# Testing the D/H ratio of alkenones and palmitic acid as salinity proxies in the Amazon Plume

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## Abstract

The stable hydrogen isotope composition of lipid biomarkers, such as alkenones, is a promising new tool for the improvement of paleosalinity reconstructions. Laboratory studies confirmed the correlation between lipid biomarker δD composition ($\delta D_{Lipid}$), water δD composition ($\delta D_{H_2O}$) and salinity. Yet, there is limited insight into the applicability of this proxy in oceanic environments. To fill this gap, we test the use of the δD composition of alkenones ($\delta D_{C37}$) and palmitic acid ($\delta D_{PA}$) as salinity proxies using samples of surface suspended material along the distinct salinity gradient induced by the Amazon Plume. Our results indicate a positive correlation between salinity and $\delta D_{H_2O}$, while the relationship between $\delta D_{H_2O}$ and $\delta D_{Lipid}$ is more complex: $\delta D_{PA}$ correlates strongly with $\delta D_{H_2O}$ ($r^2$=0.81) and shows a salinity dependent isotopic fractionation factor. $\delta D_{C37}$ only correlates with $\delta D_{H_2O}$ in a small number (n=8) of samples with alkenone concentrations >10 ng $L^{-1}$, while there is no correlation if all samples are taken into account. These findings are mirrored by alkenone based temperature reconstructions, which are inaccurate for samples with low alkenone concentrations. Deviations in $\delta D_{C37}$ and temperature are likely to be caused by limited haptophyte algae growth due to low salinity and light limitation imposed by the Amazon Plume. Our study confirms the applicability of $\delta D_{Lipid}$ as a salinity proxy in oceanic environments. But it raises a note of caution concerning regions where low alkenone production can be expected due to low salinity and light limitation, for instance, under strong riverine discharge.

# 1 Introduction

The precise reconstruction of past ocean salinity is still a pending issue in paleoclimatology (Rohling, 2007). Until recently, most paleosalinity studies have relied on foraminifera based reconstructions of the stable oxygen isotope composition of seawater, which correlates with salinity (Epstein and Mayeda, 1953). However, temperature also controls the oxygen isotope composition of foraminifera, making corrections in the estimation of paleosalinity necessary (Lea et al., 2000;Rostek et al., 1993). The imprecision associated with this approach has led to the search for alternative salinity proxies. The use of the hydrogen isotopic composition of algal lipids ($\delta D_{Lipid}$) for the reconstruction of the stable hydrogen composition of water ($\delta D_{H_2O}$) is one of such recent developments (Sessions et al., 1999;Schouten et al., 2006). As outlined in a theoretical framework by Rohling (2007), this method has the potential to lead to more precise reconstructions of surface water salinity in combination with foraminifera based $\delta^{18}O$.

So far, efforts to apply $\delta D_{Lipid}$ as a salinity proxy have mainly involved the use of long-chain alkenones. Long-chain alkenones have the advantage of being exclusively produced by specific haptophyte algae, and of showing good preservation over geologic timescales (Marlowe et al., 1984;Marlowe et al., 1990). Laboratory studies have confirmed the correlation of the D/H ratio of the $C_{37}$ alkenones ($\delta D_{C37}$) with $\delta D_{H_2O}$ (Englebrecht and Sachs, 2005;Schouten et al., 2006). Furthermore, the D/H fractionation factor between alkenones and water ($\alpha_{C_{37}}$)

$$\alpha_{C37} = \frac{\delta D_{C37} + 1000}{\delta D_{H_2O} + 1000} \qquad (1)$$

was found to be salinity dependent, leading to a potentially twofold way to reconstruct salinity (Schouten et al., 2006). There are, however, potential factors that may compromise the use of $\delta D_{C37}$ and $\alpha_{C_{37}}$ as salinity proxies. $\alpha_{C_{37}}$ is, for instance, inconsistent among different haptophyte algae species. Species preferring shelf environments have a higher $\alpha_{C_{37}}$ than species favoring open marine habitats (M'Boule et al., 2014). In some situations $\alpha_{C_{37}}$ has shown a small temperature dependency (Zhang and Sachs, 2007). Furthermore, $\alpha_{C_{37}}$ is also dependent on algal growth phase and rate (Schouten et al., 2006;Wolhowe et al., 2009;Chivall et al., 2014b). All these factors potentially exceed the effects of salinity and may impede the use of $\delta D_{C37}$ as a paleosalinity proxy. Nevertheless, paleoclimate studies have made successful use of $\delta D_{C37}$ as a paleosalinity proxy (van der Meer et al., 2008;Giosan et al., 2012;Schmidt et al., 2014;Pahnke et al., 2007;van der Meer et al., 2007). However, in some cases, factors like species variability complicated $\delta D_{C37}$ based salinity reconstructions (Kasper et al., 2015).

Apart from alkenones there is a variety of other algal lipids which feature a distinct $\delta D_{H2O} - \delta D_{Lipid}$ relationship (Zhang et al., 2009;Sauer et al., 2001;Nelson and Sachs, 2014). Among these less frequently used compounds is palmitic acid. Palmitic acid is a saturated fatty acid, which is highly abundant in most aquatic environments. The infrequent use of palmitic acid is mainly due to its ubiquitous occurrence, which does not allow linkage to a single group of producing species. Furthermore, palmitic acid is less resistant to degradation than alkenones (Sun and Wakeham, 1994). Nevertheless, δD of palmitic acid ($\delta D_{PA}$) has been successfully used as a paleoclimate indicator in several studies (Huang et al., 2002;Smittenberg et al., 2011;Shuman et al., 2006).

Although there are numerous laboratory and paleoclimate studies confirming the applicability of $\delta D_{Lipid}$ to reconstruct the past isotopic composition of water, there have been only few calibration studies in oceanic environments (Schwab and Sachs, 2011;Schwab and Sachs, 2009;Wolhowe et al., 2015). To fill this gap, we analyzed $\delta D_{C37}$ and $\delta D_{PA}$ of suspended particle samples along the salinity gradient induced by the Amazon freshwater plume and tested their applicability as salinity proxies (Fig. 1). Along with the hydrogen isotope analyses, we also tested the accuracy of the $U_{37}^{k'}$ temperature proxy (Müller et al., 1998) under the influence of the Amazon Plume. Potential impact of haptophyte species variability was monitored using the $C_{37}/C_{38}$ ratio (Rosell-Mele et al., 1994), as defined below.

$$C_{37}/C_{38} = \frac{C_{37:3}Me + C_{37:2}Me}{C_{38:3}Et + C_{38:3}Me + C_{38:2}Et + C_{38:2}Me} \qquad (2)$$

# 2 Methods

## 2.1 Study area

The study area is situated offshore northern Brazil and French Guyana close to the Amazon estuary (Fig 1). A large portion of the research area is influenced by freshwater outflow from the Amazon River, which induces a steep salinity gradient (Lentz and Limeburner, 1995). The freshwater plume is generally transported northwestwards by the North Brazil Current along the coastline of northern Brazil and French Guyana, while areas to the southeast of the Amazon River Estuary are largely unaffected by the Amazon freshwater discharge (Geyer et al., 1996). The geometry and transport of the freshwater plume are subject to large seasonal variations. The plume reaches its maximum extent during peak Amazon discharge in boreal summer (Molleri et al., 2010), while its northwestward transport is controlled by wind-stress along the shelf (Geyer et al., 1996).

## 2.2 Sampling

Sampling was conducted during the RV *Maria S. Merian* cruise MSM20/3 from February 21th to March 9th 2012 (Mulitza et al.). Samples of suspended particles were collected along a southeast to northwest transect off northeastern South America across the Amazon Plume (Fig. 1). Samples were taken via the ships seawater inlet at about 6 meters below sea level operated by a diaphragm pump. Between 100 and 500 litres of water were filtered over a period of 30 to 150 minutes on pre-combusted GFF filters. After sampling, filters were wrapped in pre-combusted aluminium foil and stored at -20°C. Along with the suspended particle samples, water samples were collected at the beginning and at the end of each filtering period. Water samples were sealed with wax and stored at 4°C before analysis. On-board salinity and temperature measurements were conducted in one second intervals by a SeaBird Electronics SBE 45 Micro thermosalinograph (accuracy 0.002°C and 0.005 psu).

## 2.3 Stable isotope analysis of water

The stable hydrogen isotope composition of seawater samples was determined at MARUM – Center for Marine Environmental Sciences, University of Bremen, with a Thermal-Conversion/Elemental-Analyser operated at 1400°C coupled to a ThermoFisher Scientific MAT 253 mass-spectrometer. Measurements were repeated ten times for each seawater sample. Four in-house water standards used for calibration were calibrated against IAEA standards VSMOW, GISP and SLAP. The maximum deviation from the calibration slope was 1.6 ‰ vs. VSMOW and the average deviation was 0.7 ‰ vs. VSMOW.

## 2.4 Lipid analysis

Suspended particle samples were freeze-dried in a Christ Alpha 1-4 freeze-dryer. Lipids were extracted in a DIONEX Accelerated Solvent Extractor (ASE 200) using a dichloromethane

(DCM): methanol (MeOH) 9 : 1 solution at 1000 psi and 100 °C for three cycles lasting 5 minutes each. Prior to extraction 2-Nonadecanone and erucic acid were added as internal standards for the ketone and acid fractions, respectively. After extraction, samples were dried in a Heidolph ROTOVAP system. The extracts were saponified using 0.1 M KOH in MeOH, yielding neutral and acid fractions. The neutral fraction was separated in three fractions using activated silica gel chromatography (1% $H_2O$). The first fraction was eluted with hexane, yielding saturated and unsaturated hydrocarbons. The second fraction was eluted with (DCM), yielding ketones, including alkenones. The third fraction was eluted with DCM:MeOH 1:1, yielding polar compounds. The acid fraction was methylized with MeOH of known isotopic composition (-156 ± 2 ‰ vs. VSMOW), yielding the corresponding fatty acid methyl esters (FAMEs). The FAMEs were subsequently cleaned over pipet columns containing two centimeters of silica. In order to remove unsaturated compounds, further cleaning over columns of two centimeters of $AgNO_3$ was conducted. Ketones and FAMEs were analyzed using a ThermoFisher Scientific Focus gas chromatograph equipped with an Rxi-5ms 30x column (30 m, 0.25 mm, 0.25 μm) and a flame ionization detector. Compounds were quantified by comparing the integrated peak areas of the compounds to external standard solutions. Precision of compound quantification is about 5% and precision of $U_{37}^{k'}$ reconstructions is 0.38°C based on multiple standard analyses. Compound-specific isotope analyses was carried out on a ThermoFisher Scientific MAT 253 Isotope Ratio Mass Spectrometer coupled via a GC Isolink operated at 1420°C to a ThermoFisher Scientific Trace GC equipped with a HP-5ms column (30 m, 0.25 mm, 1 μm). For each sample duplicate injections of $C_{37}$ and palmitic acid were conducted. Measurement accuracy was controlled by *n*-alkane standards of known isotopic composition every six measurements and by the daily determination of the $H^+_3$ factor using $H_2$ as reference gas. $H^+_3$ factors varied between 5.6 and 6.2, while the mean absolute deviation of external standards was 2.2‰. In order to prevent a bias introduced by variable alkenone distribution, the δD of alkenones was analyzed for $C_{37:2}$ and $C_{37:3}$ together rather than separately (van der Meer et al., 2013). δD values for palmitic acid were corrected for the methyl group added during methylation.

## 3 Results

Onboard sea surface temperature measurements resulted in uniform values of 28.5 ± 0.5 °C, while salinity varied between 10 and 36 psu (Fig. 1; Table 1). The hydrogen isotope analyses of seawater samples yielded δD values between 6 and -15 ‰ (all isotope values are given vs.

VSMOW). The values correlated linearly with sea surface salinity (Fig. 2a). The suspended particle samples yielded $C_{37}$ alkenone concentrations between 0.2-65.3 ng $L^{-1}$ (Table 1). Samples with a salinity >25 psu showed variable concentrations (0.2-65.3 ng $L^{-1}$), while samples with a salinity <25 psu had concentrations consistently lower than 10 ng $L^{-1}$. There were little to no alkenones (concentration <1 ng $L^{-1}$) in filter samples with a salinity <15 psu (Fig. 2c, Table 1). The fatty acid analysis yielded almost exclusively short chain compounds, of which palmitic acid had concentrations between 1.4 and 27 μg $L^{-1}$ (Fig. 2d). Variations in palmitic acid concentrations showed a weak inverse correlation with salinity (Fig. 2d). For samples with alkenone concentrations >10 ng $L^{-1}$, sea surface temperature reconstructions agreed within the calibration error of 1.5°C with onboard temperature measurements (Fig. 2b, Table 1). Samples with a concentration <10 ng $L^{-1}$ featured a larger scatter with deviations from onboard measurements of up to 10°C (Fig. 2b). The ratio of the $C_{37}/C_{38}$ alkenones resulted in values between 0.9 and 1.7 (Table 1), indicating the prevalence of open ocean haptophyte contribution throughout the transect (Rosell-Mele et al., 1994). The $C_{37:4}$ alkenone, sometimes used as a salinity proxy, was not present in our samples.

Due to the absence of alkenones in the low salinity samples, isotope analysis of the $C_{37}$ alkenone was only possible in samples with a salinity > 15 psu. For these samples, $\delta D_{C37}$ varied between -176 ‰ and -205 ‰ (Fig. 3a, Table 1). When all samples are taken into account, $\delta D_{C37}$ and $\delta D_{H_2O}$ do not correlate (Fig. 3a). If only the samples with an alkenone concentration >10 ng $L^{-1}$ were considered, linear regression yielded a correlation between $\delta D_{C37}$ and $\delta D_{H2O}$ with a slope of 1.36 ‰ $\delta D_{C37}$ per 1‰ $\delta D_{H2O}$ ($r^2 = 0.51$, $p < 0.05$; Fig. 3a). $\alpha_{C_{37}}$ varied between 0.79 and 0.84 and showed no significant salinity dependence (Fig. 3c). In contrast to $\delta D_{C37}$, $\delta D_{PA}$ strongly correlates with $\delta D_{H_2O}$, regardless of lipid concentration ($r^2 = 0.81$, $p < 10^{-7}$; Fig. 3b). The slope of the linear regression is 1.72 ‰ $\delta D_{PA}$ per 1 ‰ $\delta D_{H2O}$. The fractionation factor between palmitic acid and water ($\alpha_{PA}$) yielded values between 0.79 and 0.83, featuring a significant salinity dependency with an increase of 0.001 per salinity unit (Fig. 3d).

# 4 Discussion

## 4.1 Lipid sources

### 4.1.1 Alkenone sources

The $C_{37}/C_{38}$ ratio was used for the assessment of the dominant alkenone source (Conte et al., 1998). Open marine species like *Emiliana huxleyi* and *Gephyrocapsa oceanica* produce alkenones with a $C_{37}/C_{38}$ between 0.5 and 1.5 (Conte et al., 1998). Coastal species like *Isochrysis galbana* and *Chrysotila lamellosa* produce alkenones with a $C_{37}/C_{38}$ ratio >2, sometimes even >10 (M'Boule et al., 2014;Prahl et al., 1988;Marlowe et al., 1984). The $C_{37}/C_{38}$ ratio of the samples from the Amazon Plume varied between 0.9 and 1.7 and alkenone production was therefore likely dominated by open marine species (Conte et al., 1998). Since some of the samples feature values at the upper limit for open marine species, some (probably small) contribution by coastal haptophytes cannot be ruled out (Kasper et al., 2015). Alternatively, the small variations in the $C_{37}/C_{38}$ ratio could also be the effect of species variability within open marine haptophytes (Conte et al., 1998). In contrast to previous laboratory and field studies (Ono et al., 2009;Chu et al., 2005), we do not find a correlation between salinity and the $C_{37}/C_{38}$ ratio (not shown here).

### 4.1.2 Palmitic acid sources

Palmitic acids are not exclusively produced by aqueous organisms and are also synthesized by terrestrial plants and bacteria (Eglinton and Eglinton, 2008). Unlike aqueous organisms, terrestrial plants also synthesize long-chain fatty acids (Eglinton and Hamilton, 1967), which were not present in the filter samples. This indicates that the palmitic acids found in the Amazon Plume are exclusively produced by aquatic organisms. Also, the fast turnover rates of palmitic acid makes a contribution by riverine compounds unlikely. Furthermore, previous studies have generally confirmed that palmitic acids in marine environments are predominantly produced by marine algae (Pearson et al., 2001).

## 4.2 Temperature reconstruction

Oceanic temperature reconstructions based on alkenones are a widely used tool in paleoclimatology (Bard et al., 1997;Rühlemann et al., 1999). The global calibrations in use are based on open marine haptophyte species (Prahl and Wakeham, 1987;Müller et al., 1998).

Our reconstructed temperatures show deviations of up to 10°C from instrumentally measured temperature for samples with alkenone concentration <10 ng $L^{-1}$ (Fig. 2b). These anomalous, generally lower than expected values, could be caused by different processes. First, coastal species bear a temperature-$U_{37}^{k'}$ relationship with a markedly lower slope than open marine species (Sun et al., 2007;Versteegh et al., 2001). Hence, a larger alkenone contribution by coastal haptophyte species would lead to the observed lower temperatures. Second, lower salinity is reported to cause metabolic stress in alkenone producers leading to anomalous reconstructed temperatures (Harada et al., 2003). Third, variations in haptophyte growth rate due to nutrient or light limitation could also lead to variations in reconstructed temperatures (Epstein et al., 1998;Versteegh et al., 2001). The latter two points would also lead to lower alkenone concentrations and thus enhance the possibility of overprint by advection of allochthonous alkenones.

Variations in haptophyte algae composition recorded by changes in the $C_{37}/C_{38}$ ratio do not show a correlation with the residue

$$T_{residue} = T_{measured} - T_{reconstructed} \tag{3}$$

of the temperature reconstruction (not shown here). Hence, variations in species composition are likely insufficient to account for the $T_{residue}$. Conversely, there is a correlation between $T_{residue}$ and salinity (Fig. 4a). Salinity might therefore be an important cause for the large $T_{residue}$ (Harada et al., 2003). The riverine waters of the Amazon Plume are generally nutrient rich (Santos et al., 2008), which makes a scenario of nutrient limitation unlikely to impact temperature control of $U_{37}^{k'}$ in our study area. The high sediment load delivered by the Amazon River, however, leads to light limitation in the study area (Smith and Demaster, 1996). Light limitation is indeed reported to lower reconstructed $U_{37}^{k'}$ temperatures by up to 7°C (Versteegh et al., 2001). Since diminished alkenone production due to low salinity and light limitation would lead to smaller alkenone concentrations, this would also explain why high concentration samples feature no temperature deviation (Fig. 4b). The advection of allochthonous alkenones biasing temperature reconstructions has been suggested in other studies (Rühlemann and Butzin, 2006;Benthien and Müller, 2000). In our samples, $U_{37}^{k'}$ overprint by advected alkenones can be considered less likely, since there are no nearby areas where alkenones with a lower temperature signal could originate from.

In conclusion, there are multiple potential factors influencing the $U_{37}^{k'}$ deviation in the Amazon Plume. Given that low alkenone concentrations are consistently associated with large negative temperature deviations, reduced alkenone production due to low salinity and light limitation in the Amazon Plume might be the most important factor for the temperature deviations (Fig. 4a, b) (Versteegh et al., 2001;Harada et al., 2003).

## 4.3 Stable hydrogen isotope signals

### 4.3.1 Alkenone δD

If all samples are considered, there is no correlation between $\delta D_{C37}$ and $\delta D_{H_2O}$ (Fig. 3a). Given the relationship between $C_{37}$ concentration, $T_{residue}$ and salinity (Fig. 4a, b), we also tested whether there would be a better fit between $\delta D_{C37}$ and $\delta D_{H_2O}$ for high $C_{37}$ concentration samples. There is indeed a correlation between $\delta D_{C37}$ and $\delta D_{H2O}$ for samples with a $C_{37}$ concentration >10 ng $L^{-1}$ (Fig. 3a). However, with a p-value of 0.05 and a low sample number of n=8, this relationship has to be viewed with caution. Nevertheless, we consider it to be an important information to study the potential factors leading to the deviation between $\delta D_{C37}$ and $\delta D_{H_2O}$. Especially, since this relation reflects a generally constant $\alpha_{C37}$ of 0.81 and agrees with results obtained for open marine species cultured at different salinities (M'Boule et al., 2014). For a potential impact on $\delta D_{C37}$, factors similar to those considered for the temperature deviations have to be scrutinized: synthesis by coastal haptophyte species (M'Boule et al., 2014), changes in growth rate and phase (Schouten et al., 2006;Wolhowe et al., 2009), overprint by advected material and variations in salinity (Schouten et al., 2006). Since temperature is more or less uniform over the entire study area, a temperature effect as reported by Zhang and Sachs (2007) is not expected to play a role.

As previously mentioned, variations in the $C_{37}/C_{38}$ ratio imply only limited variation in haptophyte species composition. Moreover, the values of $\alpha_{C37}$ are between 0.795 and 0.835 and are only slightly higher than observed in laboratory experiments studying open marine haptophytes (Schouten et al., 2006), but are markedly lower than observed for coastal haptophytes (M'Boule et al., 2014). This again suggests that the studied alkenones are predominantly of open marine haptophyte origin. Although there are no signs for a full scale change from open marine to coastal haptophytes, the variability in habitat preference may still be sufficient to have a significant influence on $\alpha_{C37}$. The $C_{37}/C_{38}$ variability found in a sediment core collected offshore Mozambique by Kasper et al. (2015) was similar to the one found in our samples and the associated species variability was likely large enough to significantly influence δ$D_{C37}$. In our samples, the $C_{37}$/$C_{38}$ ratio does however not correlate with $\alpha_{C37}$ and species variations alone are therefore unlikely to be the dominant cause for the absent correlation between δ$D_{C37}$ and δ$D_{H2O}$ in low salinity samples. In contrast to laboratory studies (Schouten et al., 2006), we find no clear relationship between salinity and fractionation factor (Fig. 3c). The absence of a salinity-$\alpha_{C37}$ relationship was also reported in a field study by Schwab and Sachs (2011) who explained their findings by the presence of additional factors such as species variability and temperature, which may have counteracted the effects of salinity. If the relation between δ$D_{C37}$ and δ$D_{H2O}$ for high concentration samples is used to calculate the residue for each sample,

$$\delta D_{res\ C37} = \delta D_{C37} - \left(1.358 \times \delta D_{H_2O} - 194.558\right) \qquad (4)$$

it becomes apparent that low concentration samples have higher residuals (Fig. 4d). Furthermore, δ$D_{res\ C37}$ correlates with salinity, which indicates that δ$D_{res\ C37}$ is largely influenced by the input of low salinity Amazon freshwater (Fig. 4c). This observation would also fit with the assumption that the lower $C_{37}$ concentration in those samples were a result of lower growth rate, because lower growth rate leads to a higher fractionation factor (M'Boule et al., 2014;Schouten et al., 2006;Sachse and Sachs, 2008). Since the steep salinity gradient of the Amazon Plume leads to a wide range of surface water isotopic composition over a short geographic distance, we cannot exclude some influence of advected alkenones in samples with low or absent in situ alkenone production. As this effect is insufficient to explain the large $T_{residue}$, advection is likely not the main factor responsible for the absence of a correlation between δ$D_{C37}$ and δ$D_{H_2O}$. Although the deviation in δ$D_{C37}$ cannot be tied to a single factor, low alkenone production associated with the low salinity, suspension rich Amazon waters is likely the most important factor (Wolhowe et al., 2015). Thus, the temperature- and δ$D_{C37}$ deviations are likely caused by similar effects (Fig. 4a-d).

### 4.3.2 Palmitic acid δD

In contrast to δ$D_{C37}$, δ$D_{PA}$ correlates well with δ$D_{H2O}$ (Fig. 3b). Furthermore, $\alpha_{PA}$ correlates with salinity (Fig. 3d) and thus confirms the relationship between salinity and α observed in various laboratory and field studies for palmitic acid and other lipids (Schouten et al., 2006;M'Boule et al., 2014;Chivall et al., 2014a). Our findings imply that the limiting factors potentially leading to variations in $\alpha_{C37}$ do not influence $\alpha_{PA}$. The factors that could potentially influence $\delta D_{PA}$ are largely similar to those influencing $\delta D_{C37}$ (Chivall et al., 2014a). Unlike for alkenones there is, however, no clear evidence for a growth rate dependence of $\alpha_{PA}$ (Zhang et al. 2009).

One striking difference between palmitic acid and alkenones in our samples is the different abundance of the two compounds. Palmitic acid concentrations were about three orders of magnitude higher than alkenone concentrations (Fig. 2c, d). This is unsurprising, since palmitic acid is typically very abundant in marine environments (Pearson et al., 2001). In further contrast to the $C_{37}$ concentration, the palmitic acid concentration was not lower in low salinity samples, but featured a trend towards higher concentrations. This indicates that palmitic acid producing organisms were not negatively affected by the low salinity, sediment rich Amazon input like haptophyte algae, but rather benefited from the high nutrient supply by the Amazon (Santos et al., 2008). This marked difference supports the notion that low alkenone production rates in parts of the study area were responsible for the $\alpha_{C37}$ deviations. Furthermore, the high palmitic acid concentrations also limit the influence of a possible overprint of the in situ signal by allochthonous compounds. Apart from that, the high turnover rate of palmitic acid may further impede the influence of allochthonous compounds. This is also in contrast to alkenones, which are comparably stable towards degradation (Sun and Wakeham 1994). Therefore, the lower turnover rate of alkenones renders these compounds more susceptible to overprint by older, allochthonous compounds.

Our study shows that $\alpha_{PA}$ remains relatively stable over a range of varying environmental conditions. This finding is similar to one reached by studies along a lake transect from Southern Canada to Florida, which found a good agreement between $\delta D_{PA}$ and $\delta D_{H2O}$ over a variety of ecological environments (Huang et al., 2004, 2002). The $\alpha_{PA}$ of 0.82 observed in those studies is also in the range of $\alpha_{PA}$ observed in the Amazon Plume (0.79-0.83). This further indicates that species composition and other factors are not influencing $\alpha_{PA}$ to a large extent on an ecosystem level. Potential variations of $\alpha_{PA}$ from different contributors are either small or levelled out by integration over ecosystems. A surprising constancy in $\delta D_{PA}$ has also been observed in a sediment core from the Santa Barbara Basin (Li et al., 2009). There, the $\delta D_{PA}$ remained constant even in the presence of heterotrophic palmitic acid producers. This could indicate that the constancy in $\alpha_{PA}$ is not only limited to phototrophic organisms as observed here and by Huang et al. (2004), but also extends to heterotrophic organisms. The constancy could be caused by the very similar biosynthetic pathway for palmitic acid in bacteria and eukaryotes (Li et al., 2009).

Although there are multiple lacustrine studies successfully applying $\delta D_{PA}$ as paleoenvironmental proxy (Smittenberg et al., 2011;Shuman et al., 2006) and $\delta D_{PA}$ faithfully records $\delta D_{H2O}$ in our study, there are still multiple factors that could overprint a surface $\delta D_{PA}$ signal. Especially in open oceanic environments, palmitic acid production deeper in the water column could alter the signal recorded at the surface. After deposition, bacterial activity in the sediment could also overprint the original upper water column signal (Perry et al., 1979).

## 5 Conclusions

Our study shows that $\delta D_{PA}$ in suspended particle samples from the Amazon Plume salinity gradient records variations in salinity. For $\delta D_{C37}$, this correlation is only present in samples above a $C_{37}$ concentration of 10 ng $L^{-1}$. The low alkenone concentrations are likely caused by the sediment-rich freshwater input of the Amazon River impeding haptophyte growth and affecting $\alpha_{C37}$. Hence, the ubiquitous nature of palmitic acid proved to be highly beneficial in the study area. Moreover, palmitic acid bears the advantage of easier isotopic measurement and a high availability in most environments. The use of $\delta D_{PA}$ as a standalone salinity proxy has to be considered with caution. Potential disadvantages of palmitic acid include post depositional degradation, compound synthesis deeper in the water column, which may not record surface conditions and the bacterial overprint in the sediment. A possible way to circumvent these limitations, as well as the problems encountered for $\delta D_{C37}$, could be the alongside use of $\delta D_{PA}$ and $\delta D_{37}$. $\delta D_{PA}$ is not sensitive to the low concentration issues encountered in this study, while $\delta D_{C37}$ is only produced in surface waters and not susceptible to synthesis or degradation deeper in the water column or sediments. Therefore, the combined study of compound-specific hydrogen isotope composition of more than one compound could yield important information on influences in $\delta D_{Lipid}$ other than salinity.

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Table 1. Average geographic position, average measured sea surface temperature (SST), average sea surface salinity (SSS), $C_{37}$ concentration, palmitic acid (PA) concentration, $U_{37}^{k'}$, $C_{37}/C_{38}$ ratio, δD of water ($\delta D_{H_2O}$), δD of $C_{37}$ ($\delta D_{C37}$) and δD of palmitic acid ($\delta D_{PA}$)for each sample. Values for salinity and temperature are the average of onboard measurements taken in one second intervals during each filtering period. Errors represent the standard deviation of these measurements. δD values of water represent the mean of two samples taken at the beginning and the end of each filtering period, each sample represents the mean of ten replicate injections. Errors represent the propagated standard deviation of these measurements. δD values of $C_{37}$ and palmitic acid are the means of duplicate measurements. Errors represent the range between the duplicate measurements.

| Sample | Lat | Long | SST (C°) | SSS (psu) | Conc. $C_{37}$ (ng $L^{-1}$) | Conc. PA (μg $L^{-1}$) | $U_{37}^{k'}$ | $C_{37}/C_{38}$ | $\delta D_{H_2O}$ | δD $C_{37}$ | δD PA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PP10 | 1.9035 | -48.4169 | 28.37 ± 0.03 | 36.2 ± 0.09 | 47.7 | 1.3 | 0.98 | 1.46 | 4.8 ± 0.9 | -190.1 ± 0.5 | -170.8 ± 1 |
| PP11 | 1.7587 | -48.2568 | 28.99 ± 0.04 | 34.72 ± 0.51 | 54.2 | N/A | 0.96 | 1.56 | 6.6 ± 1.2 | -189.2 ± 3.7 | N/A |
| PP12 | 1.7123 | -48.2975 | 29.28 ± 0.05 | 31.65 ± 1.1 | 65.3 | 6 | 0.95 | 1.45 | 2.3 ± 1.1 | -185.4 ± 2.2 | -183.5 ± 0.8 |
| PP13 | 1.6655 | -48.3388 | 29.31 ± 0.18 | 28.06 ± 1.2 | 20.6 | 16.6 | 0.96 | 1.47 | -2.6 ± 1.6 | -200.8 ± 1.9 | -193.2 ± 1.7 |
| PP14 | 1.6197 | -48.3791 | 29.17 ± 0.03 | 25.79 ± 0.51 | 5.7 | 12.3 | 0.94 | 1.42 | -4.1 ± 1.1 | -206.3 ± 1.3 | -197.5 ± 0.4 |
| PP15 | 1.5724 | -48.421 | 29.28 ± 0.05 | 22.86 ± 0.47 | 8.6 | 19.4 | 0.95 | 1.44 | -6.7 ± 1 | a | -205.4 ± 0.9 |
| PP16 | 1.5676 | -48.4632 | 29.23 ± 0.05 | 20.91 ± 0.47 | 1.4 | 13.9 | 0.89 | 1.33 | -9.2 ± 0.9 | a | -209.7 ± 0.6 |
| PP17 | 1.6199 | -48.5119 | 29.02 ± 0.07 | 20.55 ± 0.41 | 1.5 | 8.7 | 0.89 | 1.19 | -11.8 ± 1.4 | -176.9 ± 0.3 | -205.9 ± 0 |
| PP19 | 2.0306 | -48.759 | 28.67 ± 0.02 | 17.84 ± 0.55 | 3.8 | N/A | 0.71 | 2.52 | -14.5 ± 1.3 | a | N/A |
| PP20 | 2.0858 | -48.7282 | 28.73 ± 0.03 | 21.15 ± 1.38 | 2.6 | N/A | 0.81 | 1.08 | N/A | a | N/A |
| PP21 | 2.1431 | -48.6728 | 28.82 ± 0.02 | 26.22 ± 1.63 | 1.3 | N/A | 0.79 | 1.12 | N/A | a | N/A |
| PP22 | 2.1815 | -48.6369 | 28.82 ± 0.05 | 30.76 ± 1.2 | 2.8 | N/A | 0.91 | 1.44 | N/A | a | N/A |
| PP23 | 2.2205 | -48.6038 | 28.9 ± 0.02 | 33.25 ± 0.5 | 2.8 | N/A | 0.95 | 1.43 | N/A | a | N/A |
| PP24 | 2.259 | -48.6055 | 28.93 ± 0.02 | 33.89 ± 0.11 | 4.9 | N/A | 0.97 | 0.99 | 3.8 ± 0.9 | -191.8 ± 1.9 | N/A |
| PP25 | 2.3389 | -48.7336 | 28.84 ± 0.04 | 27.45 ± 1.27 | 5.1 | N/A | 0.87 | 0.92 | N/A | a | N/A |
| PP26 | 2.2984 | -48.7711 | 28.82 ± 0.03 | 23.96 ± 1.09 | 0.4 | N/A | 0.87 | 1.25 | N/A | a | N/A |
| PP27 | 2.2674 | -48.7995 | 28.71 ± 0.04 | 20.8 ± 0.71 | 0.4 | N/A | 0.65 | 0.98 | N/A | a | N/A |
| PP33 | 2.0652 | -48.5919 | 28.6 ± 0.04 | 17.44 ± 0.24 | 1.1 | N/A | 0.68 | 1.01 | N/A | a | N/A |
| PP34 | 1.9301 | -48.5528 | 28.63 ± 0.04 | 16.02 ± 0.12 | 6.6 | N/A | 0.78 | 1.27 | N/A | a | N/A |
| PP35 | 1.7071 | -48.4395 | 28.45 ± 0.04 | 18.21 ± 0.39 | 0.8 | N/A | 0.76 | 1.03 | N/A | a | N/A |
| PP36 | 1.6196 | -48.4013 | 28.55 ± 0.06 | 24.34 ± 0.4 | 2.2 | 16.5 | 0.85 | 1.17 | -9.1 ± 1.2 | a | -204.3 ± 0.2 |
| PP37 | 1.7662 | -48.4925 | 28.37 ± 0.03 | 17.63 ± 1.27 | 0.6 | N/A | 0.76 | 1.2 | N/A | a | N/A |
| PP38 | 2.0088 | -48.6108 | 28.35 ± 0.05 | 14.14 ± 0.76 | 0.7 | N/A | 0.64 | 1.02 | -17.4 ± 0.9 | a | N/A |
| PP40 | 2.8827 | -49.4089 | 28.73 ± 0.03 | 33.54 ± 0.06 | 4.0 | N/A | 0.81 | 0.99 | N/A | a | N/A |
| PP41 | 2.8566 | -49.3425 | 29.08 ± 0.06 | 29.34 ± 1.32 | 0.2 | 2.1 | 0.81 | 1.8 | 0.2 ± 0.9 | a | -188 ± 1.1 |
| PP42 | 2.8342 | -49.3151 | 29.04 ± 0.03 | 26.65 ± 1.52 | 0.2 | 2.0 | 0.86 | 1.25 | -2.2 ± 1.1 | a | -197.1 ± 0.7 |
| PP43 | 3.1391 | -49.3335 | 28.46 ± 0.04 | 36.16 ± 0.11 | 16.7 | 5.5 | 0.97 | 1.55 | 5.9 ± 1.3 | -180.3 ± 0.6 | -183.4 ± 0.8 |
| PP44 | 3.0999 | -49.3064 | 28.23 ± 0.03 | 34.89 ± 0.45 | 59.1 | N/A | 0.98 | 1.54 | 6.3 ± 1.1 | -189 ± 1.4 | N/A |
| PP45 | 3.0627 | -49.4272 | 28.51 ± 0.02 | 32.83 ± 0.8 | 33.3 | N/A | 0.98 | 1.63 | 4.1 ± 0.9 | -190.8 ± 0.4 | N/A |
| PP46 | 3.0911 | -49.4337 | 28.68 ± 0.04 | 33.1 ± 0.65 | 9.2 | N/A | 0.96 | 1.42 | N/A | a | N/A |
| PP47 | 3.0554 | -49.4321 | 28.49 ± 0.01 | 29.2 ± 0.08 | 6.1 | 16.4 | 0.96 | 1.29 | 0 ± 0.9 | -177.2 ± 1.4 | -201.6 ± 0.7 |
| PP48 | 2.915 | -49.3347 | 28.03 ± 0.02 | 23.42 ± 0.27 | 7.7 | 7.2 | 0.88 | 1.14 | -9.2 ± 1.4 | -197.9 ± 0.5 | -202.3 ± 1.6 |
| PP49 | 2.8972 | -49.4713 | 28.07 ± 0.03 | 21.86 ± 0.46 | 1.3 | 16.2 | 0.89 | 1.23 | -8.4 ± 1 | a | -211.7 ± 0.3 |
| PP51 | 3.1025 | -49.7931 | 28.3 ± 0.06 | 18.31 ± 0.21 | 2.2 | N/A | 0.74 | 1.04 | N/A | a | N/A |
| PP52 | 3.098 | -49.6761 | 28.68 ± 0.03 | 24.91 ± 0.16 | 0.6 | 27.0 | 0.86 | 1.23 | -10 ± 1.3 | a | -204.9 ± 1.6 |
| PP53 | 3.5031 | -50.1667 | 28.25 ± 0.08 | 20.33 ± 1.93 | 1.0 | N/A | 0.85 | 1.38 | N/A | a | N/A |
| PP54 | 3.5576 | -50.3623 | 28.2 ± 0.1 | 18.63 ± 0.6 | 0.3 | 11.9 | 0.82 | 1.05 | N/A | a | N/A |
| PP55 | 3.9688 | -50.5373 | 28.27 ± 0.16 | 16.94 ± 1.38 | 0.7 | N/A | 0.75 | 1.04 | -16 ± 0.8 | a | N/A |
| PP57 | 4.4874 | -51.2401 | 28.04 ± 0.05 | 15.88 ± 0.09 | 0.1 | 17.7 | 0.82 | b | -18.2 ± 0.7 | a | -220.3 ± 0.8 |
| PP60 | 6.1499 | -51.2679 | 28.09 ± 0.03 | 36.16 ± 0.01 | 2.0 | 2.7 | 0.99 | b | 5.8 ± 0.8 | -183.2 ± 1.2 | -182.4 ± 0.6 |
| PP61 | 5.5698 | -51.8561 | 27.93 ± 0.09 | 32.19 ± 1.28 | 23.4 | N/A | 0.98 | 1.11 | 2.1 ± 1.3 | -191.1 ± 2.7 | N/A |
| PP62 | 5.3201 | -51.9255 | 27.9 ± 0.04 | 22.72 ± 1.32 | 3.4 | 23.2 | 0.97 | 1.1 | -8.3 ± 0.9 | -192 ± 5.4 | -209.7 ± 1.4 |
| PP65 | 4.766 | -51.5166 | 27.55 ± 0.08 | 17.58 ± 4.51 | 1.1 | 20.2 | 0.97 | 1.05 | N/A | a | N/A |
| PP66 | 6.658 | -52.8391 | 28.09 ± 0 | 36.06 ± 0 | 7.1 | 4.01 | 0.96 | 1.2 | 6.2 ± 0.7 | -195.5 ± 0.1 | -188.9 ± 0.5 |
| PP67 | 5.9423 | -52.6319 | 27.91 ± 0.07 | 25.25 ± 1.1 | 9.2 | 13.4 | 0.97 | 1.32 | -4.9 ± 1.2 | -183.7 ± 2 | -206.7 ± 0 |
| PP68 | 5.79 | -52.7484 | 27.53 ± 0.06 | 23.4 ± 0.17 | 4.6 | N/A | 0.96 | 1.16 | -7.1 ± 1.2 | -192.5 ± 0.4 | N/A |
| PP69 | 6.0839 | -53.601 | 27.47 ± 0.03 | 22.69 ± 0.24 | 2.5 | N/A | 0.8 | 1.45 | N/A | a | N/A |
| PP70 | 6.2821 | -53.1561 | 27.64 ± 0.03 | 24.96 ± 0.74 | 2.4 | N/A | 0.96 | 1.03 | N/A | a | N/A |

[N/A] No measurements conducted

[a] $C_{37}$ yield was not high enough for isotope analysis

[b] No clear peak distinction for $C_{38}$

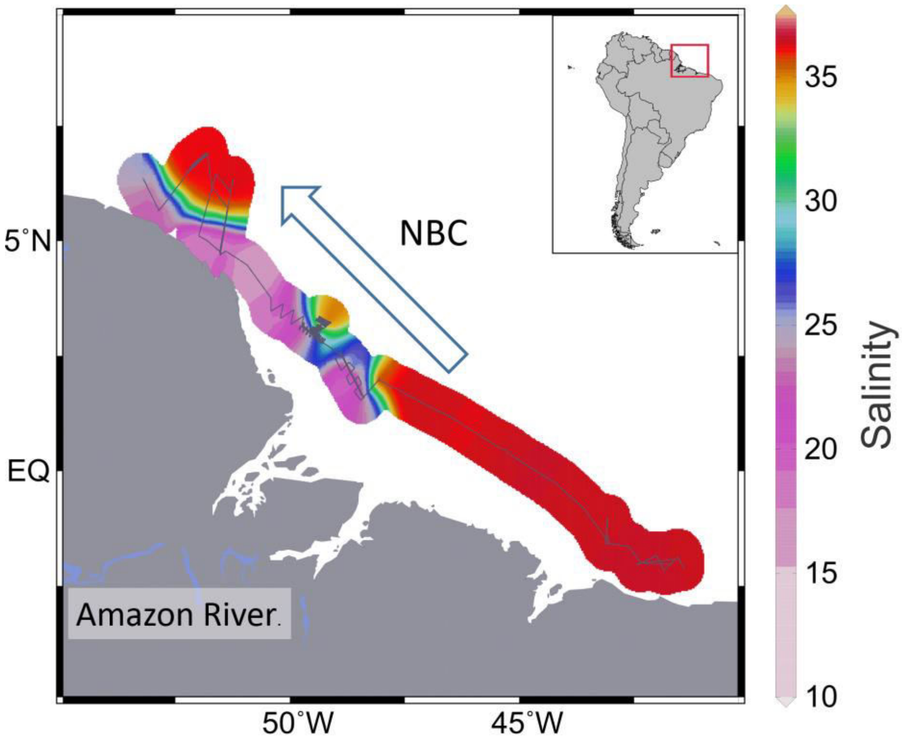

Figure 1. Map of the low salinity plume of the Amazon River outflow derived from the interpolation of onboard salinity measurements. The grey line shows RV Maria S. Merian cruise track MSM20/3 (Mulitza et al., 2013). The blue arrow depicts the North Brazil Current (NBC).

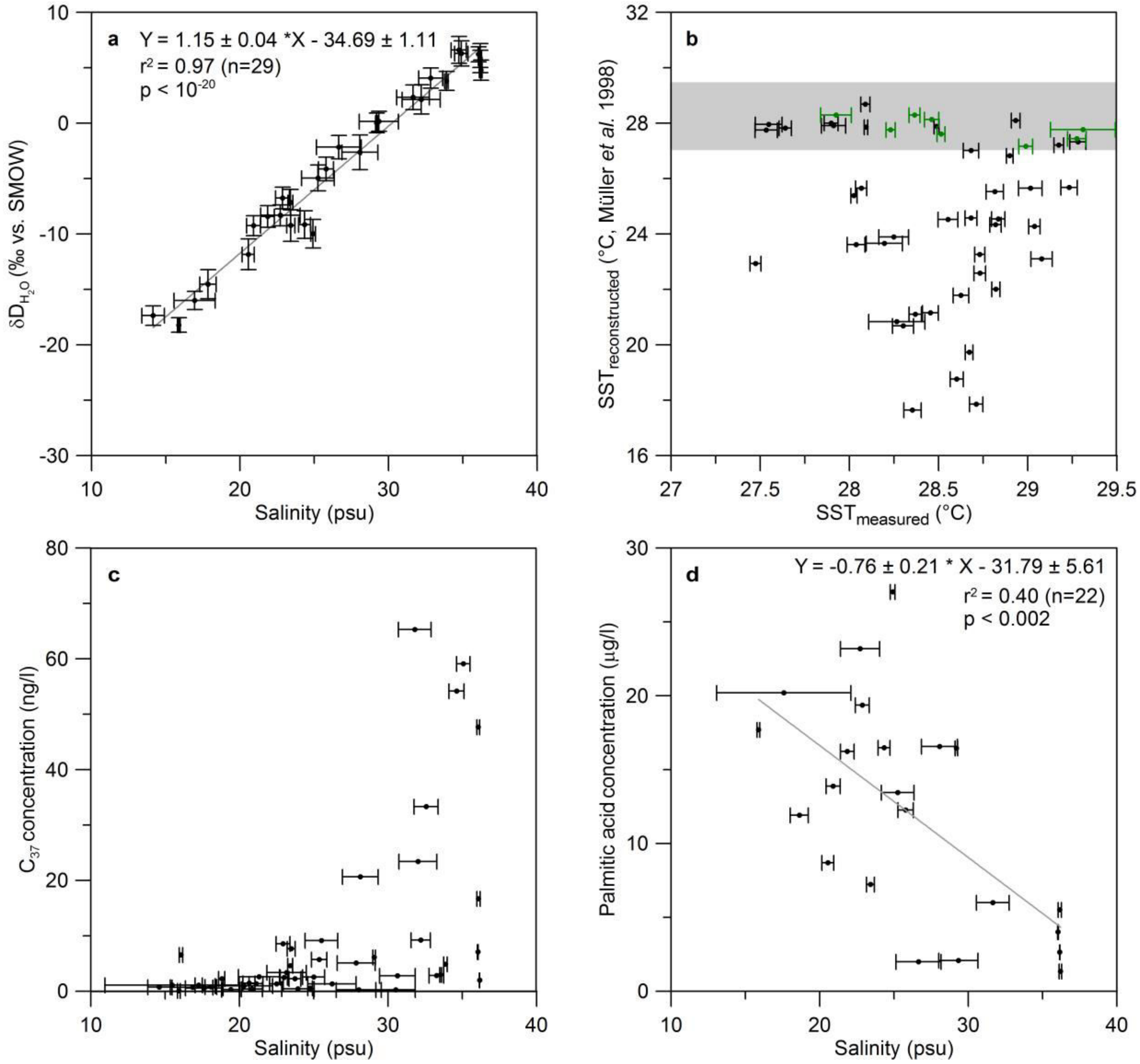

Figure 2. a) $\delta D_{H_2O}$ plotted against salinity; b) $U_{37}^{k'}$ based sea surface temperature (SST) reconstruction using the calibration by Müller et al. (1998) plotted against measured temperature. Green data points represent samples with a $C_{37}$ concentration > 10 ng $L^{-1}$. The grey bar indicates the range of measured SST; c) Concentration of the $C_{37}$ alkenones plotted against salinity; d) Palmitic acid concentration plotted against salinity.

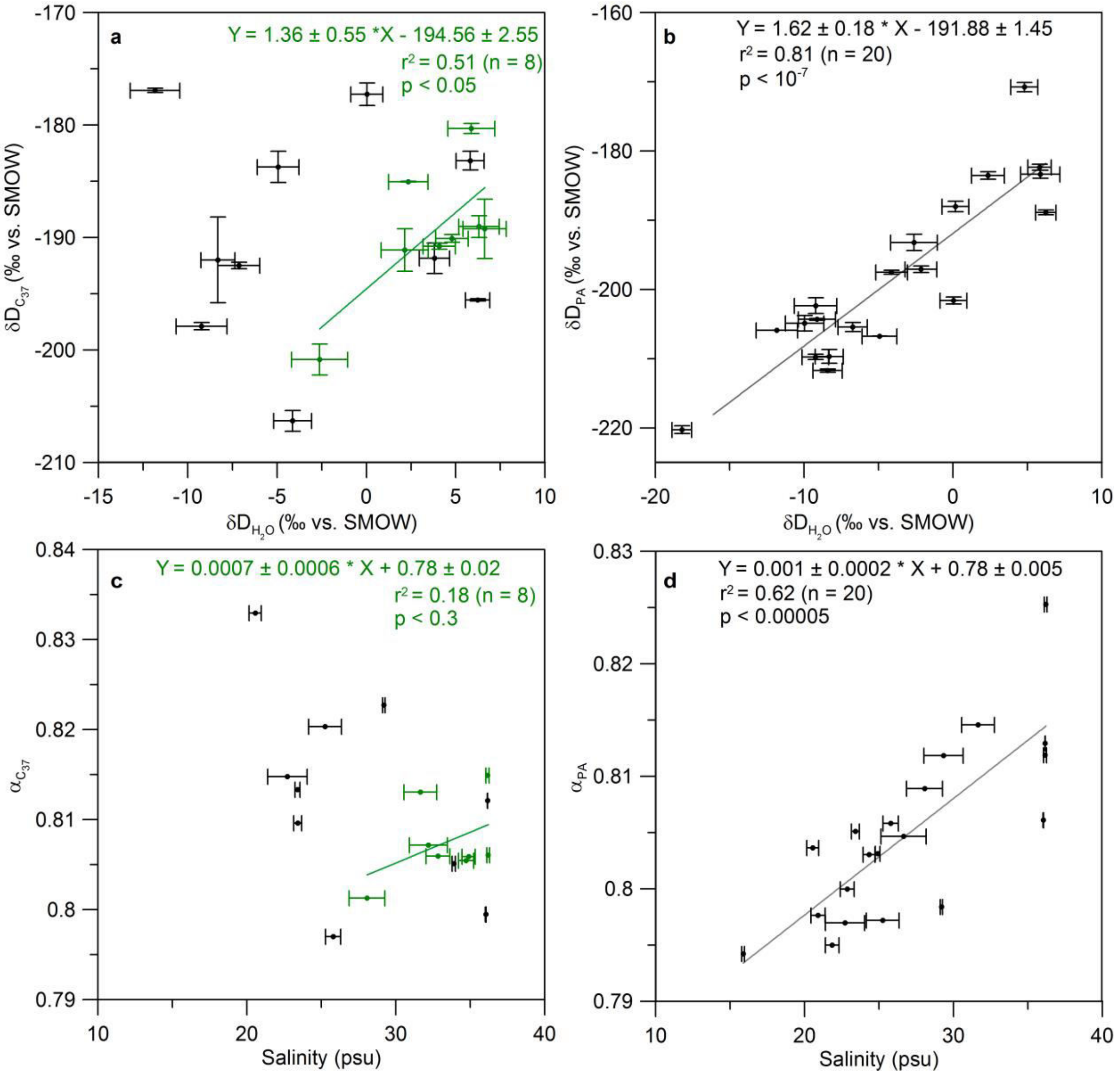

Figure 3. Results of the $\delta D_{lipid}$ analysis. a) $\delta D_{C_{37}}$ against $\delta D_{H_2O}$. Green data points represent samples with a $C_{37}$ concentration > 10 ng $L^{-1}$; b) $\delta D_{PA}$ against $\delta D_{H_2O}$; c) $\alpha_{C_{37}}$ against salinity. Green data points represent samples with a $C_{37}$ concentration > 10 ng $L^{-1}$; d) $\alpha_{PA}$ against salinity.

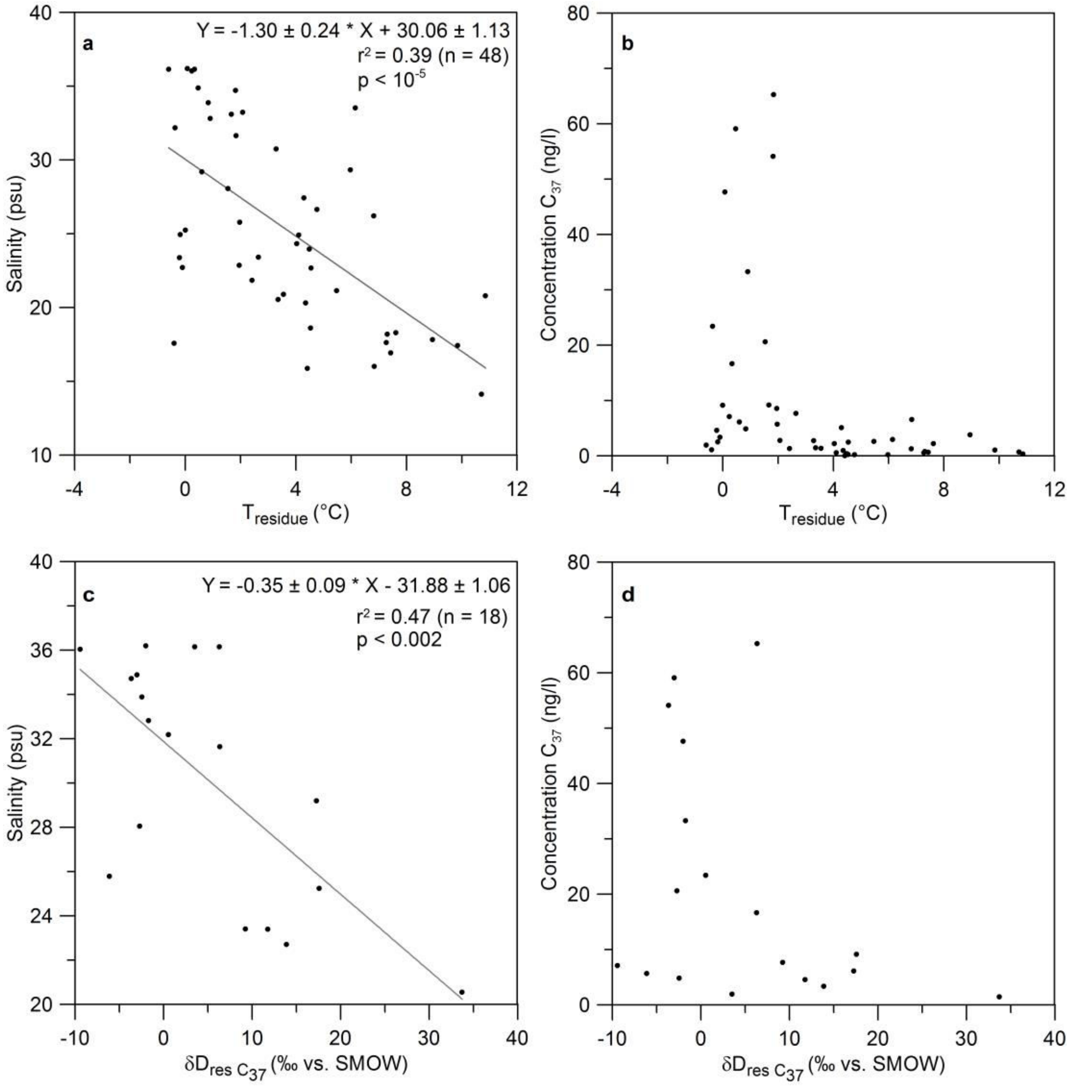

Figure 4. Residues of the $U_{37}^{k'}$ based SST reconstruction plotted against salinity (a) and $C_{37}$ concentration (b). Residues of the $\delta D_{C_{37}}$ measurement plotted against salinity (c) and $C_{37}$ concentration (d).