samples taken during the lab experiment (0.75‰). Based on these parameters, the computed uncertainty for $\varepsilon_{l/w}$ within the lab experiment accounts for 6.58‰.

3. Results and discussion

3.1. δD values of water samples

Lake Stechlin interstitial water samples had significantly higher δD values (Fig. 2) at the site without groundwater influence (C1, -28.6 ± 0.1‰) compared to values at the sites with groundwater discharge GW1 (-60.6 ± 0.1‰) and GW2 (-63.0 ± 0.2‰) (Supplementary Table S4). Values at C1 were close to the lakes surface water isotopic signature (-26.7 ± 0.3‰). Seasonal variation in δD values of the Lake Stechlin surface water is low, most likely because of its large volume and long residence time. Long term measurements from the Leibniz Institute for Freshwater

Ecology and Inland Fisheries provided a mean lake water δD value of -25‰ (August 2012 to November 2014, from different depths; J. Lewandowski, personal communication), which was in agreement with our measurement. Groundwater δD values obtained in previous studies were ca. -62‰ (83 samples from the watershed, September 2013 to 2014; J. Lewandowski, personal communication). These data are similar to our measured values for interstitial water from sites GW1 and GW2, confirming groundwater discharge at these locations.

In the lab experiment, we observed a slight increase in water δD values with increasing salinity on the first and third water sampling day, but overall isotopic variability was small within a range of ca. 2.5‰ (Supplementary Fig. S2 and Table S5). Hence, we used the average of -54.1 ± 0.8‰ as the δD signature of source water for plants and for computing ε values. Water samples from Lake Müggelsee had δD values of -52.8 ± 0.5‰ (July 2016; Supplementary Table S2) and -55.8 ± 1.3‰ (January 2016; Supplementary Table S3). These data suggest relatively little seasonal change in lake water δD values, despite a wide seasonal variability in precipitation δD values, ranging from -83‰ in winter to -48‰ in summer (Online Isotopes in Precipitation Calculator, Supplementary Fig. S1; Bowen and Revenaugh, 2003).

3.2. Lipid δD values of plant samples

The relative abundances of *n*-alkanes from *P. pectinatus* collected in Lake Stechlin were characterised by *n*-C_{25}, > *n*-C_{27}, > *n*-C_{23}. *n*-C_{29} was also abundant in significant concentrations (Supplementary Table S6). This has been observed in earlier studies (e.g. Aichner et al., 2010a) and illustrates that despite the dominance of mid-chain compounds, aquatic macrophytes could potentially contribute to long-chain *n*-alkanes in sediments. Especially in environments with sparse terrestrial vegetation, this is an important factor to consider, as recently shown by the application of compound-specific carbon isotopic analysis to sediment samples from the Tibetan Plateau (Liu et al., 2015). δD values of the three major compounds showed considerable variability of 25‰ (-146 to -169‰; Fig. 2 and Supplementary Table S7). This illustrates that intraspecies variability of leaf wax δD values can be relatively large, a phenomenon which has also been observed in terrestrial plants (Sachse et al., 2012).

No significant difference was observed between mean δD values for samples from locations with (*n*-$C_{25:}$ -160‰) and without (*n*-$C_{25:}$ -158‰) the influence of isotopically depleted groundwater (t-test p=0.71; Fig. 2). As such, groundwater is likely not a major water source for the plants sampled from this lake. Therefore, the plants sampled for our study, which were from the well-mixed littoral of the lake, took up a lake water rather than a groundwater isotopic signal. This is plausible, as many aquatic plants are able to take up and transport water both through their leaves and roots (Pedersen and Sand-Jensen, 1993; Schönherr, 1976; Van Vierssen et al., 1994).

Consequently, we used lake water δD values to calculate isotopic fractionation factors ε (see below).

During the lab experiment, all *P. pectinatus* tubers germinated, but subsequent growth differed significantly between salinity treatments, resulting in final shoot lengths between 35 and 2 cm (Fig. 3a; Supplementary Table S8). Only plants grown in salinities of 0, 5, 10, and 15 contained enough material for δD analysis of *n*-alkanes. In contrast to samples from Lake Stechlin, *n*-C_{23} was the most abundant *n*-alkane in all samples from plants grown in the lab. δD values for *n*-C_{23} ranged from -164 to -227‰ with a tendency to lower values for plants grown at higher salinities (Fig. 3b). A similar trend was observed for C_{25} *n*-alkanes (Supplementary Table S8). It is remarkable that replicates grown in the same salinity plot showed relatively large isotopic variability of up to ca. 35‰.

Since the more negative δD values at higher salinity were paralleled by lower plant growth rate (Fig. 3), an increased use of stored compounds could have influenced the measured values. During germination, *P. pectinatus* first use storage carbohydrates from tubers to build up biomass before incorporating new products of the photosynthesis (Van Vierssen et al., 1994). However, this cannot explain the wide isotopic variability in the plant samples, since Lake Müggelsee (where the tubers were collected) showed relatively little variability in lake water δD values throughout the year (ca. -56 to -53‰; Section 3.1). These values are also similar to the δD value of tap water – produced by bank filtration from Lake Müggelsee – which was used for production of the medium in our experiment. It cannot be ruled

out that the storage carbohydrates from the tubers were D-depleted relative to the plant biomass. However, this is unlikely since these substances are usually enriched in D compared to alkyl lipids (Schmidt et al., 2003). Incorporation of a slightly more negative winter signal is unlikely due to the short vegetation period of *P. pectinatus* in Lake Müggelsee, which begins around April and ends in August (Hilt et al., 2013).

3.3. Hydrogen isotopic fractionation of aquatic plant lipids

Calculated $\varepsilon_{l/w}$-values were -137 ± 9‰ (for *n*-C_{23}), -136 ± 7‰ (for *n*-C_{25}) and -131 ± 6‰ (for *n*-C_{27}) for *P. pectinatus* from freshwater Lake Stechlin and -134 ± 11‰ (*n*-C_{23}) for plants grown in the lab (Fig. 4b). These values are comparable with reported isotopic fractionation factors of aquatic plants from freshwater lakes Gunma (Japan; Chikaraishi and Naraoka, 2003) and Gannan Gahai (Tibet; Duan et al., 2014), as well as from the saline Lake Qinghai (Duan and Xu, 2012), but slightly higher than average values from three unspecified submerged plants from brackish Lake Nam Co (Mügler et al., 2008; Fig. 4a).

Samples grown at higher salinity in the lab, had $\varepsilon_{l/w}$-values ranging from -116‰ (sample 5-d) to -182‰ (sample 15-a, Fig. 4b). Average fractionation factors for salinity 10 (-163 ± 12‰) and salinity 15 (-172 ± 15‰) were ca. 30-40‰ larger than that at salinity 0 (-134 ± 11‰) and salinity 5 (-133 ± 17‰). A similar trend, i.e. greater fractionation at higher salinity, has been observed in mangroves (Ladd and

Sachs, 2012, 2017). The magnitude of isotopic depletion with increasing salinity is even larger in our dataset (2.6‰ per salinity unit; Fig. 3b) compared to the mangrove data (ca. 1‰ depletion per salinity unit; Ladd and Sachs, 2012, 2017). In contrast, smaller ε values for higher salinity were reported in a number of studies of cyanobacterial and algal lipids such as *n*-C_{17}-alkane, fatty acids, diploptene, sterols and alkenones (e.g. Schouten et al., 2006; Van der Meer et al., 2007; Sachse and Sachs, 2008; Sachs and Schwab, 2011; M´Boule et al., 2014; Chivall et al., 2014; Nelson and Sachs, 2014; Heinzelmann et al., 2015; Maloney et al., 2016).

The exact mechanism behind salinity dependent isotopic fractionation in different organisms has to be elucidated. There are two possible explanations for the observed D-depletion of *n*-alkanes at higher salinities in our data set: (I) The more negative δD values at higher salinities were a consequence of limited plant growth, as shoots just grew 3-5 cm in plots with salinities 10 and 15 (Fig. 3a). (II) D-depletion at higher salinities was caused by discrimination of the heavier isotope during water uptake or metabolic processes as a response to salt stress. Factors involving leaf transpiration processes, as discussed for mangroves (Ladd and Sachs, 2012; Ladd and Sachs, 2015a, b), can be excluded for submerged plants.

Under hypothesis I the majority of lipids would be synthesized during the early development of shoots, largely relying on substrate H rather than water H, whereas lipid synthesis ceased later due to growth inhibition under higher salinities. Lipids synthesized with H from these substrates could potentially be more D-depleted than those which were produced at later stages if either a) D-depleted substrate is used

during biosynthesis of early leaf wax lipids (as discussed above in section 3.2.) or if b) D discrimination is relatively large during that process and becomes smaller with continuing growth of the plant. We note that we do not have observational evidence for any of these processes and do not know the δD value of the substrate, but similar hypothesis has been put forward to explain observed seasonal differences in ε_{bio} for plants: the seagrass *Spartina alterniflora* has shown seasonal trends within the δD-values of its *n*-alkanes, which has been explained by usage of different substrate for synthesis throughout the vegetation period (Sessions, 2006). Similarly, differences in ε_{bio} of higher terrestrial plants (*Salix* and *Quercus*) over the season have been suggested to be the consequence of the use of carbohydrate substrates during the early season (Newberry et al. 2015, Sachse et al. 2015). These carbohydrate substances would be metabolized through the pentose phosphate cycle and therefore result in D-enriched NADPH fed into biosynthetic pathways (compared to photosynthetic NADPH), while our observation would require a strongly D-depleted H-source. It is notable that the concentrations of *n*-alkanes were higher for the here studied plants with limited growth in salinity plots "10" and "15" (20-87 µg / g d.w. for Σ*n*-alkanes compared to fully grown plants in plots "0" and "5" (3-20 µg / g. d.w.); (Supplementary Table S6). Accelerated leaf wax synthesis during the early shoot development and incorporation of a higher proportion of a D-depleted source could be an explanation for the more negative δD values of *n*-alkanes.

Evidence against limited plant growth as a driving factor behind the observed D-depletion at higher salinities comes from three plants grown at salinities 0 and 5.

Plants 0-d-1, 5-a-1, and 5-a-2 were significantly shorter (4.5 to 8.1 cm) than other replicates at lower salinities but their *n*-alkanes do not show more negative δD values (Fig. 3a; Supplementary Table S8 and Fig. S3). The variable lengths of shoots in these plots are likely not influenced by inhibited growth but rather by different sizes of tubers (Spencer, 1987). Overall, there is no significant correlation between plant length and leaf wax δD values in salinity plots "0" and "5" ($R^2 = 0.22$; $p = 0.2$).

Under hypothesis II the drivers of larger ε_{bio} under higher salinities are associated with strategies of the plants to combat salt stress. These include a) downregulation of water permeability of the cell under higher salinities, resulting in isotopically different intra- and extracellular water, or b) metabolic processes which involve hydrogen transport within the $NADP^+$ system and the enzymatically driven leaf wax synthesis itself (Sachs and Schwab, 2011; Sachse et al., 2012; Chivall et al., 2014; Heinzelmann et al., 2015). The exact mechanism used by *P. pectinatus* for adaption to higher salinities is unclear (Casagranda and Boudouresque, 2007). Strategies could include a downregulation of the exchange between extra- and intracellular water to avoid osmotic stress. Aquatic plants including *Potamogeton* sp. have highly permeable cell membranes to enable water exchange (Schönherr, 1976). Increased D-discrimination during restricted water uptake, resulting in more negative δD values of intracellular water, would be a plausible consequence. Another metabolic response, thought to explain the trend towards lower δD values of leaf wax lipids at higher salinities in mangroves, is the formation of osmolytes, also referred to as "compatible solutes" (Ladd and Sachs, 2012). These are compounds, often sugars,

which are formed in many kinds of organisms to combat intracellular salt stress. It is unclear to what extent this process is relevant for *P. pectinatus*, and which isotopic signature these compounds have. In case these compounds are relatively enriched in D, which is common for carbohydrates (Schmidt et al., 2003) their biosynthesis would lead to more depleted remaining substrate available for lipid synthesis.

4. Conclusions

Alkanes from the common submerged plant species *P. pectinatus* which has been grown under freshwater conditions (Lake Stechlin and lab experiment salinity 0; n = 13) showed $\varepsilon_{l/w}$-values of -136 ± 9‰ for *n*-C_{23}. Isotopic fractionation for plants grown under higher salinities was larger (salinity 10, -163 ± 12 and salinity 15, -172 ± 15) but plants in these plots were clearly inhibited in their growth compared to those at salinities 0 and 5. Even though there are indications that limited plant growth is not the driving factor behind the observed trend, this needs further examination by analyzing δD values of fully developed plants, which have been grown over a broad salinity gradient. Additional experiments are also needed to determine the effect of salinity on isotopic fractionation for other aquatic plant species, for calibration, and to understand the underlying mechanisms behind the measured data.

The results indicate that salinity changes must be considered as an additional influencing factor when interpreting δD values of aquatic biomarkers in paleoclimatic records from lakes. This includes applications such as using leaf wax δD as a proxy for lake water δD, or dual approaches which combine δD of aquatic and terrestrial biomarkers to reconstruct past humidity conditions.

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Figures

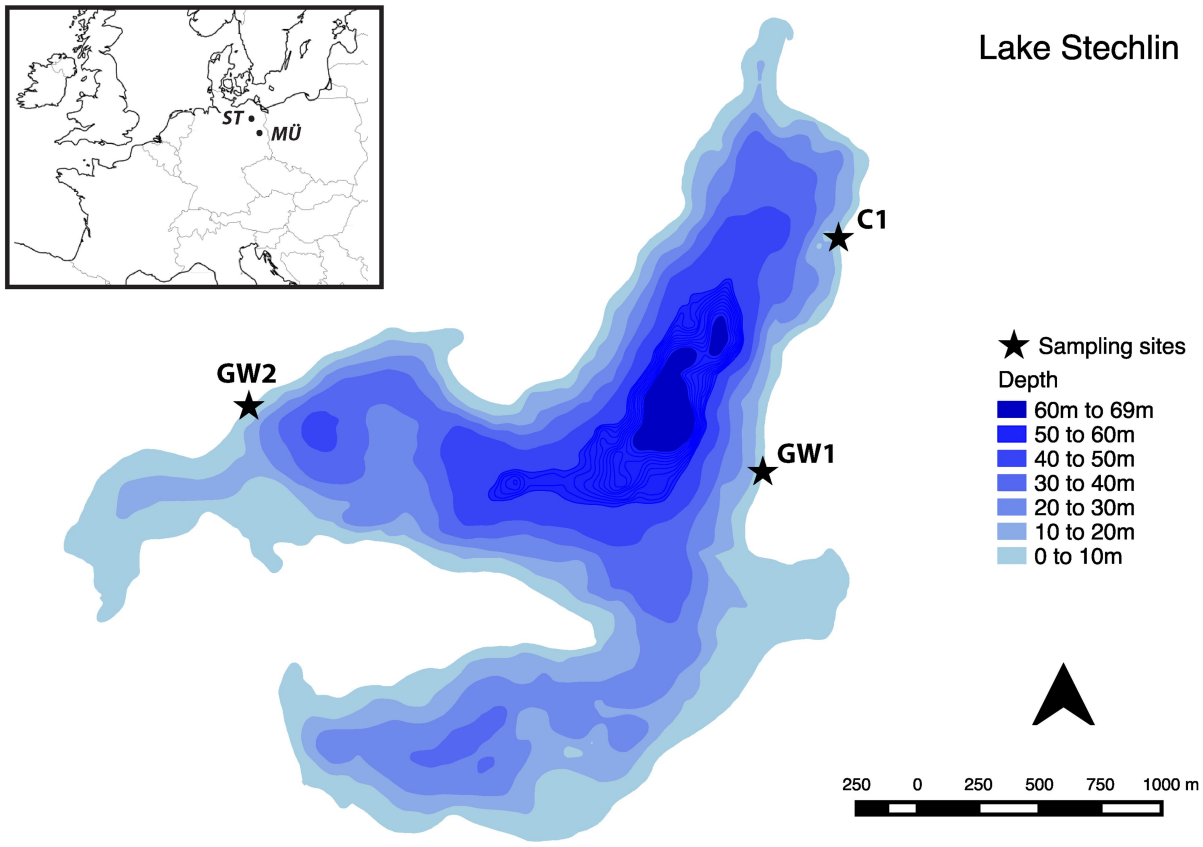

Fig. 1. Locations of lakes Stechlin (ST) and Müggelsee (MÜ). Sampling locations with (GW 1 and 2) and without groundwater discharge (C1) in Lake Stechlin known from detailed analyses by Périllon et al. (2017).

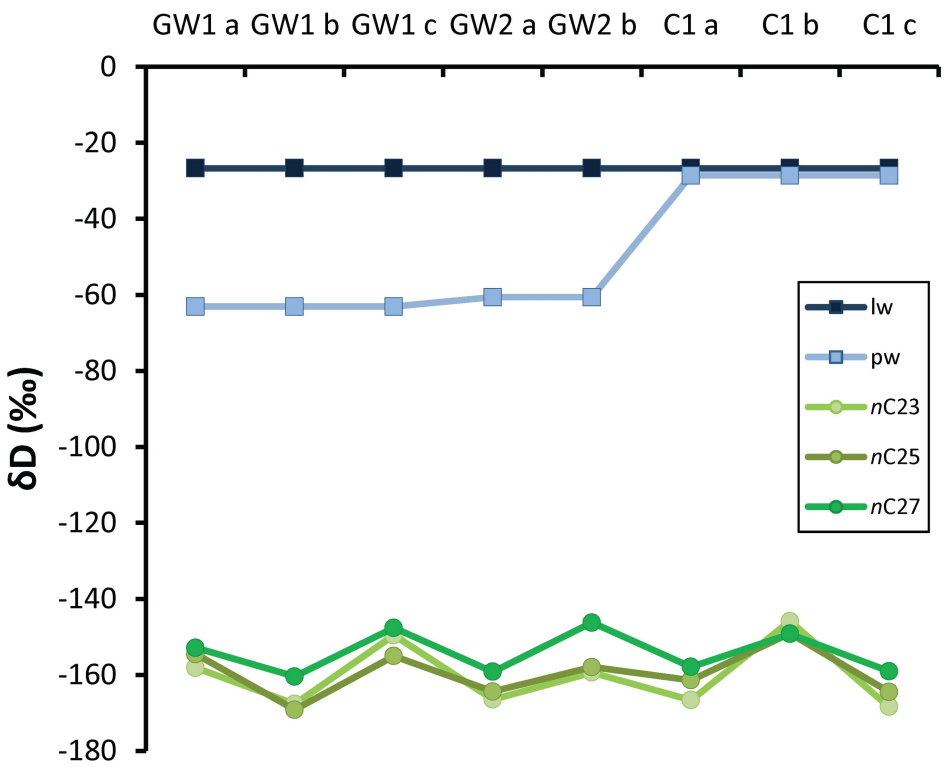

Fig. 2. δD values of lake water (lw), pore water (pw) and *n*-alkanes extracted from macrophytes collected in Lake Stechlin. Standard deviations of replicate measurements were 0.32‰ for lw, <0.2‰ for pw and 1-7‰ for *n*-alkanes (Supplementary Tables S4 and S7).

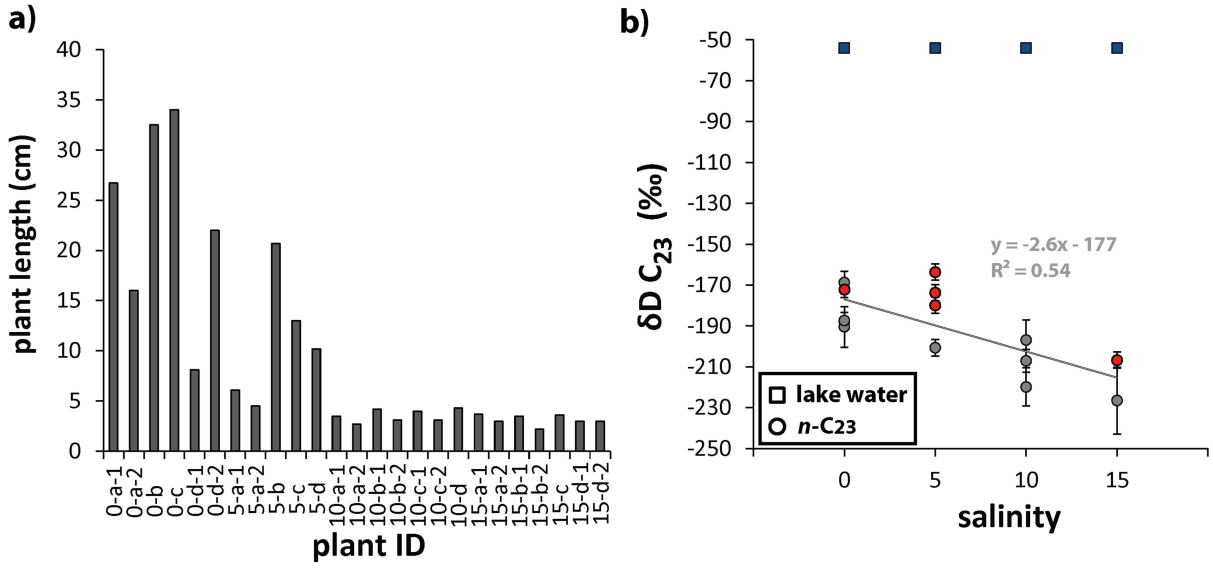

Fig. 3. (a) Length of plants growing in plots with salinity 0, 5, 10, and 15. Plots with salinity 20 and 30 are not shown because plants did not grow at highest salinities. Replicate beakers are labelled a-d. Plants grown in one beaker (labelled 1 and 2) were in some cases combined for IRMS analysis (12) to provide a large enough signal for reliable isotope measurement. (b) Circles indicate δD of *n*-C_{23} plotted vs. salinity. Red dots mark samples for which only a single measurement was possible. Error bars combine the standard deviations of duplicate measurements and the root mean square error of the A5-standard-mix (3.96‰). Blue squares indicate δD values of source water (standard deviation: 0.75‰).

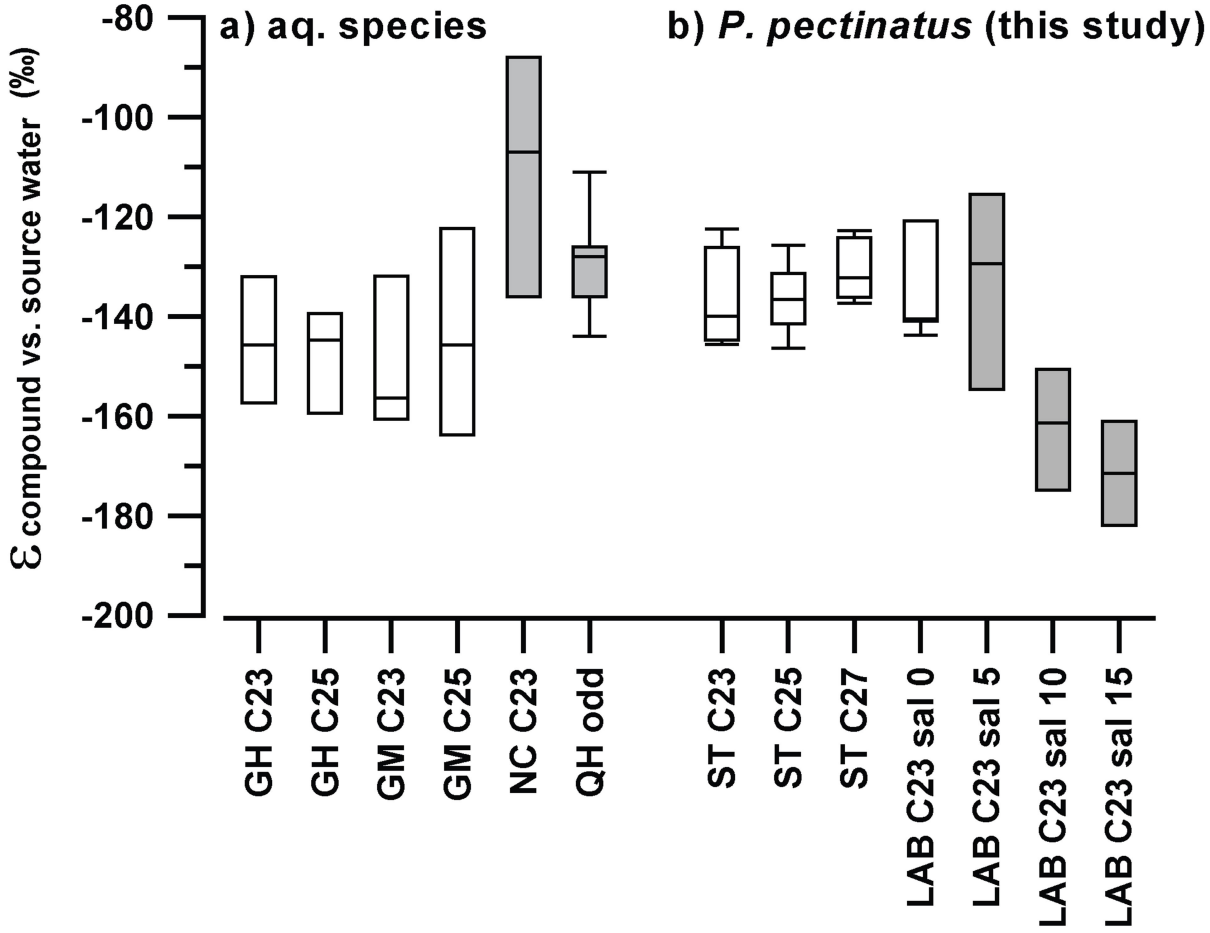

Fig. 4. Isotopic fractionation factor ε for target compounds vs. source water. (a) Literature data for submerged aquatic species collected at Nam Co (NC; unspecified macrophyte; Mügler et al., 2008), Lake Gahai (GH, *Spirogyra intorta, Myriophyllum verticillatum*; Duan et al., 2014), Lake Qinghai (QH; *Spirogyra intorta, Potamogeton gramineus*; Duan and Xu, 2012), and Lake Gunma (GM; *Vallisneria asiatica, Potamogeton perfoliatus, Hydrilla verticillata;* Chikaraishi and Naraoka, 2003). (b) Results for *P. pectinatus* (this study) derived from field samples (Lake Stechlin; ST) and lab experiment conducted at different salinity values (SAL 0 - 15). Shaded boxes indicate non-freshwater samples.

Supplementary material to

Aichner et al., Organic Geochemistry, 2017 "*Biosynthetic hydrogen isotopic fractionation factors during lipid synthesis in submerged aquatic macrophytes: Effect of groundwater discharge and salinity*"
https://doi.org/10.1016/j.orggeochem.2017.07.021

Table S1: Lake Stechlin characteristics.

Location	53°09'N 13°02'E
Area (km^2)	4.3
Mean/maximum depth (m)	23/69.5
Water residence time (yr)	> 40
Conductivity (µS/cm)	268 ± 17[a]
Total P concentration [µg/l]	14.2 ± 4.7[a]

[a] Monthly sampling of surface water, April-September 2013 and 2014. Conductivity measured at plant sampling locations (see Périllon et al. 2017). Total P determined above deepest point of the lake.

Fig. S1. Seasonal δD-values of precipitation in our study area. Data from Online Isotope Precipitation Calculator computed forLake Stechlin (Bowen and Revenaugh, 2003).

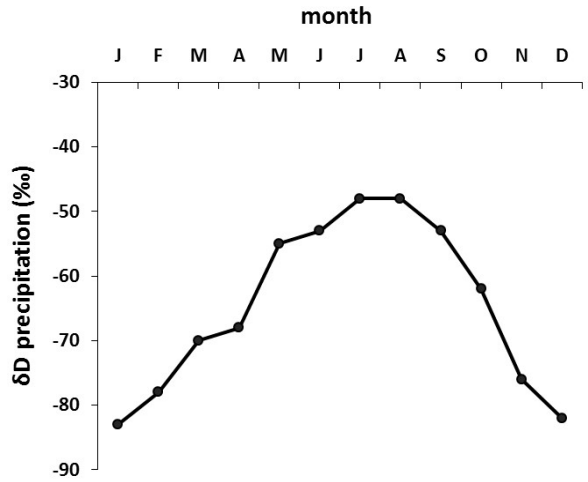

Table S2: δD-values of water samples from different locations in Lake Müggelsee in July 2016.

Sample	Depth	δD (‰)	St dev
Müggel_1	shore	-53.87	0.36
Müggel_2	shore	-53.05	0.17
Müggel_3	shore	-52.87	0.11
Müggel_4	2.7 m	-52.85	0.14
Müggel_5	2.7 m	-52.36	0.14
Müggel_6	2.7 m	-52.66	0.08
Müggel_7	shore	-52.68	0.06
Müggel_8	2.7 m	-52.61	0.10
Avg.		**-52.87**	
St dev		**0.45**	

Table S3: δD values of water samples collected at Lake Müggelsee in January 2016 at three locations in ca. 100 cm depth.

Sample	δD (‰)	St dev	Location	
1_1	-56.3	0.10	52°26'52.40"N	13°38'54.10"E
1_2	-56.5	0.15		
1_3	-56.9	0.06		
1_4	-56.9	0.08		
2_1	-54.7	0.09	52°26'18.30"N	13°37'17.90"E
2_2	-54.6	0.13		
2_3	-54.5	0.13		
2_4	-54.3	0.07		
3_1	-54.5	0.12	52°25'46.20"N	13°37'24.60"E
3_2	-55.4	0.07		
3_3	-56.9	0.05		
3_4	-57.9	0.05		
Avg.	**-55.8**			
St dev	**1.3**			

Table S4: δD values of interstitial and lake water sampled in Lake Stechlin. Standard deviation derived from replicate measurements

Sample	**Depth**	**δD (‰)**	**St dev**
Stechlin C1	70 cm below sediment	-28.59	0.14
Stechlin GW1	70 cm below sediment	-60.60	0.06
Stechlin GW2	70 cm below sediment	-63.04	0.16
Stechlin lake water	50 cm above sediment	-26.70	0.32

Fig. S2. δD-values of water samples taken during the salinity experiment. Error bars indicate standard deviation of replicate measurements. See also Supplementary Table S5.

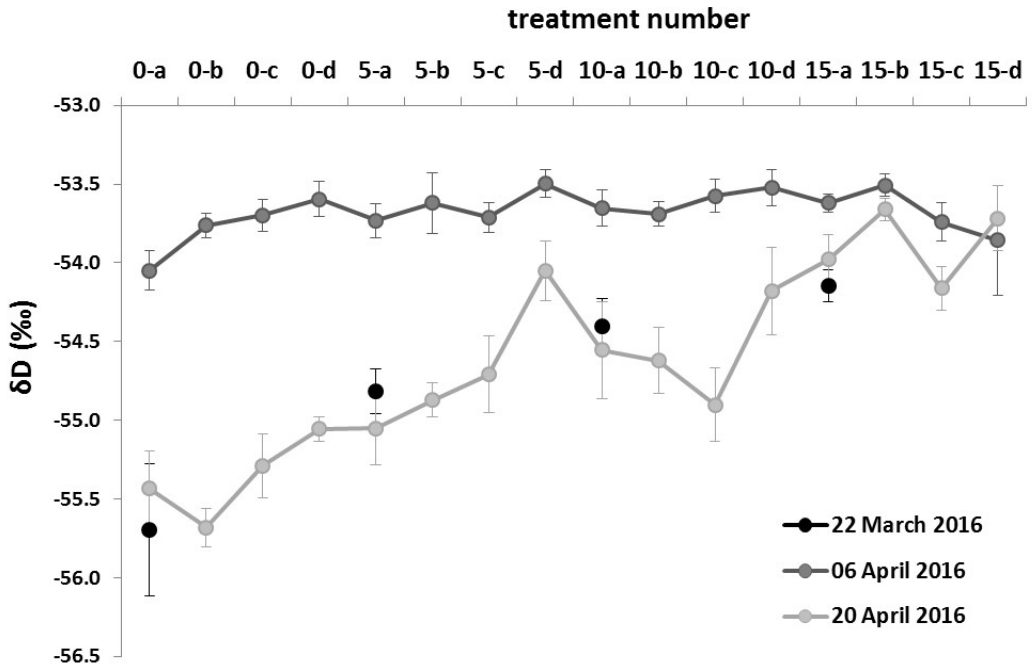

Table S5: δD-values of water samples taken during the salinity experiment. Data represent average and standard deviation of seven replicate measurements.

	22 Mar 16		06 Apr 16		22 Apr 16	
Sample	**δD [‰]**	**St dev**	**δD [‰]**	**St dev**	**δD [‰]**	**St dev**
0-a	-55.70	0.42	-54.05	0.12	-55.43	0.24
0-b			-53.76	0.08	-55.68	0.12
0-c			-53.70	0.10	-55.29	0.21
0-d			-53.59	0.11	-55.06	0.08
5-a	-54.82	0.14	-53.73	0.11	-55.05	0.23
5-b			-53.62	0.19	-54.87	0.11
5-c			-53.71	0.10	-54.71	0.24
5-d			-53.50	0.09	-54.05	0.19
10-a	-54.40	0.18	-53.65	0.12	-54.56	0.31
10-b			-53.69	0.08	-54.62	0.21
10-c			-53.57	0.10	-54.90	0.23
10-d			-53.52	0.12	-54.18	0.28
15-a	-54.15	0.10	-53.62	0.06	-53.98	0.16
15-b			-53.51	0.07	-53.66	0.07
15-c			-53.74	0.12	-54.16	0.14
15-d			-53.86	0.35	-53.72	0.21

Table S6: Concentration of *n*-alkanes in *P. pectinatus* from (a) Lake Stechlin and (b) the lab experiment (d.w., dry weight).

(a)

Sample ID	Dry weight (mg)	Concentration (µg / g d.w.)							Abundance [%]					
		C_{21}	C_{23}	C_{25}	C_{27}	C_{29}	C_{31}	Sum	C_{21}	C_{23}	C_{25}	C_{27}	C_{29}	C_{31}
GW1 a	175	0.8	5.2	6.1	6.2	3.4	1.1	22.8	3.3	22.8	27.0	27.0	15.0	4.9
GW1 b	109	3.4	10.5	16.7	15.8	8.2	2.5	57.2	6.0	18.4	29.2	27.6	14.4	4.4
GW1 c	106	0.9	4.9	8.3	7.4	4.5	1.6	27.5	3.4	17.7	30.0	26.9	16.2	5.7
C1 a	186	1.0	7.4	10.5	10.4	5.9	2.1	37.2	2.6	19.9	28.3	27.9	15.7	5.6
C1 b	198	0.7	2.0	3.4	4.8	2.6	0.9	14.4	5.2	14.2	23.7	33.0	18.0	5.9
C1 c	409	1.2	10.2	13.8	12.5	6.6	2.3	46.5	2.6	21.8	29.6	26.9	14.1	5.0
GW2 a	212	15.6	15.7	21.6	23.2	14.2	6.9	97.2	16.0	16.2	22.2	23.9	14.6	7.1
GW2 b	496	1.4	1.1	1.4	1.3	1.0	0.4	6.7	15.8	18.0	22.1	21.3	15.8	7.0
								Avg.	**6.9**	**18.6**	**26.5**	**26.8**	**15.5**	**5.7**
								St dev.	**5.7**	**2.8**	**3.3**	**3.4**	**1.2**	**1.0**

(b)

		C_{21}	C_{23}	C_{25}	C_{27}	C_{29}	C_{31}	Sum	C_{21}	C_{23}	C_{25}	C_{27}	C_{29}	C_{31}
0-a-12	18.6	0.6	1.1	0.6	0.5	0.3	0.3	3.0	19.0	36.6	18.5	15.2	10.6	9.7
0-b	8.4	2.7	3.3	2.1	1.5	0.8	0.6	10.3	25.8	31.6	20.7	14.3	7.5	5.4
0-c	7.4	2.7	2.0	1.3	1.1	0.6	0.6	7.8	35.1	26.2	16.5	14.4	7.8	7.2
0-b-1	3.3	6.5	4.3	1.9	1.2	0.6	0.0	14.6	44.9	29.3	13.1	8.3	4.4	0.0
0-b-2	12.5	1.6	2.9	1.5	0.8	0.5	0.5	7.4	22.2	39.6	20.5	11.3	6.4	6.5
5-a-12	3.2	4.1	5.8	3.9	1.9	1.0	1.0	16.6	24.3	34.7	23.4	11.3	6.2	5.9
5-b	4.7	1.7	5.0	2.5	0.9	0.7	0.4	10.7	15.9	46.3	23.2	8.5	6.1	4.2
5-c	3.3	6.8	7.1	3.4	1.9	0.7	0.5	19.9	34.3	35.4	16.9	9.6	3.8	2.3
5-d	5.0	2.9	3.6	1.6	1.0	0.4	0.3	9.5	30.6	37.3	16.6	10.8	4.7	3.0
10-a-12	1.4	5.9	15.6	9.6	3.0	2.1	1.2	36.3	16.4	43.1	26.5	8.4	5.7	3.2
10-b-12	0.6	18.3	15.9	9.1	3.6	3.3	2.3	50.1	36.4	31.7	18.2	7.1	6.6	4.7
10-c-12	1.1	1.6	10.8	5.4	1.3	0.8	0.9	19.9	8.2	54.0	27.0	6.6	4.2	4.3
10-d	1.7	7.0	18.8	11.2	3.0	1.8	1.2	41.8	16.7	45.0	26.9	7.2	4.3	2.8
15-a-12	1.5	7.0	32.5	17.7	4.9	3.0	1.5	65.1	10.7	50.0	27.2	7.5	4.7	2.3

15-b-12	1.0		8.6	24.7	13.9	3.7	2.4	1.5	53.3	16.2	46.4	26.1	6.9	4.5	2.8
15-d-12	0.6		13.3	33.7	24.3	9.2	6.5	3.8	87.1	15.3	38.7	27.9	10.6	7.5	4.3
									Avg.	**23.2**	**39.1**	**21.8**	**9.9**	**5.9**	**4.3**
									St dev:	**10.4**	**7.8**	**4.8**	**2.8**	**1.8**	**2.3**

Table S7: δD-values for *Potamogeton pectinatus* from Lake Stechlin, as well as δD values (‰) of source water (lw, lake water; pw, pore water) and isotopic fractionation factor (ε) of lw vs. target compounds *n*-$C_{23,}$ *n*-C_{25} and *n*-C_{27} (n.d.., no data; s.m., single measurement; STD, standard deviation of duplicate measurements).

Sample	δD C_{23}	STD	δD C_{25}	STD	δD C_{27}	STD	δD C_{29}	STD	δD C_{31}	STD	δD lw	δD pw	ε lw/C_{23}	ε lw/C_{25}	ε lw/C_{27}	ε lw/C_{29}
GW1 a	-158	3	-154	3	-153	5	-145	1	n.d.	s.m.	-26.7	-63.0	-135	-131	-130	-122
GW1 b	-168	2	-169	1	-160	2	-136	s.m.	n.d.	s.m.	-26.7	-63.0	-145	-146	-137	-112
GW1 c	-150	s.m.	-155	s.m.	-148	s.m.	-143	s.m.	n.d.	s.m.	-26.7	-63.0	-126	-132	-124	-120
GW2 a	-166	7	-164	3	-159	0	-150	2	-152	6	-26.7	-60.6	-144	-141	-136	-127
GW2 b	-159	s.m.	-158	2	-146	4	-136	s.m.	n.d.	s.m.	-26.7	-60.6	-136	-135	-123	-112
C1 a	-167	1	-161	2	-158	1	-154	1	-151	2	-26.7	-28.6	-144	-138	-135	-131
C1 b	-146	1	-149	1	-149	1	-144	0	n.d.	s.m.	-26.7	-28.6	-122	-126	-126	-121
C1 c	-168	4	-164	1	-159	2	-149	0	-142	4	-26.7	-28.6	-146	-142	-136	-126
												Avg.	**-137**	**-136**	**-131**	**-121**
												St dev:	**9**	**7**	**6**	**7**

Table S8: Plant samples from the lab experiment measured for δD-values of *n*-alkanes. Plants grown in one beaker (1 and 2) were in some cases combined for IRMS-analysis (12) to gain a strong enough signal for reliable isotope measurement. Standard deviation, (STD) from duplicate measurements; ε values calculated vs. a constant δD value of water of -54.1‰; EC, measured electrical conductivity; s.m., single measurement; n.d., no data.

Salinity	EC (mS/cm)	pH	Sample	Plant length (cm)	Dry weight (mg)	Sample IRMS	δD C_{23} (‰)	STD	δD C_{25} (‰)	STD	ε C_{23} (‰)	ε C_{25} (‰)
0	0.6	8.4	0-a-1	26.7	12.7	0-a-12	-188	2	-175	s.m.	-141	-128
0			0-a-2	16.0	5.9							
0	0.6	8.3	0-b	32.5	8.4	0-b	-190	9	n.d.		-144	
0	0.6	8.2	0-c	34.0	7.4	0-c	-172	s.m.	n.d.		-125	
0	0.6	8.3	0-d-1	8.1	3.3	0-d-1	-169	4	n.d.		-121	
0			0-d-2	22.0	12.5	0-d-2	-187	0	-177	0	-141	-130
5	10.0	8.2	5-a-1	6.1	2.0	5-a-12	-174	s.m.	n.d.		-126	
5			5-a-2	4.5	1.2							
5	10.0	8.4	5-b	20.7	4.7	5-b	-201	0	-190	6	-155	-144
5	10.0	8.3	5-c	13.0	3.3	5-c	-180	s.m.	n.d.		-133	
5	10.0	8.3	5-d	10.2	5.0	5-d	-164	s.m.	n.d.		-116	
10	18.4	8.1	10-a-1	3.5	0.7	10-a-12	-207	4	-203	s.m.	-162	-157
10			10-a-2	2.7	0.7							
10	18.3	8.1	10-b-1	4.2	0.3	10-b-12	n.d.		n.d.			
10			10-b-2	3.1	0.2							
10	18.3	8.1	10-c-1	4.0	1.1	10-c-12	-220	8	-216	11	-175	-171
10			10-c-2	3.1	1.0							
10	18.4	8.1	10-d	4.3	1.7	10-d	-197	9	n.d.		-151	
15	25.9	8.1	15-a-1	3.7	0.6	15-a-12	-227	16	-206	s.m.	-182	-160
15			15-a-2	3.0	0.9							
15	25.9	8.0	15-b-1	3.5	0.7	15-b-12	-207	s.m.	n.d.		-161	
15			15-b-2	2.2	0.3							
15	25.8	8.1	15-c	3.6	1.2	15-c	n.d.		n.d.			
15	25.8	8.1	15-d-1	3.0	0.4	15-d-12	n.d.		n.d.			
15			15-d-2	3.0	0.2							

Fig. S3: δD-values of plant samples grown in the laboratory experiment plotted vs. plant length. Error bars indicate standard deviation of replicate measurements. Error bars combine the standard deviation of replicate measurements and the root means square error of the A5-standard-mix (3.96‰).