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Sources and proxy potential of long chain alkyl diols in lacustrine environments

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Abstract

Long chain 1,13- and 1,15-alkyl diols form the base of a number of recently proposed proxies used for climate reconstruction. However, the sources of these lipids and environmental controls on their distribution are still poorly constrained. We have analyzed the long chain alkyl diol (LCD) composition of cultures of ten eustigmatophyte species, with three species from different families grown at various temperatures, to identify the effect of species composition and growth temperature on the LCD distribution. The results were compared with the LCD distribution of sixty-two lake surface sediments, and with previously reported LCD distributions from marine environments. The different families within the Eustigmatophyceae show distinct LCD patterns, with the freshwater family Eustigmataceae most closely resembling LCD distributions in both marine and lake environments. Unlike the other two eustigmatophyte families analyzed (Monodopsidaceae and Goniochloridaceae), C_{28} and C_{30} 1,13-alkyl diols and C_{30} and C_{32} 1,15-alkyl diols are all relatively abundant in the family Eustigmataceae, while the mono-unsaturated C_{32} 1,15-alkyl diol was below detection limit. In contrast to the marine environment, LCD distributions in lakes did not show a clear relationship with temperature. The Long chain Diol Index (LDI), a proxy previously proposed for sea surface temperature reconstruction, showed a relatively weak correlation ($R^2 = 0.33$) with mean annual air temperature used as an approximation for annual mean surface temperature of the lakes. A much-improved correlation ($R^2 = 0.74$, p-value<0.001) was observed applying a multiple linear regression analysis between LCD distributions and lake temperatures reconstructed using branched tetraether lipid distributions. The obtained regression model provides good estimates of temperatures for cultures of the family Eustigmataceae, suggesting that algae belonging to this family have an important role as a source for LCDs in lacustrine environments, or, alternatively, that the main sources of LCDs are similarly affected by temperature as the Eustigmataceae. The results suggest that LCDs may have the potential to be applicable as a palaeotemperature proxy for lacustrine environments, although further calibration work is still required.

1. INTRODUCTION

After the first discovery of long chain alkyl diols (LCD) in Black Sea sediments (De Leeuw et al., 1981), it has become clear that they are widely distributed in both marine and freshwater environments (Versteegh et al., 1997). LCDs occur as several structurally related series, and are derived from distinct sources. The marine diatom genus *Proboscia*, for example, has been identified as a major source for both saturated and mono-unsaturated C_{28} and C_{30} 1,14-alkyl diols (Sinninghe Damsté et al., 2003), whereas the freshwater fern *Azolla* has been linked to C_{30} – C_{36} 1,ω20-alkyl diols (Speelman et al., 2009). However, the major sources of the most common long chain diols in both marine and freshwater environments, i.e. C_{28} – C_{32} 1,13- and 1,15-alkyl diols (also indicated as 1,ω18- and 1,ω16-alkyl diols; Versteegh et al., 1997), have not been unambiguously identified yet. Eustigmatophyte algae are the only organisms known to produce both 1,13- and 1,15-alkyl diols (Méjanelle et al., 2003; Shimokawara et al., 2010; Volkman et al., 1992; 1999) but their importance as a source for LCDs, particularly in marine environments, is uncertain. These algae are rarely reported in the marine environment and the LCD distribution of cultured marine eustigmatophytes is generally dominated by saturated and mono-unsaturated C_{32} 1,15-alkyl diols (Volkman et al., 1992; 1999), in contrast to the marine environments, where the mono-unsaturated C_{32} 1,15-alkyl diol is not detected and the saturated C_{32} 1,15-alkyl diol is present in relatively low abundances (~0 - 30% of all 1,13- and 1,15-alkyl diols; Rampen et al., 2012). For lake environments, the major sources of these LCDs are also uncertain. The similarity of the LCD distribution of a *Nannochloropsis* sp. and Lake Baikal sediments suggests Eustigmatophyceae as a possible source in lake environments (Shimokawara et al., 2010). In addition, Villanueva et al. (2014) observed similar patterns between 18S rRNA gene copy numbers of Eustigmatophyceae and the concentration of LCDs in the water column of the stratified Lake Challa (East Africa). The results from that study suggested that not-yet described Eustigmatophyte groups are the major producers of the LCDs. The occurrence of uncultivated Eustigmatophyte groups is in agreement with a study on the phylogeny of Eustigmatophyceae isolates obtained from lakes, ponds and bogs from Itasca State Park, revealing the existence of several new genera and species (Prior et al., 2009), indicating that our knowledge on this algal group is still fairly limited.

LCDs are increasingly used as palaeoenvironmental indicators. In 1997, Versteegh et al. proposed the diol index, a ratio between C_{30} and C_{32} 1,15-alkyl diols, as a marker for upwelling or freshwater influence in marine environments. More recently, ratios between 1,14-alkyl diols and 1,13-

or 1,15-alkyl diols have also been applied as indicators for upwelling (Rampen et al., 2008; Seki et al., 2012; Willmott et al., 2010), while 1,ω20-alkyl diols have been proposed as markers for the past occurrence of *Azolla* and, consequently, freshwater conditions (Speelman et al., 2009). Furthermore, a ratio between LCDs and tetrahymanol (Romero-Viana et al., 2012), and the deuterium isotopic composition of the C_{32} 1,15-alkyl diol (Romero-Viana et al., 2013) have been introduced as indicators for rainfall variability. Rampen et al. (2012) introduced the Long chain Diol Index (LDI), based on a ratio between C_{30} 1,15-alkyl diol and C_{28} and C_{30} 1,13-alkyl diols, as a proxy for the reconstruction of past sea surface temperature. The LDI has subsequently been used in paleoceanographic studies and shows sea temperature reconstructions that are similar to those obtained with other organic proxies (Lopes dos Santos et al., 2013; Rampen et al., 2012; Rodrigo-Gámiz et al., 2014). Consequently, as most of these LCD proxies involve C_{28} – C_{32} 1,13- and 1,15-alkyl diols, it becomes increasingly relevant to identify their sources in both marine and lacustrine environments.

In order to shed further light on the role of eustigmatophyte algae as a potential source for LCDs, we analyzed LCD distributions in ten different species, selected from all phylogenetic branches within the Eustigmatophyceae for which algal cultures were available. Furthermore, three species from different groups were grown at a range of temperatures to determine how growth temperature affects the LCD composition. The culture results obtained were compared with published data from the marine natural environment (Rampen et al., 2012). Furthermore, we analyzed LCD distributions in surface sediments from sixty-two lakes, previously used for a study on branched glycerol dialkyl glycerol tetraethers (branched GDGTs; Blaga et al., 2010). This lacustrine LCD dataset was compared with the LCD distributions from the cultures and the marine dataset, and finally used to test whether LCDs could also be utilized as lake temperature proxies.

2. MATERIALS AND METHODS

Unialgal cultures of ten eustigmatophyte species were grown in batch cultures with an appropriate culture medium, using a 12/12 h light/dark cycle, and harvested at the end of the log growth phase (Table 1 and Supplementary data). The cultures of *Goniochloris sculpta* grown at 8 and 14°C , which did not grow well and never reached the exponential growth phase, were harvested after 49 days. Algal material was collected either by filtration on pre-combusted Whatman GF/C 47 mm filters or centrifugation (Table 1). The samples were saponified according to Rampen et al. (2011) and the extracts obtained were fractionated into apolar and polar fractions using a glass pipette column filled with activated alumina and elution with hexane/dichloromethane (9/1; vol./vol.) and dichloromethane/methanol (1/1; vol./vol.), respectively. Aliquots of the polar fractions of *Nannochloropsis gaditana*, grown at 14°C, and *G. sculpta*, grown at 16°C were dissolved in hexane and derivatized with dimethyl-disulfide (DMDS) (activated with iodine in diethyl ether at 40°C overnight) to determine the position of the double bond (Buser et al., 1983). Prior to analysis by gas chromatography/mass spectrometry (GC/MS), polar fractions were silylated by adding *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine and heating the mixture in an oven at 60°C for 20 min. GC/MS analyses were performed using a Thermofinnigan TRACE gas chromatograph equipped with a fused silica column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness 0.12 µm) and He as carrier gas, using GC conditions as described by Rampen et al. (2008). The gas chromatograph was coupled to a Thermofinnigan DSQ quadrupole mass spectrometer scanning a mass range of *m/z* 50-800 at 3 scans per second and operating at an ionization energy of 70 eV. LCDs were quantified using the relative intensities of characteristic fragments in their mass spectra (cf. Versteegh et al., 1997). 18S rRNA gene sequences from the same algal species as analyzed in this study were obtained from Genbank (http://www.ncbi.nlm.nih.gov/genbank/, Table 1) and a Neighbor Joining phylogenetic tree was created using distances calculated according to Kimura (1980) and clustering described by Saitou and Nei (1987), which was transformed into an ultrametric tree using the PATHd8 program (Britton et al., 2007).

Sixty-two lake surface sediments were analyzed for their LCD content. Sediments had been extracted previously and branched GDGT compositions have been reported by Blaga et al. (2010). All polar fractions were silylated as described above, and analyzed for LCD distributions using an Agilent Technologies 7890A GC system equipped with a fused silica column (30 m x 0.32 mm) coated with HP-5MS (film thickness 0.25 µm) and He as carrier gas. The gas chromatograph was coupled to an Agilent Technologies 5975C VL MSD operating with an ionization energy of 70 eV using GC conditions as described by Rampen et al. (2008). LCDs were identified using a full scan method, scanning a mass range of 50-800 and quantified using a SIM method, using characteristic fragments in the mass spectra, i.e. *m/z* 299, 313, 327, 341 and 355 (cf. Rampen et al., 2008; 2012). LCD distributions were correlated with annual mean air temperatures obtained from nearby climate observation stations (KNMI, 1998) instead of temperatures provided by Blaga et al. (2010), following the approach of Pearson et al. (2011). SigmaPlot 12.0 was used for regression analyses, while analyses of covariance were performed using SYSTAT 13.

3. RESULTS

3.1. LCDs in eustigmatophyte cultures

For this study we analyzed LCD distributions in cultures of ten eustigmatophyte species, classified within the families Eustigmataceae, Monodopsidaceae and Goniochloridaceae (classification according to Fawley et al., in press, and references cited therein). Although the major environmentally significant groups of eustigmatophytes are probably not yet available in culture (Villanueva et al., 2014), we attempted to obtain the broadest phylogenetic variety of eustigmatophyte species currently possible. From each of the three available eustigmatophyte families, one species was grown at four different temperatures (8, 14, 16 and 20°C; see Table 1).

All eustigmatophyte species analyzed were found to contain LCDs (long chain diol composition of the eustigmatophyte species are available online in supplementary Table 1 and at http://pangaea.de). The LCD distributions of species of the family Monodopsidaceae were dominated by the C_{32} 1,15-alkyl diol, and contained lower fractional abundances (relative to the sum of all LCDs) of C_{30} and C_{31} 1,15-alkyl diols and the C_{30} 1,13-alkyl diol (Fig. 1). The two marine species in this study, *Nannochloropsis gaditana* and *N. oceanica*, contained relatively high fractional abundances of the mono-unsaturated (i.e. with the double bond at C-27) C_{32} 1,15-alkyl diol. *N. oceanica* also contained a mono-unsaturated C_{32} 1,17-alkyl diol, but unsaturated LCDs were not detected in *Monodopsis subterranea*.

Goniochloris sculpta and *Microtalis aquatica*, both belonging to the Goniochloridaceae, showed an LCD distribution similar to those of the studied species of the Monodopsidaceae, with predominantly saturated and mono-unsaturated C_{32} 1,15-alkyl diols, together with C_{30} and C_{31} 1,15-alkyl diols (Fig. 1). The double bond position for *G. sculpta*, grown at 16°C, was determined to be at C-27 as observed for the Monodopsidaceae. However, unlike the Monodopsidaceae, *G. sculpta* and *M. aquatica* contained only 1,15-alkyl diols and no C_{30} 1,13-alkyl diol. *Pseudostaurastrum enorme*, classified in the same family as *G. sculpta* and *M. aquatica*, had a substantially different LCD distribution, comprising saturated and mono-unsaturated C_{32} 1,15-alkyl diols, but also C_{28} and C_{30} 1,13-, 1,14- and 1,15-alkyl diols and only a low contribution of C_{31} 1,15-alkyl diol (<1%).

Eustigmataceae showed a distinct LCD distribution, dominated by the C_{28} 1,13-alkyl diol, C_{30} 1,15-alkyl diol and C_{32} 1,15-alkyl diol, together with lower abundances of C_{29} and C_{30} 1,13-alkyl diols and C_{31} 1,15-alkyl diol (Fig. 1). In addition, *Vischeria stellata* contained a mixture of C_{34} alkyl diols, dominated by the 1,19-diol isomer. A mono-unsaturated C_{32} 1,15-alkyl diol was not detected in any of the Eustigmataceae cultures.

The LCD distributions of the three different families do not substantially change with growth temperature, with the exception of a decrease in the fractional abundance of the monounsaturated C_{32} 1,15-alkyl diol compared to the saturated C_{32} 1,15-alkyl diol with increasing growth temperature as observed for both *N. gaditana* and *G. sculpta* (Figs. 1 and 2f).

3.2. LCDs in lake sediments

We also analyzed the LCD composition of surface sediments of sixty-two lakes, mainly from Europe and South America (Blaga et al., 2010). LCD concentrations in these lakes were generally low, requiring the use of a selective ion monitoring (SIM) method (Rampen et al., 2012). Even when using SIM, LCDs were below detection limit in four lake sediments. The remaining fifty-eight lakes show significant variation in their LCD composition (Fig. 3; supplementary Table 2). LCDs predominantly consist of C_{28}, C_{30} and C_{32} chain lengths (Fig. 3a). On average, C_{30} alkyl diols were most abundant in the studied lake sediments, contributing 20 – 50% of all LCDs and being the most dominant LCDs in 50% of all lakes. However, in the South American lakes, C_{32} alkyl diols were the most dominant LCDs in 56% of all lakes investigated, while C_{28} alkyl diols were relatively more abundant in the European lakes compared to South American lakes. Most lakes also contained small amounts of C_{34} alkyl diols, but these were not quantified.

The C_{32} 1,15-alkyl diol was present in all of the lake sediments, generally contributing >10% of all LCDs, and the most abundant LCD in almost 75% of all South American lakes and 30% of all European lakes. C_{30} 1,13- and 1,15-alkyl diols were also present in all lakes, together with relatively low C_{30} 1,14- and 1,16-alkyl diols. In the European lakes, C_{28} alkyl diols mainly consisted of the 1,13-alkyl diol, which was the most dominant LCD in 50% of these lakes, while C_{28} 1,13- and 1,14-alkyl diol fractional abundances in the South American lakes were similar, but low. The mono-unsaturated C_{32} 1,15-alkyl diol was only present in low abundances or below detection limit (not shown). Concentrations were too low to determine the exact position of the double bond, but mass spectral fragmentation pattern of this sedimentary LCD indicated that the double bond was positioned between C_{16} and C_{32}.

4. DISCUSSION

4.1. Comparison of LCD distributions in cultures, and lake and marine sediments

C_{28} and C_{30} 1,13-alkyl diols and C_{30} and C_{32} 1,15-alkyl diols were the dominant LCDs in Eustigmatophyceae cultures, but the three phylogenetic clusters of Eustigmatophyceae did show clear differences in their LCD patterns (Figs. 1, 2 and 3). Most obvious is the presence of the mono-unsaturated C_{32} 1,15-alkyl diol in Monodopsidaceae and Goniochloridaceae and its absence in the Eustigmataceae. It is also remarkable that 1,13-alkyl diols were not detected in the *Goniochloris* and *Microtalis* species, and species of the family Monodopsidaceae contained relatively low amounts of the C_{30} 1,13-alkyl diol, while Eustigmataceae and the species *Pseudostaurastrum enorme* contained both C_{28} and C_{30} 1,13-alkyl diols. The species *P. enorme* differed from all other studied algae in containing relatively high amounts of 1,14-alkyl diols (>30% of the total LCD composition). Long chain alkyl diol distributions in Monodopsidaceae and Eustigmataceae are comparable to those previously reported by Volkman et al (1992; 1999), Méjanelle et al. (2003) and Shimokawara et al., (2010), while this is the first report on long chain alkyl diol distributions of Goniochloridaceae. It was not possible to grow all cultures in the same culture medium, and the effect of the different culture media (Table 1) on the LCD distributions remains untested. However, differences between phylogenetically related species, grown in different media, are relatively small, while species, belonging to different clusters but grown in the same culture media, show substantially different LCD distributions.

Most (i.e. >90%) of the lake sediments studied contained LCDs, indicating that these lipids are common compounds in lacustrine environments. LCDs in lakes predominantly comprise C_{28} and C_{30} 1,13-alkyl diols and C_{30} and C_{32} 1,15-alkyl diols (Fig. 3), as observed in marine environments (Rampen et al., 2012; Versteegh et al., 1997). The relative abundances of the three most abundant LCDs, the C_{28} 1,13-alkyl diol, C_{30} 1,15-alkyl diol and C_{32} 1,15-alkyl diol, are plotted in a ternary diagram to compare LCD distributions from the different eustigmatophyte families, marine sediments and lake sediments (Fig. 4). This shows that marine surface sediments generally have different LCD distributions compared to the lake sediments and the studied eustigmatophyte species. In marine surface sediments, the C_{32} 1,15-diol rarely contributes >30% to the sum of these three LCDs, while this LCD is often the dominant alkyl diol in lake surface sediments and Eustigmatophyceae. It is remarkable that four of the marine sediments containing >30% C_{32} 1,15-alkyl diol are from the Hudson Bay, a large inland sea strongly influenced by riverine input (e.g. Déry et al., 2011). Indeed, it has already been noted that the abundance of C_{32} 1,15-alkyl diol is higher in brackish and freshwater areas compared to the marine environment (Versteegh et al., 1997), suggesting that a high abundance of this LCD may signify freshwater/brackish conditions. However, a substantial number of freshwater lakes are characterized by a relatively low C_{32} 1,15-alkyl diol abundance, similar to those in the marine environment (Fig. 4) indicating that a low relative abundance of this LCD may not in itself signify marine conditions. It remains uncertain what determines the C_{32} 1,15-alkyl diol abundance; no significant correlations were observed between C_{32} 1,15-alkyl diol abundances and lake size or depth, or pH (data not shown), but the different LCD distributions of the different eustigmatophyte families (Fig. 1) suggest that this could be species-related.

LCD distributions in species belonging to the family Eustigmataceae are, compared to the other families, relatively similar to those in lake and marine sediments (Fig. 4). In contrast, species of the families Monodopsidaceae and Goniochloridaceae contain high fractional abundances of the mono-unsaturated C_{32} 1,15-alkyl diol, which is not detected or present at low relative abundances in marine and lacustrine environments. Possibly, the mono-unsaturated C_{32} 1,15-alkyl diol may be preferentially degraded compared to the saturated LCDs, or transformed into saturated LCDs, potentially explaining this discrepancy. However, the mono-unsaturated C_{32} 1,15-alkyl diol is also absent or present in low abundance in suspended particulate matter and descending particles in marine and lacustrine environments (e.g. Rampen et al., 2008; 2007; Villanueva et al., 2014), suggesting that the Monodopsidaceae and Goniochloridaceae species are not major sources for LCDs in aquatic environments. Although their LCD distributions indicate that species of the family Eustigmataceae could be producers of these lipids in aquatic environments, a recent gene-based study by Villanueva et al. (2014) revealed that most of the Eustigmatophyceae clones in the East African Lake Challa belonged to novel Eustigmatophyceae groups, suggesting that the major sources for LCDs in the environment may not yet be available in culture.

4.2. Impact of temperature on LCD distributions

Nannochloropsis gaditana and *G. sculpta*, grown at temperatures between 8 and 20°C, show a decrease in the fractional abundance of mono-unsaturated C_{32} 1,15-alkyl diol, while the fractional abundance of the saturated C_{32} 1,15-alkyl diol increased with increasing temperature (Fig. 2). A similar increase in fractional abundance of unsaturated long chain 1,14-alkyl diols with increasing temperature was observed for *Proboscia* diatoms (Rampen et al., 2009). Changing the degree of unsaturation is a well-known environmental adaptation mechanism, often used by organisms to maintain a constant lipid fluidity and membrane permeability (Russell and Fukunaga, 1990; Suutari and Laakso, 1994). However, as discussed above, in both marine (Rampen et al., 2008; Rampen et al., 2007; Versteegh et al., 1997) and lake sediments (Villanueva et al., 2014; this study), mono-unsaturated C_{32} 1,15-alkyl diols are rarely abundant. Thus, in aquatic environments, probably Eustigmatophyceae species other than Monodopsidaceae and Goniochloridaceae are the major sources for LCDs. These species may have adopted other methods to adapt their lipid composition to growth temperature. In marine surface sediments, the relative abundance of both C_{28} and C_{30} 1,13-alkyl diols decrease, and that of the C_{30} 1,15-alkyl diol increases with increasing temperature, which forms the basis of the LDI paleotemperature proxy (Rampen et al., 2012). LDI-values for the *G. sculpta* cultures grown at different temperatures are 1 due to the absence of 1,13-alkyl diols, while LDI values for *N. gaditana* cultures show a negative trend with increasing growth temperature (Fig. 2e). Eustigmataceae do show similar temperature trends for the fractional abundance of individual LCDs and the LDI as those observed for marine sediments (Fig. 2), but with significant offsets, which may be due to different growth conditions (e.g. salinity as the Eustigmataceae species cultivated are freshwater species). Alternatively, it could suggest that species of this family are not a major source for LCDs in the marine environment.

We also investigated the influence of temperature on the distribution of LCDs in lake sediments, by plotting relative abundances (i.e. the abundance of an individual LCD divided by the summed abundances of C_{28} and C_{30} 1,13-alkyl diols and C_{30} and C_{32} 1,15-alkyl diols) of individual 1,13- and 1,15-alkyl diols in the lake surface sediments versus temperature. Preferably, measured in-situ lake temperatures should be used but these were often not available for the lakes studied. As an alternative, mean annual air temperatures (MAAT) obtained from nearby weather stations were used (KNMI, 1998), as lake water temperatures are often strongly correlated with MAAT (Livingstone and Lotter, 1998). Only weak or no correlations are observed for the relationship between relative LCD abundances and temperature (Fig. 5a-d), which is in contrast to correlations observed for the relative abundances of C_{28} and C_{30} 1,13-alkyl diols and C_{30} 1,15-alkyl diols with sea surface temperature in marine surface sediments (Rampen et al., 2012). Nevertheless, LDI values of the LCDs in the lake sediments do show a weak but significant correlation with MAAT ($R^2 = 0.33$, p-value <0.001, n = 56; Fig. 5e). For branched GDGTs it has been shown that improved correlations may be obtained between branched GDGT relative abundances and MAAT when multiple linear regression is used (Loomis et al., 2012; Tierney et al., 2010). Therefore, we applied a multiple linear regression on the relative abundances of the four most dominant LCDs, e.g. C_{28} and C_{30} 1,13-alkyl diol, C_{30} and C_{32} 1,15-alkyl diol and MAAT. The following model was obtained (Fig. 5f):

$$\text{Temperature (°C)} = 24.6 - 32.0 * RA_{C28\ 1,13\text{-alkyl diol}} - 59.0 * RA_{C30\ 1,13\text{-alkyl diol}} - 7.4 * RA_{C30\ 1,15\text{-alkyl diol}} \quad (1)$$

where RA indicates the relative abundance of an individual LCD. Because of the closed sum effect, the relative abundance of the C_{32} 1,15 alkyl diol is not expressed in formula (1) but does affect the outcome of the equation. The correlation observed for this model ($R^2 = 0.53$, p-value <0.001, n = 56; Fig. 5f) is stronger than observed for the LDI, but still much weaker than that observed between the LDI and sea surface temperature for the marine environment (i.e., $R^2 = 0.97$, Rampen et al., 2012). Part of the scatter may be due to inaccuracies in the lake temperature data used, due to differences in location and altitude of the lakes and the weather stations, but the correlation did not improve after correcting for altitude differences using lapse rates of 6.5 or 5.1 °C km^{-1} (Stone and Carlson, 1979). Another reason for the observed scatter may be that LCDs are produced in a specific time period during the annual cycle. Other proxies are known to be affected by seasonal production maxima (e.g. Castañeda and Schouten, 2011 and references cited therein). However, we also did not observe a significant improvement of the correlation between LCDs and temperature when using the different monthly mean air temperatures instead of MAAT.

As an alternative approach, we used branched GDGT distributions of the lake sediments previously reported by Blaga et al. (2010) to reconstruct summer lake temperature by application of the calibration of Pearson et al. (2011). It is likely that the algae in the lake are growing predominantly in summer and, in this way, the calibration becomes independent from the data of the weather stations, which, as explained, have their limitations. Interestingly, the use of branched GDGT-derived temperatures results in an improved correlation of temperature with LDI-values ($R^2 = 0.51$, p-value <0.001, n = 55, Fig. 6e). The data point from Lago di Albano (branched GDGT-derived temperature of 10.6 and an LDI value of 0.95) was identified as an outlier in the lake dataset (studentized residual = 5.027). Although the properties of Lago di Albano are not clearly different from other lakes in this study (Blaga et al., 2010), the LCD fraction is characterized by an unusual dominance of C_{30} 1,15-alkyl diol (>50%) and a corresponding high LDI value, not observed in any other lake. Exclusion of this data point strongly improves the correlation with LDI, resulting in an R^2 value of 0.64 (n = 54). Comparison with the marine LDI-temperature correlation shows a difference in slope (Fig. 6e). This may be partially explained by the fact that the LDI calibration for marine environments is based on annual mean sea surface temperatures, while the LDI calibration in lakes is based on branched GDGT-derived summer temperatures.

We also applied a multiple linear regression on relative abundances of the four most dominant LCDs, e.g. C_{28} and C_{30} 1,13-alkyl diol and C_{30} and C_{32} 1,15-alkyl diol and branched GDGT derived summer temperatures. Again, data from Lago di Albano is identified as an outlier (studentized residual = 4.577) and when excluding this data point, the following model is obtained (Fig. 6f):

$$\text{Temperature (°C)} = 26.8 - 25.9 * RA_{C28\ 1,13\text{-alkyl diol}} - 54.3 * RA_{C30\ 1,13\text{-alkyl diol}} + 7.4 * RA_{C30\ 1,15\text{-alkyl diol}} \quad (2)$$

The correlation observed for this model ($R^2 = 0.74$, p-value <0.001, n = 54) does suggest that also in lacustrine environments, LCD distributions are affected by temperature and may therefore also have potential for lacustrine temperature reconstruction. The improved correlation with proxy temperature rather than measured annual mean temperatures may be that the proxy temperature is more representative of the lake temperature during production of the LCDs. Nevertheless, the scatter remains substantial, and may have several causes. First of all, the branched GDGT-derived temperature has a much larger error than instrumentally meausured temperatures that are typically used for proxy calibration. In addition, the source organisms for the lipids used in both proxies may bloom at different times or depths, thereby registering different temperatures. The scatter may also be due to the occurrence of different eustigmatophyte species in the different lakes, which may have different blooming periods, but may also possess different LCD adaptations to temperature, something also observed for alkenones in lacustrine haptophyte algae (see Castaneda and Schouten, 2011 and references cited therein).

When the obtained regression model is applied to the LCD data of the eustigmatophyte culture analyzed in this study, the temperature values obtained for the *N. gaditana* and *V. stellata* cultures also show a positive correlation with growth temperature. Remarkably, the correlation slopes for *N. gaditana* and *V. stellata* are not significantly different from the lake dataset (Fig. 6f, p-values of 0.502 and 0.665, respectively). In addition, although the intercept for *N. gaditana* clearly differs from the lake dataset (p-value < 0.001), the y-intercepts for *V. stellata* and the lake dataset are not significantly different (p-value = 0.541). The similarities observed between LCD distributions in lakes and *Vischeria* species, and their correlations with temperature, may indicate that *Vischeria* species, and possibly species of the family Eustigmataceae in general, are an important source for lacustrine LCDs. Alternatively, and perhaps more likely, LCD distributions in lacustrine sources may be similarly affected by environmental conditions as in *Vischeria* species.

The results of this study show the potential of LCDs for lacustrine temperature reconstruction. However, calibration of this new proxy requires a much larger lake dataset, including well-constrained annual and seasonal lake water temperatures, but also pH, alkalinity and other factors, which may affect LCD distributions. In addition, analysis of sediment trap and suspended particulate matter samples, and new eustigmatophyte isolates may provide valuable information.

5. CONCLUSIONS

LCDs were detected in ten eustigmatophyte species with distinct LCD patterns for different eustigmatophyte families. Identification of LCDs in fifty-eight out of sixty-two studied lake sediments indicates that these lipids are common biomarkers in lacustrine environments. Comparison of our results with previously published LCD distributions in marine sediments indicates higher relative abundances of C_{32} 1,15-alkyl diol in cultures and most lake sediments, indicating different sources. From all eustigmatophyte species analyzed in this study, LCD distributions from the algal family Eustigmataceae are most similar to the LCD distributions of aquatic environments. Cultivation of several species at a range of temperatures show an increase in saturated C_{32} 1,15-alkyl diol and a corresponding decrease in mono-unsaturated C_{32} 1,15-alkyl diol with increasing temperature in *N.*

gaditana and *G. sculpta*. In natural environments, however, the mono-unsaturated C_{32} 1,15-alkyl diol is rarely abundant, whereas relative abundances of C_{28} and C_{30} 1,13- and C_{30} 1,15-alkyl diols in marine environments do correlate with temperature. Relative abundances of these individual LCDs in lakes show no correlation with mean annual air temperature. However, the Long chain Diol Index, (LDI) based on these three lipids, does correlate with temperature, and an even stronger correlation is observed using a multiple linear regression. The strongest correlation was obtained by performing multiple linear regression on relative abundances of C_{28} and C_{30} 1,13- and C_{30} 1,15-alkyl diols and branched GDGT-derived temperatures from the same lakes. The R^2 value of 0.74 obtained for this model suggests that LCDs may also be applicable as palaeotemperature proxies for lacustrine environments. However, more validation studies are needed before this proxy can be applied with confidence.

ACKNOWLEDGEMENTS

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Figure captions

Fig. 1. LCD carbon number distributions (expressed as fractional abundances) of the cultures of the ten studied species of Eustigmatophyceae. Three species were grown at four different temperatures as indicated. For each carbon number the various LCD isomers are indicated by a colour code. For reference, the molecular phylogeny of the Eustigmatophyceae based on the 18S rRNA gene, is provided.

Fig. 2. Fractional abundances (F) of individual LCDs (a-d; C_{28} 1,13-alkyl diol, C_{30} 1,15-alkyl diol, C_{30} 1,13-alkyl diol, C_{32} 1,15-alkyl diol, respectively), the LDI ratio (e) and the fractional abundances of unsaturated LCDs (f) of Eustigmatophyceae cultures plotted versus growth temperature. Data of Rampen et al. (2009) for *Proboscia* diatoms is also plotted in (f).

Fig. 3. Bar plots showing the chain length distributions (a) and distributions of the different LCD isomers with chain lengths of 28 - 32 (b – f, respectively) carbon atoms for the eustigmatophyte cultures and the lake sediments.

Fig. 4. Ternary diagram showing relative abundances of C_{28} 1,13-alkyl diol, C_{30} 1,15-alkyl diol and C_{32} 1,15-alkyl diol from cultures (this study), lake surface sediments (this study) and marine surface sediments (data published by Rampen et al., 2012). The green line indicates 30% C_{32} 1,15-alkyl diol, which is a maximum value for most marine sediments.

Fig. 5. Cross plots of the relative abundances (RA) of C_{28} 1,13-alkyl diol, C_{30} 1,15-alkyl diol, C_{30} 1,13-alkyl diol, C_{32} 1,15-alkyl diol vs. mean annual air temperatures (MAAT) (a – d, respectively), LDI values vs. MAAT (e) and (f) MAAT vs. LCD inferred temperatures (eq. 1), obtained from lake sediments. Light blue lines indicate the relationships between the fractional abundance of LCDs (a-d) and LDI (e) in marine surface sediments and sea surface temperature as reported by Rampen et al. (2012).

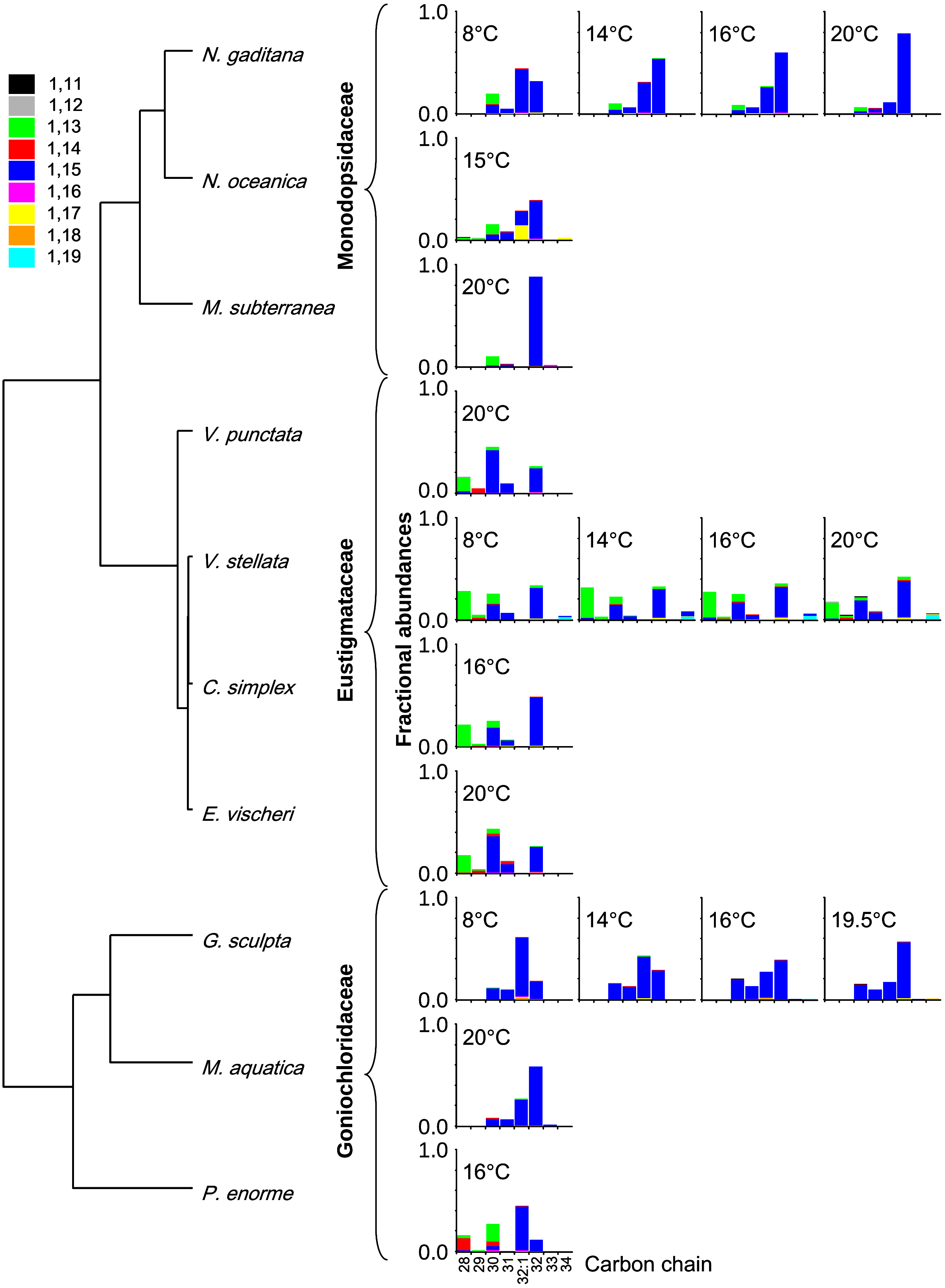
Fig. 6. Cross plots of the relative abundances of C_{28} 1,13-alkyl diol, C_{30} 1,15-alkyl diol, C_{30} 1,13-alkyl diol, C_{32} 1,15-alkyl diol (a – d, respectively), and LDI values (e) obtained from cultures and lake sediments vs. temperature and branched GDGT-derived summer temperatures, respectively, and (f) temperature and branched GDGT-derived summer temperatures vs. LCD inferred temperatures from cultures and lake sediments (eq. 2), respectively. Light blue lines indicate the relationships between

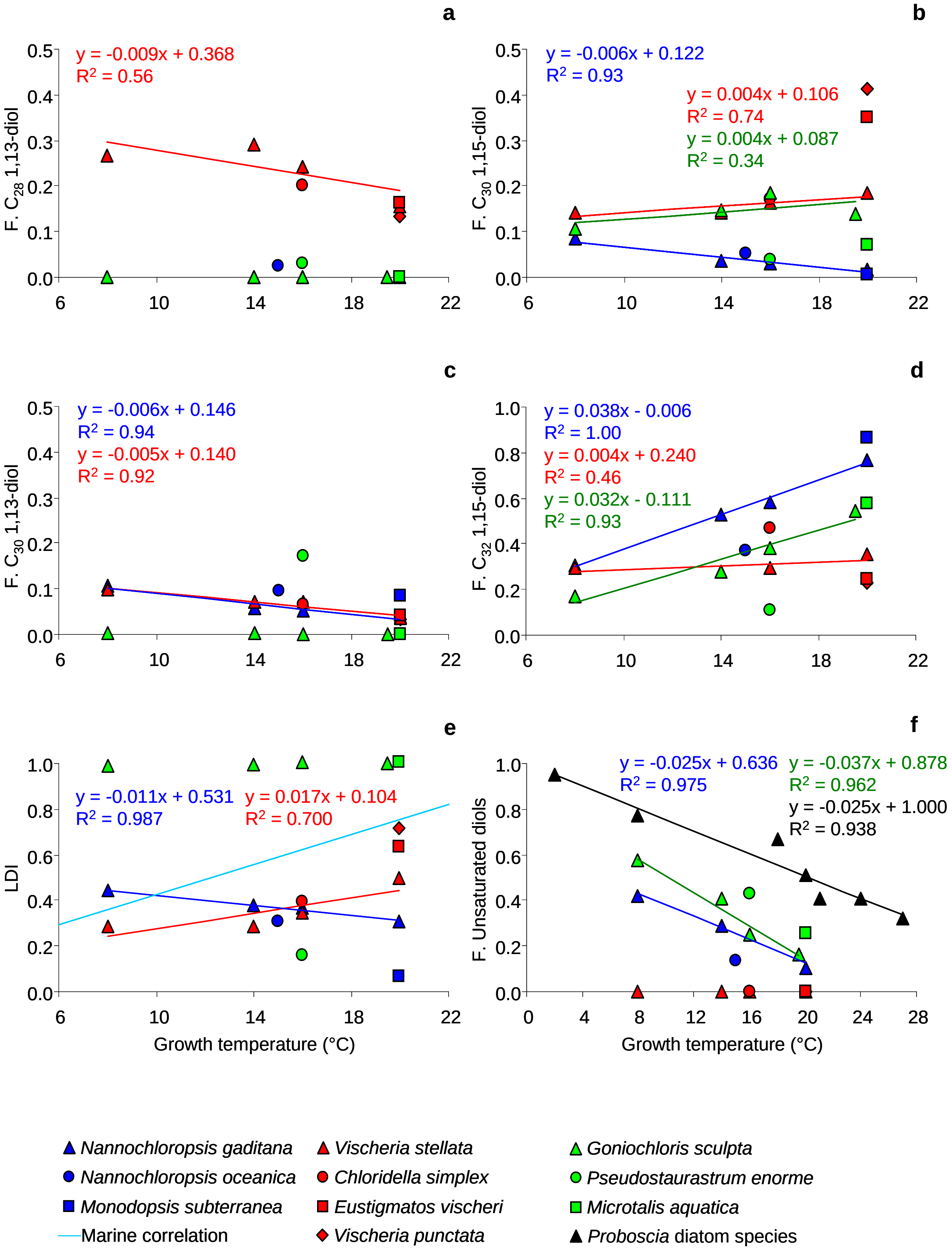
the fractional abundance of LCDs (a – d), LDI (e) and equation 2-inferred temperatures (f) in marine surface sediments and sea surface temperature as reported by Rampen et al. (2012). The red encircled lake sample points in (e) and (f) indicate an outlier sample from Lago di Albano.

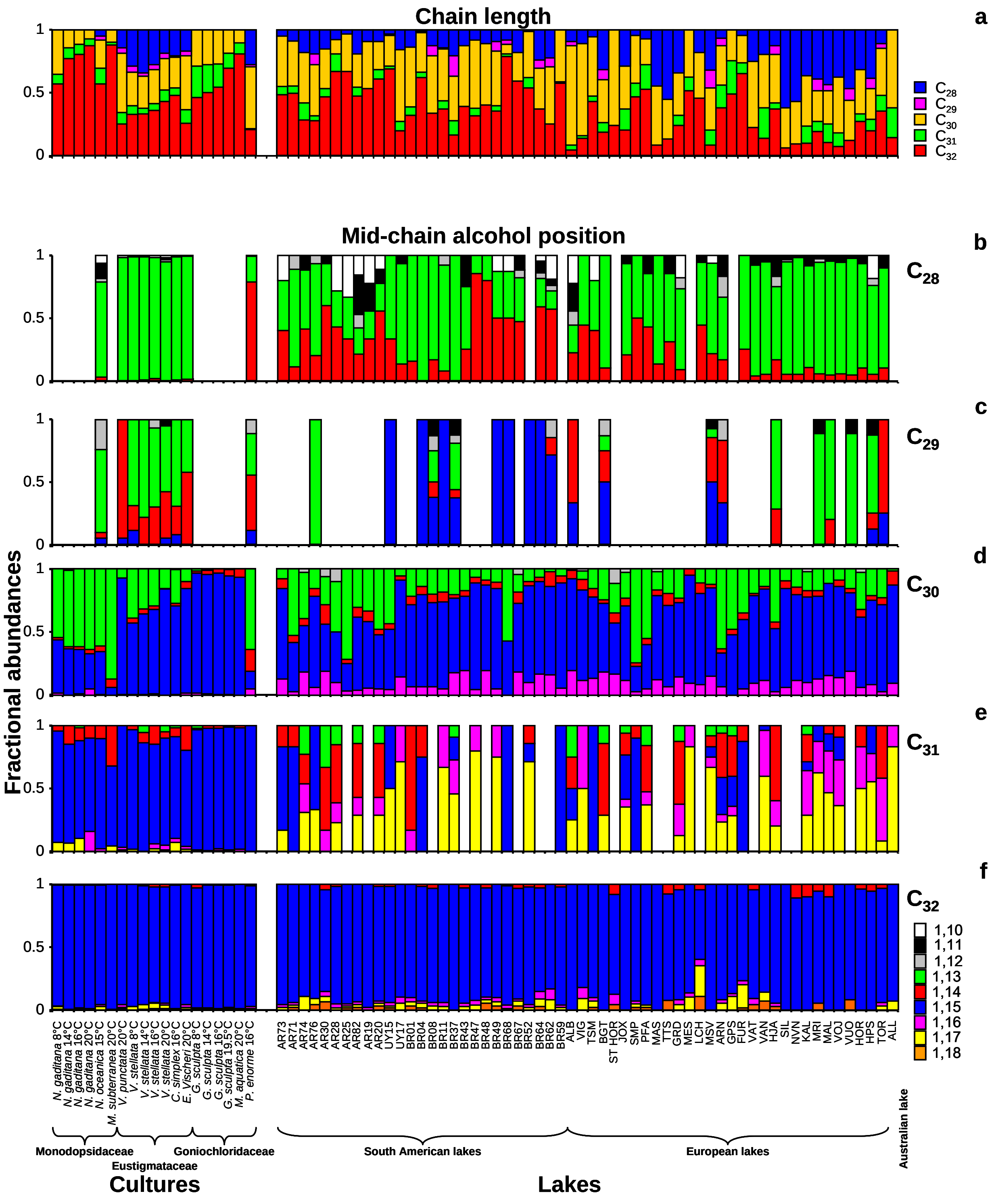
Table 1: Eustigmatophyte culture information

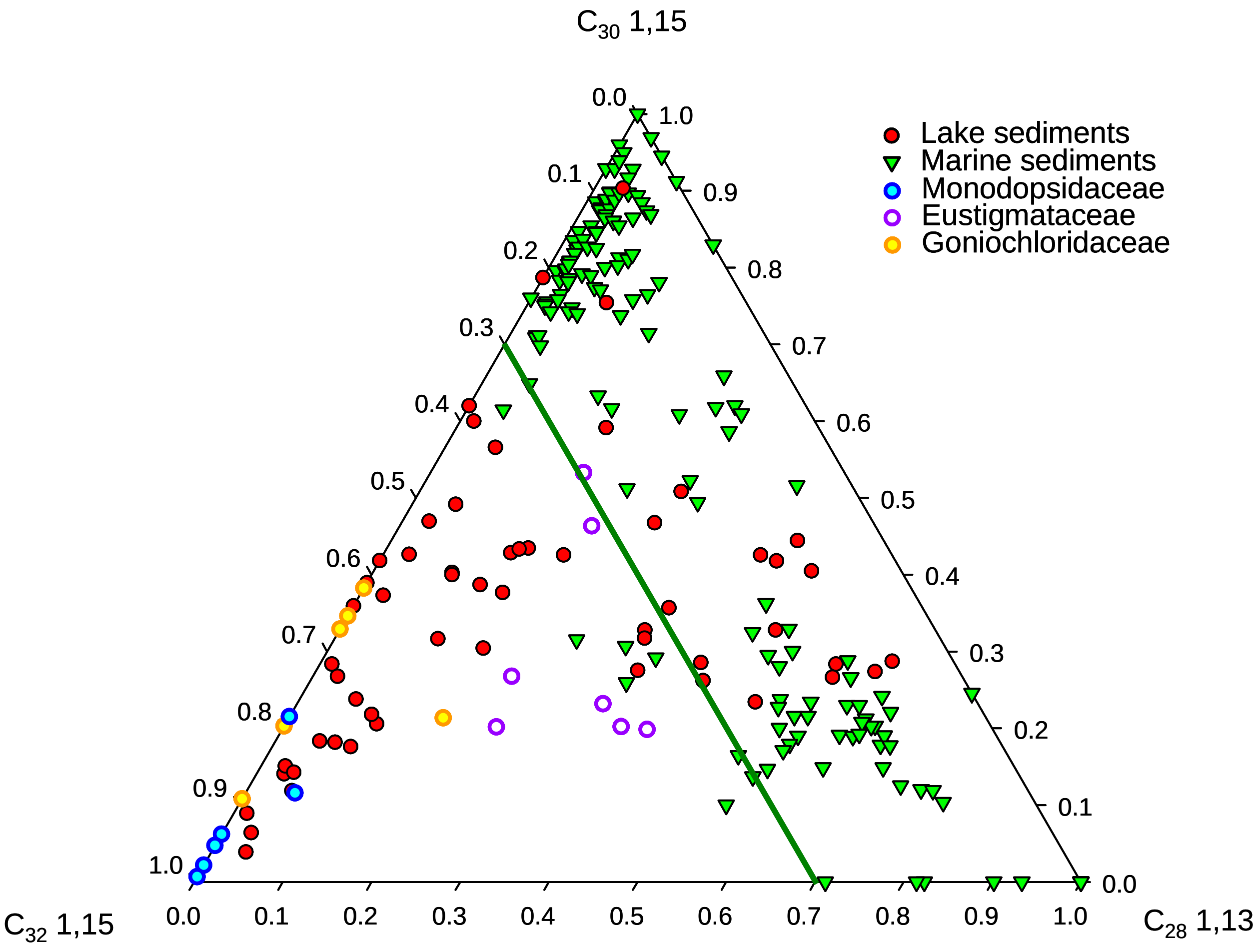
Culture	Original environment	Medium[1]	Temperature (°C)	Duration (days)	Harvest method	18S rRNA gene sequence
Monodopsidaceae						
Nannochloropsis gaditana (CCAP 849/5)	Marine	F/2	8,14,16, 20	38, 32, 31, 35	filter	AF045036
Nannochloropsis oceanica (CCAP 849/10)	Marine	F/2	15	9	filter	JF489982
Monodopsis subterranea (CCAP 848/1)	Freshwater	3N BBM + V	20	35	filter	U41054
Eustigmataceae						
Vischeria punctata (CCAP 887/3)	Soil	3N BBM + V	20	50	filter	FJ858972
Vischeria stellata (CCAP 887/4)	Soil	3N BBM + V	8,14,16, 20	123, 56, 56, 29	filter	HQ710570
Chloridella simplex (SAG 51.91)	Snow	JM	16	87	filter	KF848923
Eustigmatos vischeri (CCAP 860/7)	Soil	3N BBM + V	20	41	filter	FJ858973
Goniochloridaceae						
Goniochloris sculpta (SAG 29.96)	Freshwater	JM	8, 14,16, 19.5	68, 21, 49, 49	centrifuge	FJ858970
Microtalis aquatica (CCMP 3153	Freshwater	MDY	20	35	filter	HQ710564
Pseudostaurastrum enorme (SAG 11.85)	Freshwater	Modified DesMid	16	53	filter	EF044312

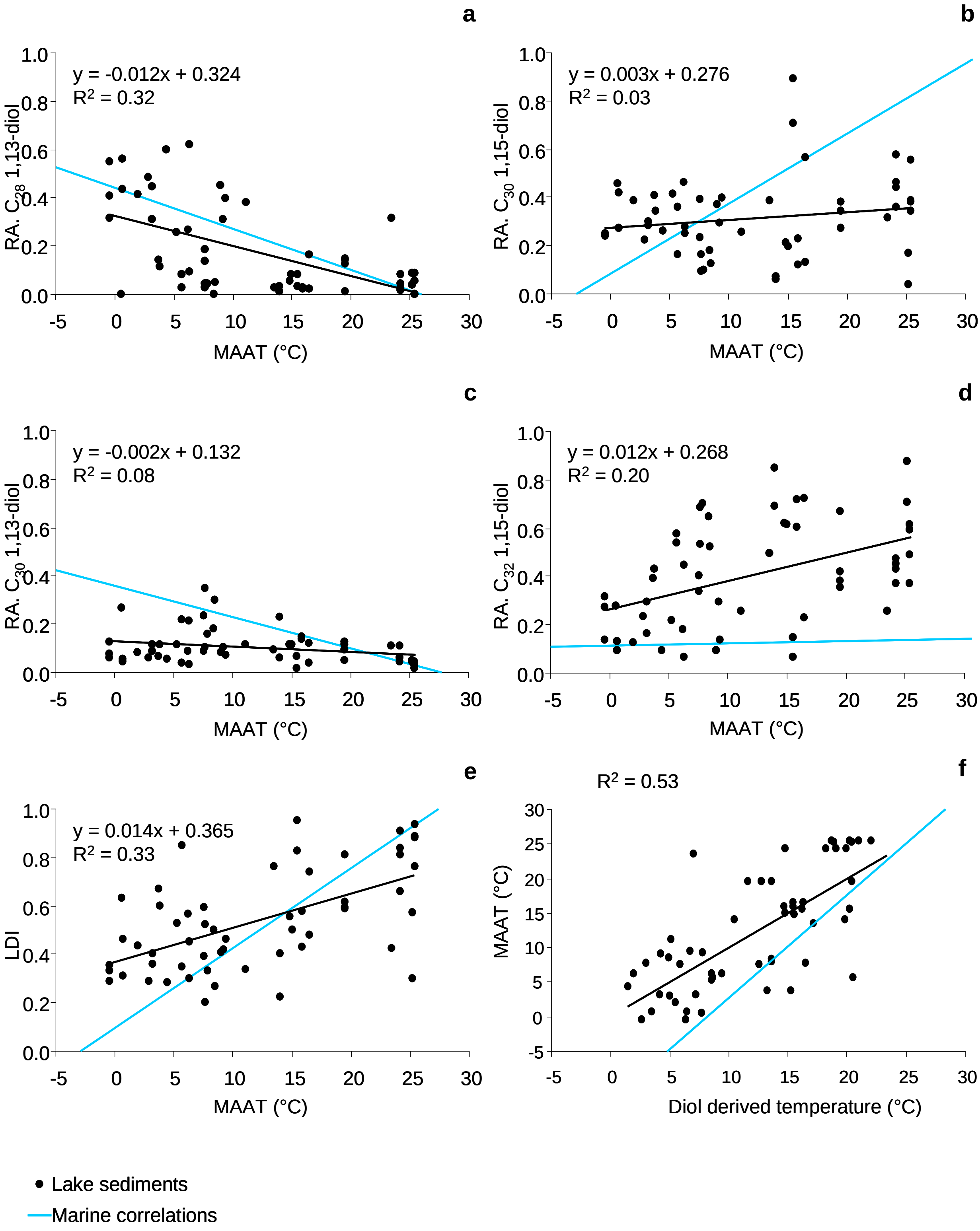
[1]: detailed information on culture media can be obtained from http://www.ccap.ac.uk/

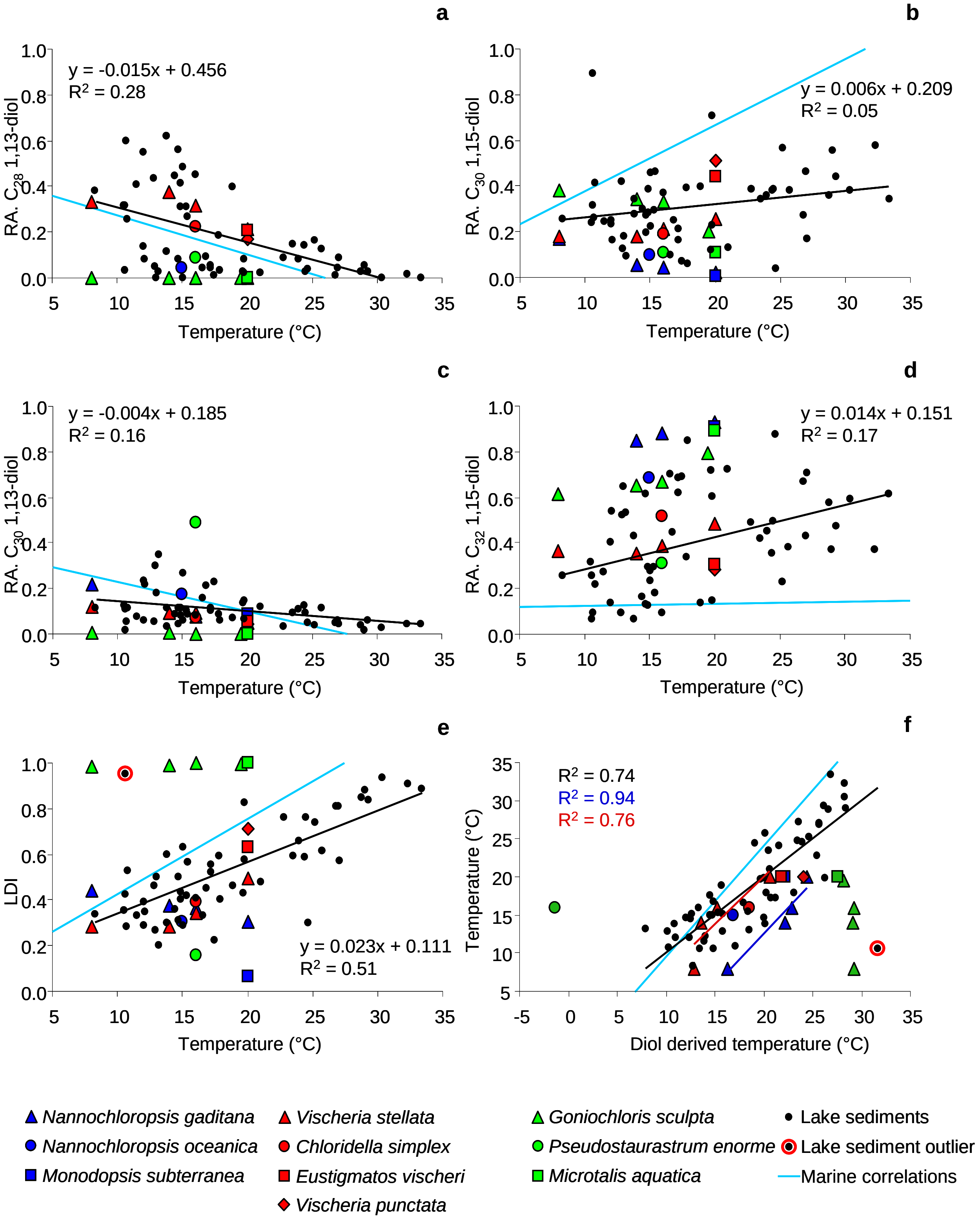

1,11
1,12
1,13
1,14
1,15
1,16
1,17
1,18
1,19
N. gaditana
N. oceanica
M. subterranea
V. punctata
V. stellata
C. simplex
E. vischeri
G. sculpta
M. aquatica
P. enorme
Monodopsidaceae
Eustigmataceae
Goniochloridaceae
Fractional abundances
1.0
0.0
8°C
14°C
16°C
20°C
15°C
20°C
20°C
8°C
14°C
16°C
20°C
16°C
20°C
8°C
14°C
16°C
19.5°C
20°C
16°C
28
29
30
31
32:1
32
33
34
Carbon chain


a
F. C28 1,13-diol
y = -0.009x + 0.368
R2 = 0.56
b
F. C30 1,15-diol
y = -0.006x + 0.122
R2 = 0.93
y = 0.004x + 0.106
R2 = 0.74
y = 0.004x + 0.087
R2 = 0.34
c
F. C30 1,13-diol
y = -0.006x + 0.146
R2 = 0.94
y = -0.005x + 0.140
R2 = 0.92
d
F. C32 1,15-diol
y = 0.038x - 0.006
R2 = 1.00
y = 0.004x + 0.240
R2 = 0.46
y = 0.032x - 0.111
R2 = 0.93
e
LDI
y = -0.011x + 0.531
R2 = 0.987
y = 0.017x + 0.104
R2 = 0.700
f
F. Unsaturated diols
y = -0.025x + 0.636
R2 = 0.975
y = -0.037x + 0.878
R2 = 0.962
y = -0.025x + 1.000
R2 = 0.938
Growth temperature (°C)
Growth temperature (°C)
0.0 0.1 0.2 0.3 0.4 0.5
0.0 0.2 0.4 0.6 0.8 1.0
6 10 14 18 22
0 4 8 12 16 20 24 28
Nannochloropsis gaditana
Nannochloropsis oceanica
Monodopsis subterranea
Marine correlation
Vischeria stellata
Chloridella simplex
Eustigmatos vischeri
Vischeria punctata
Goniochloris sculpta
Pseudostaurastrum enorme
Microtalis aquatica
Proboscia diatom species


Chain length
Mid-chain alcohol position
a
b
c
d
e
f
1
0.5
0
Fractional abundances
🟦 C_{28}
🟪 C_{29}
🟨 C_{30}
🟩 C_{31}
🟥 C_{32}
C_{28}
C_{29}
C_{30}
C_{31}
C_{32}
⬜ 1,10
⬛ 1,11
▨ 1,12
🟩 1,13
🟥 1,14
🟦 1,15
🟪 1,16
🟨 1,17
🟧 1,18
N. gaditana 8°C
N. gaditana 14°C
N. gaditana 16°C
N. gaditana 20°C
N. oceanica 15°C
M. subterranea 20°C
V. punctata 20°C
V. stellata 8°C
V. stellata 14°C
V. stellata 16°C
V. stellata 20°C
C. simplex 16°C
E. vischeri 20°C
G. sculpta 8°C
G. sculpta 14°C
G. sculpta 16°C
G. sculpta 19.5°C
M. aquatica 20°C
P. enorme 16°C
AR73
AR71
AR74
AR76
AR30
AR28
AR25
AR82
AR19
AR20
UY15
UY17
BR01
BR04
BR08
BR11
BR37
BR43
BR47
BR48
BR49
BR68
BR67
BR52
BR64
BR62
BR59
ALB
VIG
TSM
BGT
ST HOR
JOX
SMP
PFA
MAS
TTS
GRD
MES
LCH
MSV
ARN
GPS
FUR
VAT
VAN
HJA
SIL
NVN
KAL
MRI
MAL
VOJ
VUO
HOR
HPS
TOR
ALL
Monodopsidaceae
Eustigmataceae
Goniochloridaceae
South American lakes
European lakes
Australian lake
Cultures
Lakes


C30 1,15
C32 1,15
C28 1,13
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Lake sediments
Marine sediments
Monodopsidaceae
Eustigmataceae
Goniochloridaceae


a
y = -0.012x + 0.324
R2 = 0.32
RA. C28 1,13-diol
MAAT (°C)
b
y = 0.003x + 0.276
R2 = 0.03
RA. C30 1,15-diol
MAAT (°C)
c
y = -0.002x + 0.132
R2 = 0.08
RA. C30 1,13-diol
MAAT (°C)
d
y = 0.012x + 0.268
R2 = 0.20
RA. C32 1,15-diol
MAAT (°C)
e
y = 0.014x + 0.365
R2 = 0.33
LDI
MAAT (°C)
f
R2 = 0.53
MAAT (°C)
Diol derived temperature (°C)
0.0
0.2
0.4
0.6
0.8
1.0
-5
0
5
10
15
20
25
30
● Lake sediments
Marine correlations


a
y = -0.015x + 0.456
R² = 0.28
RA. C28 1,13-diol
Temperature (°C)
b
y = 0.006x + 0.209
R² = 0.05
RA. C30 1,15-diol
Temperature (°C)
c
y = -0.004x + 0.185
R² = 0.16
RA. C30 1,13-diol
Temperature (°C)
d
y = 0.014x + 0.151
R² = 0.17
RA. C32 1,15-diol
Temperature (°C)
e
y = 0.023x + 0.111
R² = 0.51
LDI
Temperature (°C)
f
R² = 0.74
R² = 0.94
R² = 0.76
Temperature (°C)
Diol derived temperature (°C)
0.0
0.2
0.4
0.6
0.8
1.0
5
10
15
20
25
30
35
-5
0
▲ Nannochloropsis gaditana
● Nannochloropsis oceanica
■ Monodopsis subterranea
▲ Vischeria stellata
● Chloridella simplex
■ Eustigmatos vischeri
◆ Vischeria punctata
▲ Goniochloris sculpta
● Pseudostaurastrum enorme
■ Microtalis aquatica
● Lake sediments
◉ Lake sediment outlier
— Marine correlations