

## Biomarker indicators of past climate

Rienk H. Smittenberg, Department of Geological Sciences, Stockholm University, Stockholm, Sweden

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### Abstract

The molecular remainders of living organisms, found in sedimentary records, reflect past ecological, environmental and climatic conditions. Occurrence, abundance, relative distribution and even isotopic composition of these lipid biomarkers can be linked to climatic conditions. This chapter reviews organic proxies for past temperature, salinity, hydrology, sea ice conditions and biomass burning.

Keywords Glycerol dialkyl glycerol tetraethers Hydrogen isotopes IP25 Leaf wax hydrogen isotopes Lipid biomarkers Long Chain Alkenones MBT Paleofire Paleohydrology Paleosalinity Paleotemperature Past sea ice TEX86

### Key points

Overview of lipid biomarker proxies for paleotemperature, paleohydrology, paleosalinity, and paleofire. Historical context.

### Introduction

Molecular fossils, or ‘lipid biomarkers,’ are organic compounds specific for certain types of organisms. These molecules add to the growing arsenal of climate indicators scientists are using to reconstruct climate history helping to understand climate change and dynamics. Analysis is normally performed on ocean and lake sediment cores but also peat deposits, while pre-Quaternary climate can be reconstructed from sedimentary rocks. The lipid biomarkers are extracted from the samples using organic solvents and then typically identified and quantified using chromatography and mass spectrometry, also including analysis of their stable isotope composition. It is organized around proxies for paleotemperature, paleosalinity, paleohydrology, past sea ice, and biomass burning. More lipid biomarker proxies exist that can be related to past climatic changes in a more indirect manner, by virtue of them reflecting changes in ecosystems that are affected by climatic change. This article is a major update from the two earlier encyclopedia editions, with the newest insights.

### Biomarker proxies of temperature

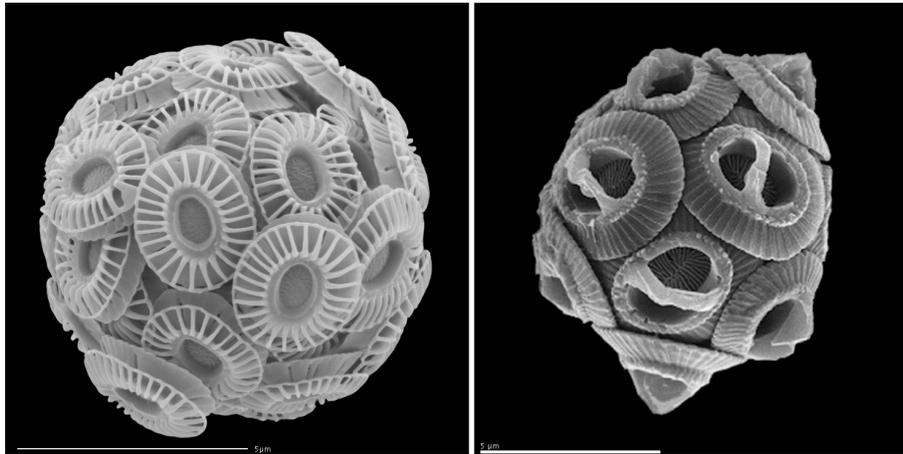
Temperature reconstruction is central to our understanding of past climatic changes. Impressive advances have been made in organic geochemical approaches to temperature reconstructions over recent decades. Initially, only sea surface temperature could be reconstructed, but the technique has expanded also to lakes and even land. All of the temperature proxies have in common that they use a ratio of two or more lipids from the same biological source, predominantly cell membrane lipids, whose relative abundance is dependent on the growth temperature.

### Long chain alkenones

The discovery of temperature-controlled unsaturation patterns in long-chain ketones (alkenones, LCA) ( $nC_{35}$ – $nC_{42}$  methyl and ethyl ketones) in the mid-1980s and their identification as biomolecules unique to prymnesiophyte algae (a class of predominantly unicellular algae), principally the coccolithophorid *Emiliania huxleyi* (a single celled algae that secretes a calcareous shell, an important constituent of the phytoplankton) (Fig. 1) was a watershed event in paleoclimate research. A sizeable advantage of the alkenone paleotemperature technique, in comparison to other temperature proxies such as Mg/Ca ratios in planktonic Foraminifera or faunal (foraminifera, radiolarians, diatoms) assemblage changes, is the fact that alkenones are preserved in the sediments even after the dissolution of the calcareous remains of their producers. The technique can, therefore, be applied in regions where other techniques would fail (e.g., the Southern or Arctic Oceans). Alkenones have been found in sediments as old as the early Cretaceous and they have been used to reconstruct SSTs on time scales ranging back to millions of years. The  $U^{K'}_{37}$  index expresses the relative abundance of the di-unsaturated  $C_{37}$  alkenone ( $C_{37:2}$ ) compared to that of both the di- and tri-unsaturated ( $C_{37:3}$ ) alkenone:

$$U^{K'}_{37} = [C_{37:2}] / [C_{37:2} + C_{37:3}]$$

An ever-growing amount of ocean sediment core-top samples has been added to a global database over the last decades, allowing calibration of the  $U^{K'}_{37}$  index against SST.



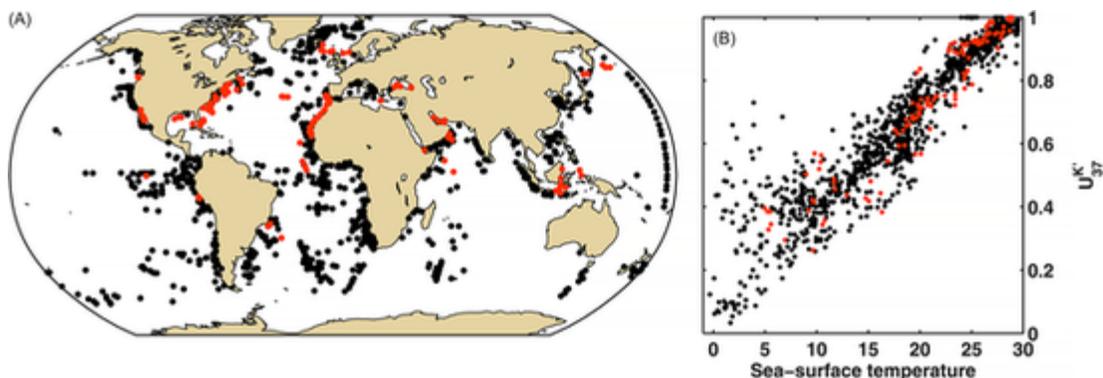
**Fig. 1** Scanning electron micrographs of the haptophyte algae *Emiliana huxleyi* (left) and *Gephyrocapsa oceanica* (right), the primary producers of alkenones in the modern ocean that stand at the base of several paleoclimate proxies. Source: www.microtax.org.

The most recent calibration by (Tierney and Tingley, 2018) (Fig. 2) uses a dataset of over 1300 core-top  $U^{K'}_{37}$  measurements, and is based on a Bayesian regression model. References to earlier calibrations can be found in this publication. For the two decades prior, a linear global calibration based on core-tops from the eastern southern South Atlantic and the global ocean (60°N–60°S) ( $U^{K'}_{37} = 0.033 T + 0.044$ ) was the one most widely used. Although the alkenone SST proxy generally gives good results, there is evidence that the coupling between the core-top alkenone temperature signal and overlying surface waters can be significantly compromised in regions with strong deeper currents and where there are large temperature and productivity gradients. Also of note is that the production of LCA is strongly seasonal. (Tierney and Tingley, 2018) found significant attenuation of the  $U^{K'}_{37}$  response to SST at warm temperatures (>24 °C), with the slope reduced by nearly 50% as  $U^{K'}_{37}$  approaches unity.

Long chain alkenones have also been detected in lakes, where they are produced by several species of haptophyte algae, which can now be phylogenetically separated into Group II alkenone-producers that are common in saline and brackish environments, and Group I haptophytes that are the common freshwater-dwelling alkenone-producers. A range of lacustrine temperature calibrations exist but many are influenced by local effects of lake stratification, large seasonal temperature changes and blooming period, and haptophyte species diversity. However, the newest phylogenetic insights, use of ancient DNA techniques, and improved separation of the entire suite of unsaturated alkenones by gas chromatography now allow further development of new indexes based on unsaturated alkenones also in fresh water systems, for instance leading to calibrations for polar lakes and using different combinations of the various LCA beyond the traditional  $C_{37:2}$  and  $C_{37:3}$ .

### Glycerol dialkyl glycerol tetraether (GDGT)-based paleothermometry

Over the last two decades, glycerol dialkyl glycerol tetraethers (GDGTs) (Fig. 3.Fig. 3) have become one of the most investigated lipid classes in marine and terrestrial organic geochemical research. GDGTs are microbial membrane core lipids that are biosynthesized as mul-



**Fig. 2** (A) Locations of the core-top samples in the alkenone calibration database ( $n = 1344$ ). (B) Core-top  $U^{K'}_{37}$  values versus observed sea surface temperature. Red points denote new data as a part of the study of Tierney and Tingley (2018). Reproduced with permission from (2018).

multiple homolog series of isoprenoid or methyl-branched isomers (isoGDGTs and brGDGTs, respectively), whose relative abundance depends on a range of environmental parameters, in particular temperature. This has led to the development of GDGT-based temperature proxies, but it is also expanding to other biogeochemical parameters.

### Archaeal derived isoprenoid GDGTs and the $TEX_{86}$ proxy

Archaea, and more specifically *Thaumarchaeota*, are an abundant and diverse group of prokaryotes, inhabiting a range of marine, freshwater, terrestrial and extreme environments. They have a deeply branching phylogeny emanating from ancient hot environments. Thaumarchaeal cell membranes are predominantly comprised of isoprenoid glycerol dialkyl glycerol tetraethers (isoGDGTs, Fig. 3.Fig. 3) lipids which are biosynthetically regulated in response to environmental change (temperature, pH, buffering capacity) and are typically resistant to chemical decomposition after cell death. Commercial availability of high performance liquid chromatography - mass spectrometry (HPLC-MS) around the turn of the millennium made it possible to detect these lipids in environmental samples, and (Schouten et al., 2002) was the first to suggest that the number of cyclopentane moieties in isoGDGTs within marine sediments was related to SST. Sediments from colder regions contained mostly GDGT-0 and crenarchaeol whereas warmer settings had a higher relative concentration of isoGDGTs with one to three pentacyclic rings and a crenarchaeol isomer. This led to the formulation of the tetraether index with 86 carbon atoms ( $TEX_{86}$ ) (Box 1).

GDGT-0 and crenarchaeol were excluded from  $TEX_{86}$  because their inclusion dilutes the influence of other minor GDGTs. In addition, GDGT-0 can also have additional sources (e.g., methanogenic archaea), as discussed further below. Similar to the  $U^{K'}_{37}$  proxy, the initial temperature calibration based on only 44 surface sediment samples was successively superseded by newer calibrations based on a growing amount core-top samples, and some specific for certain settings like very warm or very low SST regions. The most recent one is also based on a Bayesian-based calibration, using 1095  $TEX_{86}$  values from marine surface sediments (Tierney and Tingley, 2015). The  $TEX_{86}$  paleothermometer has been used to reconstruct SST over the past 190 million years of Earth history.

There are a number of constraints and considerations to be made when using the  $TEX_{86}$  proxy. Its variation in marine sediments is explained for 70% by SST (Tierney and Tingley, 2015), meaning that 30% of its variation is caused by other environmental parameters and effects. Below follows a summary of these ().

#### Box 1

. Selected equations that are the basis for GDGT-based paleotemperature calibrations. For some equations, measured concentrations are used (indicated by square brackets), while more recently more use is being made of the fractional abundance (FA, indicated by round brackets) of GDGTs compared to the sum. A somewhat overwhelming amount of other GDGT-based equations and proxies exist that relate to specific environmental factors or sources. The reader is referred to the specific GDGT literature.

Box 1

$$TEX_{86} = \frac{[GDGT - 2] + [GDGT - 3] + [Cren']}{[GDGT - 1] + [GDGT - 2] + [GDGT - 3] + [Cren']}$$

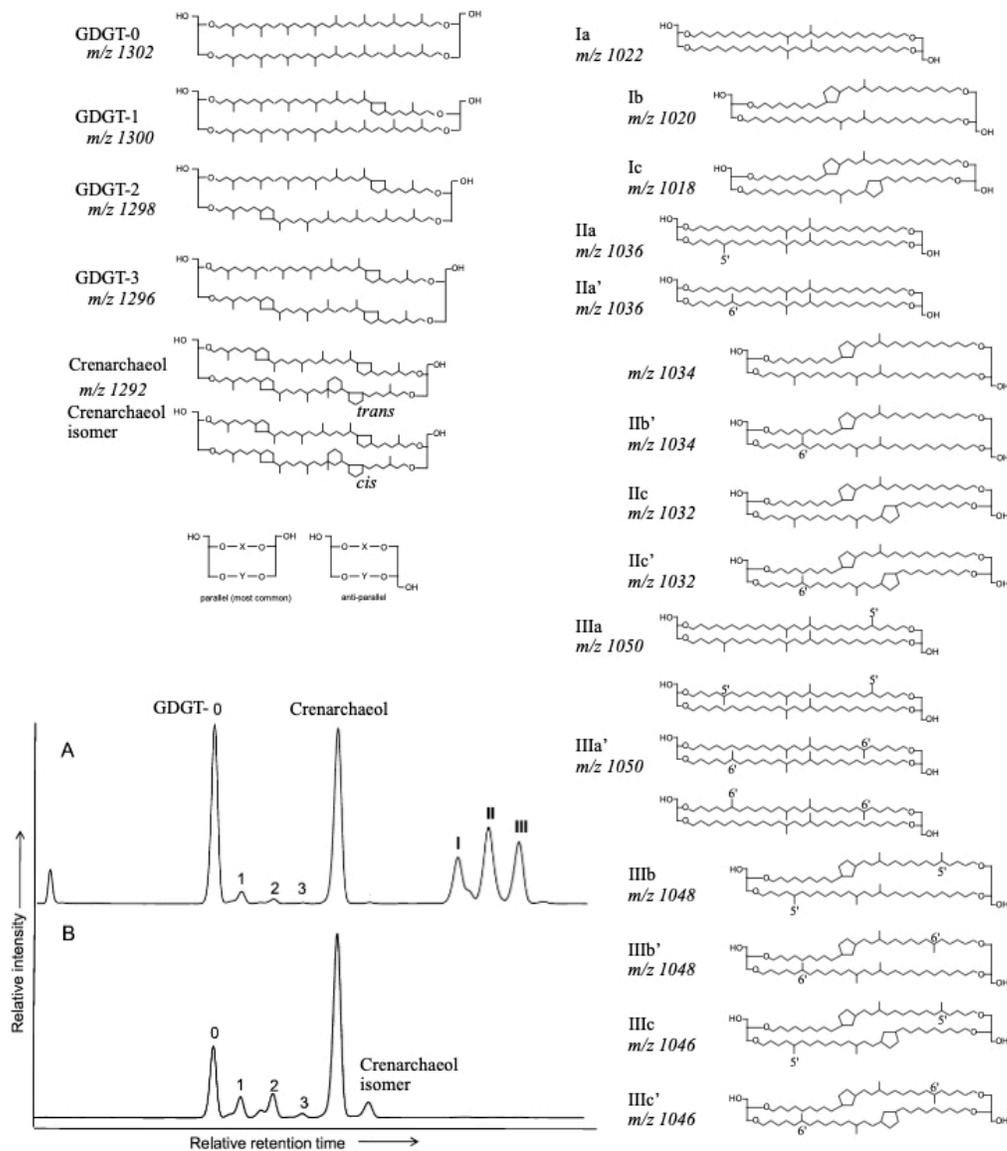
$$\%GDGT - 0 = \frac{[GDGT - 0]}{[GDGT - 0] + [Cren]}$$

$$\begin{aligned} \text{Ring Index (RI)} &= 0 \times (GDGT - 0) + 1 \times (GDGT - 1) + 2 \\ &\quad \times (GDGT - 2) + 3 \times (GDGT - 3) + 4 \times (Cren + Cren') \\ &\quad \text{(using the FA of the indicated GDGTs)} \end{aligned}$$

$$\text{Methane Index (MI)} = \frac{[GDGT - 1] + [GDGT - 2] + [GDGT - 3]}{[GDGT - 1] + [GDGT - 2] + [GDGT - 3] + [Cren] + [Cren']}$$

$$\text{BIT index} = \frac{[Ia + IIa + IIIa + IIa' + IIIa']}{[Ia + IIa + IIIa + IIa' + IIIa' + cren]}$$

$$MBT'_{5ME} = \frac{(Ia + Ib + Ic)}{(Ia + Ib + Ic + IIa + IIb + IIc + IIIa)} \text{ using the FA based on all brGDGTs shown in Fig 3.}$$



**Fig. 3** Core structures of GDGTs commonly found in marine and lake sediments, with the associated mass-to-charge ratio ( $m/z$ ) and common numeration used in the literature and in this article. On the left isoprenoidal GDGTs that are at the basis of the  $\text{TEX}_{86}$  proxy, on the right the branched GDGTs, been found 'At the bottom left two high-performance liquid chromatography–mass spectrometry (HPLC-MS) chromatograms of a 'cold' and 'warm' sedimentary GDGT distribution are shown. A: Sample from the Norwegian Drammensfjord, with a  $\text{TEX}_{86}$  value of 0.42 and a BIT index value of around 0.6. B: Sample from the Arabian Sea, with a  $\text{TEX}_{86}$  value of 0.68 and a BIT index of  $<0.05$ . Modified after Schouten et al. (2009) and (De Jonge et al., 2014).

- 1) Although the  $\text{TEX}_{86}$  proxy correlates strongly to SST or temperatures present between 0 and 200 m water depth, *Thaumarchaeota* are chemolithoautotrophic ammonium oxidizers that are most abundant just below the upper photic zone (i.e., where nitrification rates are highest) with a maximum abundance between 50 and 300 m, that is, within and just below the mixed layer of the ocean. Since there is a high correlation between subsurface temperatures and SSTs,  $\text{TEX}_{86}$  remains useful as an SST proxy even if GDGTs are produced lower in the mixed layer. However, in locations with a shallow thermocline water, subsurface variations can be decoupled from SSTs, and in such situations  $\text{TEX}_{86}$  is better treated as a thermocline proxy. The same is true for locations with a distinct oxygen minimum zone where high ammonium concentrations result in a strong subsurface production of isoGDGTs and related temperature signal.
- 2) Exogenous sources of GDGTs (i.e., those other than marine *Thaumarchaeota*) can complicate the interpretation of  $\text{TEX}_{86}$  values. However, several indices have been developed to screen for such secondary inputs. The branched-to-isoprenoidal tetraether (BIT) index can be used to assess whether terrestrial input of isoGDGTs impacts  $\text{TEX}_{86}$ . Early work argued that  $\text{TEX}_{86}$  estimates with BIT values  $>0.4$  should not be used for SST reconstruction (Weijers et al., 2007). Methanogenic and methanotrophic archaea synthesize

isoGDGTs with zero to three cyclopentane rings and thus can influence TEX<sub>86</sub> values in places where there is substantial methane production and/or oxidation. The %GDGT-0 index is a qualitative indicator for the contribution of methanogenic archaea to the sedimentary GDGT pool: when %GDGT-0 values exceed 67% The Methane Index (MI) can be used to assess the impact of anaerobic methanotrophy on TEX<sub>86</sub> where low values (<0.3) indicate normal, marine conditions and high values (>0.5) indicate high rates of anaerobic methane oxidation. The Ring Index (RI) – which represents the weighted average of cyclopentane moieties in GDGT compounds – can also distinguish soil and/or methane-impacted samples. It can also help to quantify the extent to which samples deviate from the modern TEX<sub>86</sub>–RI relationship ( $\Delta$ RI). Samples with  $\Delta$ RI values >0.3 may indicate potentially problematic TEX<sub>86</sub> values.

- 3) In paleoclimate studies, a warm summer bias (especially in the high latitudes) is often cited to account for mismatches between temperature proxies (e.g.,  $\delta^{18}\text{O}$ , Mg/Ca) or conflicting climate model outputs. However, the relatively deep habitat of the thaumarchaeota and their chemoautotrophic lifestyle appear to limit the effect of seasonality on TEX<sub>86</sub> values. The TEX<sub>86</sub>–temperature relationship is not improved when using seasonal mean ocean temperatures and calibration residuals are not suggestive of a strong seasonal influence (Tierney and Tingley, 2015).
- 4) TEX<sub>86</sub> can also be influenced by physiological and environmental controls. It has been found that oxygen (O<sub>2</sub>) concentration greatly influences membrane lipid composition, leading to significant increases in TEX<sub>86</sub>-derived temperatures with increasing O<sub>2</sub> limitation. This finding necessitates a reassessment of archaeal lipid-based paleoclimate proxies, particularly when applied to environments where O<sub>2</sub> is depleted. TEX<sub>86</sub> data may thus have a warm bias in oxygen depleted settings like oxygen minimum zones at locations with much upwelling and surface productivity, but may also have been more widespread in the geological past under greenhouse conditions when oceanic anoxia was more prevalent. Note that especially these settings produce organic rich sediments from where organic proxies can readily be extracted.
- 5) The TEX<sub>86</sub> paleothermometer can be influenced by thermal maturation in deeply buried sediments; TEX<sub>86</sub> values decline above 240–260 °C due to preferential degradation of core GDGTs with an increasing number of cyclopentane moieties.

To summarize, the entire GDGT distribution should always be investigated before blindly applying the TEX<sub>86</sub> proxy to reconstruct SST.

To summarize, over the last two decades the TEX<sub>86</sub> paleothermometer has led to important inferences concerning past temperature variability on Earth over the last 190 million years. A major outstanding challenge is the large uncertainty in TEX<sub>86</sub>-derived SSTs in past warm climates, for which no present-day analog exists. There remains significant value in employing TEX<sub>86</sub> alongside other SST proxies (e.g.  $\delta^{18}\text{O}$ ,  $\Delta$ 47, Mg/Ca), as this allows more accurate and robust quantification of temperature trends and anomalies.

### Branched GDGTs

Branched GDGTs (brGDGTs) contain core alkyl chains with one or more methyl groups (Fig. 2), and were originally attributed to a pure terrestrial source as they were thought to be produced by an unknown group of soil bacteria (Weijers et al., 2007). It has later been found that they are also produced in aquatic systems albeit predominant in fresh waters. The distribution of brGDGTs in soils, peats and lacustrine sediments has been found to relate to mainly to mean annual air temperature, but to a certain degree also to pH, lake conductivity and alkalinity. This has led to the development of a terrestrial paleotemperature proxy, and has been a major development in paleoclimate research. Most terrestrial-based paleotemperature proxies are transfer functions based on biological parameters, e.g., pollen assemblages that are semi-quantitative at best, and are heavily biased geographically to the mid latitudes.

Initially, the Methylation of Branched Tetraether (MBT) index was used, with a correction for the influence of pH on the GDGT distribution via the Cyclization Index of tetraether (CBT) index (Weijers et al., 2007). Improved separation of the brGDGTs led to the identification of positional isomers with the methyl groups at different locations on the alkyl chain (De Jonge et al., 2014). The relative distribution of 5-methyl-brGDGTs, expressed in the MBT'<sub>5me</sub> index (see Box 1) was subsequently found to correlate best with mean annual air temperature (MAAT), which removed the need of a correction for pH. By now, large suite of calibrations have seen the light of day, sometimes for specific regions or settings. This started for the original MBT and CBT indices for soils, and later for the MBT'<sub>5me</sub> index. Separate calibrations now exist for soils, peats and lakes. Despite the good correlation between MBT'<sub>5me</sub> and temperature, and relative insensitivity toward pH, there is a considerable residual error in all calibrations. There have been findings that shifts in environmental conditions can also cause changes in MBT'<sub>5me</sub>, decoupled from temperature variations. This has been proposed to be caused by changes in the brGDGT producing bacterial community, which is influenced by the source environment. Fractional abundances of brGDGTs, and thus the temperature calibrations, are distinct in lakes compared to soils or peats in the same ambient temperatures. Current research focuses on finding out significant relationships between the (relative) concentrations of specific brGDGTs and specific sources and environmental factors. Here, the relative abundance of brGDGT structures other than shown in Fig. 3 appears promising, for instance those with a central carbon-carbon bond between the two alkyl chains (H-shaped GDGTs).

This indicates that it is critical to understand the influence of different GDGT sources to a particular sedimentary setting - which can have mixed source - before applying one or the other calibration. Moreover, there is a large bias in the global brGDGT datasets toward the mid latitudes, while barely any brGDGT studies in soils and peats have been conducted in tropical rainforest ecosystems.

To conclude, the brGDGT-based paleotemperature proxy offers a unique way to reconstruct terrestrial temperature. However, a careful assessment of potential sources needs to be made, because changes therein can have a strong overprint over any temperature effect on the brGDGT distribution. This can be obtained by investigating other sedimentary information, for instance carbon content, bulk density, or X-Ray scanning. As best practice, sedimentary sequences should be selected that are monotonous in their source (e.g., only lake or peat) and if possible, any calibration that is used should be confirmed by comparing estimates from recent sediments with the instrumental record.

The brGDGT-based paleotemperature proxy has not been as widely used as the SST proxies described above, due to the confounding factors mentioned here, but is increasingly resulting in insights the evolution of and temperatures from the last glacial maximum to the present.

### Long-chain diols

Long chain alkyl diols (LCDs) consist of linear alkyl chains with one terminal alcohol group and one at a midchain carbon position. They predominantly consist of even-numbered carbon chains with 28 to 32 carbon atoms and midchain alcohol groups positioned at C-13, C-14, or C-15 (Rampen et al., 2022). Their relative distribution has over the last decade been investigated as another potential sea surface temperature. Recently, (Rampen et al., 2022) found that an earlier proposed LDI index:

$$\text{LDI} = (C_{30} \text{ 1, 15 - diol}) / ((C_{25} \text{ 1, 13 - diol}) + (C_{30} \text{ 1, 13 - diol}) + (C_{30} \text{ 1, 15 - diol}))$$

using fractional abundances of the long-chain diols, primarily registers temperatures from the warmest month when mixed-layer depths, salinity, and nutrient concentrations are low. The lipids are produced by a globally occurring group of marine eustigmatophyte algae (a relatively small group of unicellular photosynthetic algae, of which only some are marine dwelling). According to (Rampen et al., 2022), the LDI may not be applicable in areas where *Proboscia* diatoms contribute 1,13-diols, but this can be recognized by enhanced contributions of C<sub>28</sub> 1,12 diol. Freshwater input may also affect the correlation between temperature and the LDI, but here, the relative abundance of C<sub>32</sub> 1,15-diol helps to identify and correct for this effect. Details about the calibration between SST and LDI, and the proposed correction, can be found in (Rampen et al., 2022). The calibration error of the LDI is 2.4 °C, which compares well with other SST proxies, and the LDI thus holds promise to provide the previously inaccessible paleoclimate information of warmest month temperature, which could especially be useful in combination with other proxies for mean annual temperature, thus allowing to estimate past seasonality as well.

### Paleosalinity

Along with temperature, salinity is the most important oceanographic parameter. Its reconstruction can shed light on changes in ocean stratification, water mass circulation, and precipitation, which are tightly linked to climate. Two paleosalinity proxies exist: one based on the relative abundance of long chain alkenones (LCA), in particular that of the tetra-unsaturated ones, and the hydrogen isotopic composition (<sup>2</sup>H/<sup>1</sup>H ratio, normally expressed in delta notation as δ<sup>2</sup>H or δD against Vienna Standard Mean Ocean Water) of organic compounds produced by phytoplankton.

#### Paleosalinity using long chain alkenone distributions

Besides being related to temperature (see above), the relative distribution of tetra-unsaturated (C<sub>37:4</sub>) long chain alkenones (LCA) was initially found to be related to seawater salinity, but this could later not be confirmed for open ocean settings. Interest remained however, once it became clearer that alkenones are also common in brackish and even fresh water systems. For instance, utilizing the salinity gradient of the Baltic Sea proper, (Kaiser et al., 2017) found that the relative abundance of C<sub>37:4</sub> LCA (%C<sub>37:4</sub>) did correlate with salinity. By comparing LCA distributions and combining this with genetic analysis, further evidence was found that LCA distributions relate to salinity, and mainly so because of the existence of different groups of LCA-producing *Isochrysidales* algae: Group III *Isochrysidales* is represented by *E. Huxleyi* and *G. Oceanica* strains found only in the marine environment. Group II *Isochrysidales* were found in marine (coastal) and lacustrine environments, and Group I *Isochrysidales* comprises exclusively uncultured haptophytes that are known only from lacustrine environments. These phylotypes occupy different salinity ranges, and produce a wide range of LCA, also including isomers with different position of the unsaturations. The mechanism that restricts phylotypes to specific salinity ranges is unknown but may be related to different evolutionary transitions of the phylotypes from marine to terrestrial environments. Kaiser et al. (2019) propose a new index based on the ratio of isomeric ketones with 37 carbon atoms, which reflects the relative abundance of the different groups, because they found that this correlates well with salinity in oligohaline environments:

$$\text{RIK}_{37} = [C_{37:3aMe}] / [C_{37:aMe} + C_{37:3b}]$$

where the ‘a’ is the common Δ<sup>7,14,21</sup> isomer and the ‘b’ is a novel Δ<sup>14,21,28</sup> isomer (i.e., with unsaturations at different position in the carbon chain).

To summarize, there have been promising developments in the use of long chain alkenone distributions to reconstruct past salinity, especially in combination with genetic analysis.

### The lipid $\delta^2\text{H}$ paleosalinity proxy

The hydrogen isotopic composition ( $\delta^2\text{H}$  or  $\delta\text{D}$ , expressed in delta notation against Vienna Standard Mean Ocean Water) of lipids from plants and algae reflect the  $\delta^2\text{H}$  value of environmental water (Sachse et al., 2012), and in marine environments this is closely related to salinity: fresh water is isotopically depleted compared to ocean water. Early studies found that hydrogen isotope ratios of long-chain alkenones ( $\delta^2\text{H}_{\text{C}_{37}}$ ) reflect salinity in culture studies and proposed to use this as a tool to reconstruct sea surface salinity (SSS) in the geologic record. Initially, isotopic fractionation against deuterium ( $\alpha_{\text{C}_{37}}$ ) during biosynthesis was also shown to increase with salinity in several culture studies (which would thus make the proxy more sensitive), but this was later not observed when testing this proxy using marine sediments: (Weiss et al., 2019) found a significant linear correlation between  $\delta^2\text{H}_{\text{C}_{37}}$  and mean annual SSS ( $r = 0.75$ ,  $p < 0.0001$ ). Other studies have also found good correlations between  $\delta^2\text{H}_{\text{water}}$ , salinity, and other lipids, for instance dinosterol, produced by dinoflagellates. This follows the general observation (further detailed below) that the  $\delta^2\text{H}$  value of algal lipids reflect that of the source water and other lipids could thus potentially be used as well, granted that salinity is the main driver of changes in source water  $\delta^2\text{H}$ . In all cases, a local calibration of the between  $\delta^2\text{H}_{\text{lipid}}$ ,  $\delta^2\text{H}_{\text{water}}$  and salinity needs to be made.

### Paleohydrology using compound-specific $\delta^2\text{H}$

The hydrogen isotopic composition ( $\delta^2\text{H}$ ) of lipid biomarkers has become an established hydroclimatic proxy over the last decade (Freimuth, 2018; Sachse et al., 2012). The method has predominantly been applied using two types of lipids produced by photosynthetic organisms: algal lipids and plant leaf waxes. Plant waxes and algal lipids are common in lacustrine and ocean sediments, providing widely distributed records of hydrologic change in locations where other water isotope proxies (e.g., ice sheets, cave deposits) do not exist. The principle is the same as for other recorders of the oxygen and hydrogen isotopic composition of environmental water: the hydrological cycle causes fractionation and distillation of the heavy water isotopes upon cycles of evaporation and precipitation. This, in turn, can be used to trace back these processes. Sachse et al. (2012) provide a good review about the basic principles, while subsequent investigations (e.g., Freimuth, 2018) provide further insight in the factors that influence the transmission of the original source water signal from the plant level to the catchment scale and ultimately the sedimentary record. Photosynthetic organisms use their ambient water as their primary source of hydrogen for their biosynthesis, and the lipids they produce reflect, at first order, the source water signal. However, biosynthesis and evapotranspiration induce a net fractionation between source water and lipids that can range between approximately  $-100\text{‰}$  to  $-270\text{‰}$ , depending on organism and lipid type.

A range of studies have confirmed that algal lipid  $\delta^2\text{H}$  generally tracks that of the source water, but biosynthetic fractionation between source water and lipid ( $\alpha_{\text{lipid/water}}$ ) can differ substantially between lipids produced by the same organism, but also between the same lipids produced by different organisms (Zhang and Sachs, 2007). As a consequence, interpretation of  $\delta^2\text{H}$  records from common lipids like  $\text{C}_{16}$  fatty acids is treacherous, and highly specific algal lipids are a better choice: for instance, the botryococenes - hydrocarbons made by the common fresh water green alga *Botryococcus Braunii*; dinosterol - which is specific for dinoflagellates; or highly branched isoprenoids - specific for diatoms.

The biosynthetic fractionation of leaf wax-derived *n*-alkanes is more constrained, and together with the fact that these hydrophobic lipids are both abundant in the sedimentary record and readily isolated, has made them a primary target for compound-specific  $\delta^2\text{H}$  analysis. The  $\delta^2\text{H}$  values of mid-chain *n*-alkanes (odd numbered  $\text{C}_{17}\text{-C}_{25}$ ) are mainly produced by aquatic organisms including aquatic plants, and therefore reflect lake or wetland water  $\delta^2\text{H}$ . This in turn is dictated by the weighted average of incoming precipitation and runoff, and evaporation that causes residual enrichment of deuterium.  $\delta^2\text{H}$  values of terrestrial plant leaf wax-derived longer chain homologs (mainly odd-numbered  $\text{C}_{27}\text{-C}_{31}$  alkanes) reflect that of the xylem water in the stems and leaves, ultimately derived from rainfed groundwater. The xylem water and leaf wax  $\delta^2\text{H}$  can be isotopically enriched by evapotranspiration.

The offset between  $\delta^2\text{H}$  values of terrestrial and aquatic lipids has been proposed as a possible proxy for relative humidity, arguing that lake derived lipids are not affected by (soil and leaf) evapotranspiration, in contrast to lipids from terrestrial plants. However, much depends on the local conditions like catchment size and geography (depth/surface area), turnover time of the lake water influenced by influx and evaporation, and therefore response time of its isotopic signal, groundwater hydrology, etcetera.

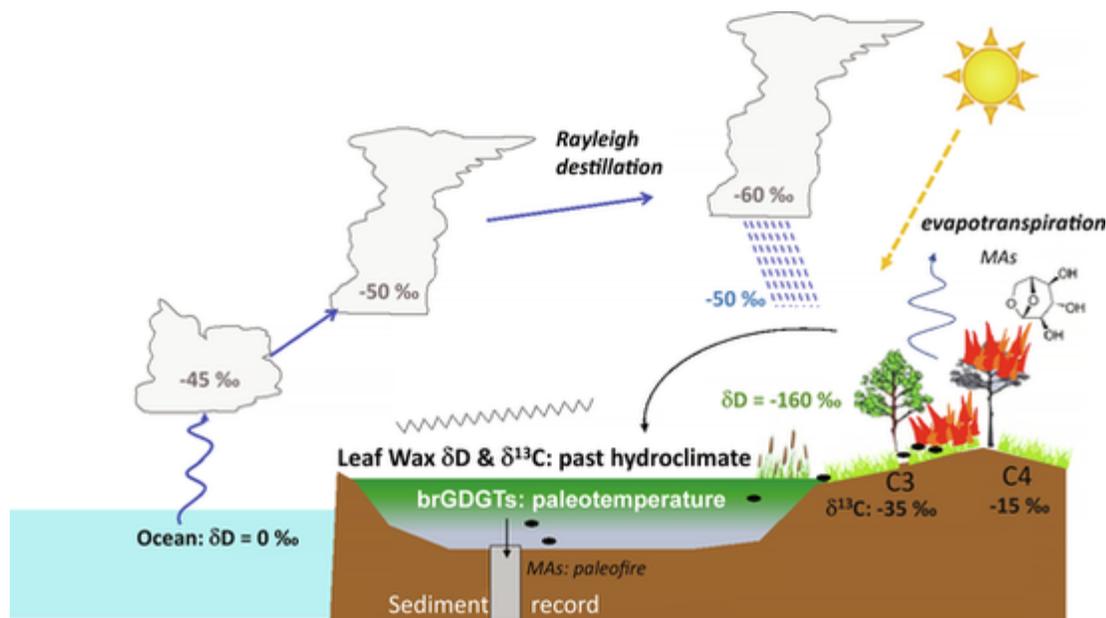
Quantitative interpretation of  $\delta^2\text{H}_{\text{wax}}$  as a proxy for past precipitation should be done with caution, due to a multitude of confounding biological, ecological and environmental factors such as the primary season of lipid production, light intensity, growth rate, growth stage, salinity and vegetation changes. However, a number of studies have shown that despite large variations on the local and individual plant level scale, that this signal noise is to large degree cancelled out on the catchment scale. Careful analysis and comparison of individual lipids compared to the mean may give insight in changes in the provenance of these lipids (e.g.,  $\text{C}_4$  and  $\text{C}_3$  grasses versus trees, aquatic versus terrestrial plants), and can further be used to constrain the original source water signal. For certain environments, where vegetation changes can be well constrained and found to be minor compared to changes in hydrological conditions,  $\delta^2\text{H}_{\text{wax}}$  can be used semi-quantitatively. For instance, in humid tropical forest systems  $\delta^2\text{H}_{\text{wax}}$  is typically depleted by  $130\text{‰}$  relative to the source water (Feakins et al., 2016). In any case, if lipids derived from different sources show consistent trends and their  $\delta^2\text{H}$  values covary with sediment core depth, then it can be assumed that this reflects changes in the primary source water signal. On the other hand, the changes in the  $\delta^2\text{H}$  depth profiles of lipids from different sources can provide insight in more detailed hydrological changes, for instance past seasonality of rainfall and drought.

To conclude, the hydrogen isotopic composition of algal lipid and leaf wax derived *n*-alkanes can provide valuable paleohydrological information, but some critical assumptions need to be tested: (1) The compound-specific  $\delta^2\text{H}$  values are correlated to the study region's meteoric water  $\delta^2\text{H}$  values, combined with evaporative enrichment; (2) In case of leaf waxes, modulation of the primary climate signal by vegetation change is limited or well constrained. (3) Seasonal bias remains constant through time: the signal of leaf waxes typically reflects that of the early growth season, while the  $\delta^2\text{H}$  value of algal lipids reflects the water signal from the period of main algal bloom. To interpret conclusions that are based on lipid  $\delta^2\text{H}$  values as a paleohydrological proxy, complementary multiproxy analysis is critical. In almost all cases a qualitative assessment can be made about past conditions, which include changes in source moisture, rainfall amount, and rainfall seasonality (Fig. 4).

### Molecular indicators of paleofire

Biomass burning leads to the release of carbon dioxide ( $\text{CO}_2$ ), methane ( $\text{CH}_4$ ) and other greenhouse gases, aerosols and particulates such as soot. These emissions affect global climate in various ways. Biomass burning influences the carbon cycle by altering vegetation growth and succession trajectories. Fire-vegetation interactions can determine whether an ecosystem is a net source or sink of atmospheric carbon for a given time interval, in which the long-term fate of pyrogenic Carbon is a critical component. Fire is episodic in nature and thus needs to be investigated from a long-term perspective. Nearly all information about past biomass burning comes from sedimentary charcoal records. However, charcoal forms only part of the pyrogenic Carbon (pyC) continuum, and charcoal records are biased toward lower intensity fires and woody substrates. A range of thermal-chemical and molecular methods exist to investigate other parts of the pyC continuum, which in sedimentary records can be used as paleofire indicators. The majority estimates the abundance of 'Black Carbon' (Hammes et al., 2007), and three molecular ('biomarker') methods exist: (1) The concentration of monosaccharide anhydrides (MAs, anhydrosugars) – produced by the burning of cellulose and thus this is a true marker for biomass burning. (2) the analysis of Benzene Polycarboxylic Acids (BPCAs) that are produced from macromolecular polyaromatic matter (charcoal, soot) upon hot nitric acid digestion of a sample. (3) Polyaromatic Hydrocarbons (PAHs) are derived from the incomplete combustion of organic matter, which in modern times includes fossil fuels.

The MAs levoglucosan, galactosan and mannosan are generated exclusively by the pyrolysis and combustion of cellulose and hemicellulose. MAs are some of the only unique organic markers for fire and used extensively in aerosol research. They are even regarded as a valuable biomass waste product. However, they have not yet seen wide use as tracers in terrestrial or marine research because our understanding of their biogeochemistry and transport through the Earth system is poorly constrained. MAs are chemically reactive in all phases (gaseous, aqueous and particulate), semivolatile, water-soluble, and biodegradable. This means that MAs are not conservative tracers, and



**Fig. 4** Cartoon of the application of four different molecular paleoclimate proxies obtained from a lake sediment record. The hydrogen isotopic composition ( $\delta\text{D}$  or  $\delta^2\text{H}$ ) of leaf wax-derived long chain alkanes buried in the sediment reflects that of the compounded isotope effects taking place in the hydrological cycle. BrGDGTs can be used to reconstruct past temperature. Monosaccharide anhydrides (MAs) produced by biomass burning can be used as a proxy for past fire. Drought and fire-induced vegetation changes between a dominance of C3 (trees) to C4 (grasses) vegetation can be traced using the stable carbon isotopic composition ( $\delta^{13}\text{C}$ ) of the leaf waxes.

changes in organic matter transport and degradation need to be considered when interpreting sedimentary records. A few studies exist where MAs have been used as tracers of paleofire, for instance in Lake Bosumtwi, Ghana, marine sediments south of Java, and lake El Lake El'gygytgyn in Eastern Siberia, in conjunction with other paleoclimate proxies. Although MAs are thus a promising new organic marker specifically for biomass burning (contrasting fossil fuel burning), their relatively high degradability and solubility needs to be taken into account especially for sedimentary records with changing transport and burial conditions. Also, the studies mentioned above used methods that were not thoroughly tested for potential analytical biases.

While MAs are only formed at low burning temperatures (150–350 °C) and thus reflect low intensity fires, pyrogenic PAHs are predominantly produced via incomplete combustion of organics between 300 and 600 °C (Karp et al., 2020). In the present day, PAHs are also massively produced by the burning of fossil fuels, but this was obviously not the case earlier and they thus convey signatures of vegetation burned in the geologic past. In a detailed study of PAHs as paleofire markers (Karp et al., 2020) found that PAHs tend to be smaller in smoke that is larger in residues, consistent with molecular physical and chemical properties. Retene and dimethyl phenanthrene ratios distinguished gymnosperm from angiosperm sources, and woody angiosperms from grasses and woody gymnosperms. PAHs also reflect the carbon isotopic signatures of their source material, e.g., C3 and C4 plant types. PAH size, alkylation, and isotope characteristics can thus differentiate combusted plant types and distinguish between air-borne and sedimentary transport mechanisms. These new proxy approaches using PAH amounts, distributions, and isotope signatures can aid and refine interpretations of paleofire ecology in the geologic record. On the other hand, PAHs in sedimentary and geologic records may also derive from increased erosion of PAH-rich soil and thereby not related to biomass burning in the near environment.

A third molecular method to trace the presence of pyC in environmental samples, including sedimentary records, is benzenepolycarboxylic acid (BPCA) analysis. These monomers are produced from polyaromatic material upon hot nitric acid digestion (Wiedemeier et al., 2016). Despite having minimally overlapping analytical windows and traditionally serving different research purposes, both PAH and BPCA analysis are both highly informative for pyC characterization as they provide valuable information about its molecular composition, in particular the degree of condensation. This is strongly linked to combustion conditions (temperature and oxygen availability). In a sedimentary record, the sum of BPCAs indicates changes in influx of black carbon, whereas the composition of BPCAs, i.e., the ratio of pentacarboxylic acid to mellitic acid (B5CA to B6CA, with five respectively six carboxylic acid groups), indicates changes in the general fire regime such as the fire temperature. The combination of both PAH and the BPCA method can lead to a better source apportionment of PyOM in soils and sediments and illuminate the pathways and fluxes of differently sized and pyrolyzed PyOM through environmental compartments. Even more information about PyOM in the environment can be retrieved from compound-specific isotopic analysis of both PAH and BPCA molecular markers.

## Past sea ice

Significant decrease in Arctic and Antarctic sea ice in recent decades has prompted the development and application of novel approaches to the reconstruction of past sea ice conditions over much longer timeframes. One such approach is based on the variable distribution of certain source-specific highly branched isoprenoid (HBI) lipid biomarkers in well-dated marine sediment records. C<sub>25</sub> and C<sub>30</sub> HBIs are ubiquitous components in recent sediments that appear to originate from four genera of marine diatoms. However, in 2007, a unique monounsaturated C<sub>25</sub> HBI was identified to be produced by certain diatoms that live under (especially seasonal) sea ice conditions. This molecule was coined the IP<sub>25</sub> proxy (ice proxy with 25 carbons). Since then, more studies confirmed the validity of this proxy which is now well established, and a southern hemisphere sister proxy, the IPSO<sub>25</sub> proxy (Ice Proxy for Southern Oceans with 25 carbon atoms), was added later. In contrast to most other proxies, IP<sub>25</sub> is not a ratio of lipids, but is an on/off proxy: detection of this lipid indicates past sea ice margin conditions. Absence, however, either indicates permanent thick sea ice that does not allow algal growth, or year-round open ocean, or limited export and/or preservation. To assess the extent to which open ocean conditions prevailed, the IP<sub>25</sub> proxy can be analyzed together with other, more general lipid biomarkers indicative of algal growth, like diatomsterol (brassicasterol) from diatoms, dinosterol from dinoflagellates, or long-chain alkenones.

In addition to the proxies based on long chain alkenones, described above, the relative abundance of C<sub>37:4</sub> alkenones (note: also used for sea surface salinity) might also be a proxy for annual mean sea ice concentrations. (Wang et al., 2021) found that a specific lineage of *isochrysoales* (Group 2i) is responsible for elevated C<sub>37:4</sub> methyl alkenones in the northern high latitude oceans. This *isochrysoales* lineage co-occurs widely with sea ice in marine environments and is distinct from other known marine alkenone-producers. In sediment cores from the Svalbard region, the %C<sub>37:4</sub> concentration aligns with the Greenland temperature record and other qualitative regional sea ice records spanning the past 14 thousand years, reflecting sea ice concentrations quantitatively.

## Concluding remarks

As can be deduced from the overview given above, the toolbox of an organic geochemist/paleoclimatologist is rather expansive, and still growing. Besides the lipid biomarkers described above, there are many more biomarker methods that can be used to reconstruct past environmental conditions - which are often influenced by climatic changes. These second order climatic effects on changes in vegetation, ecosystems, limnology, ocean stratification, runoff and erosion, nutrient status, etc., all leave their imprint in the biological remainders preserved in lake and ocean sediments and that can be traced using lipid biomarkers as well. For instance, the stable carbon isotope com-

position ( $\delta^{13}\text{C}$ ) of plants and plant waxes is influenced by water availability, but also by the relative abundance of C3 vs C4 plants, which in turn are influenced by atmospheric  $\text{CO}_2$  level, temperature and rainfall seasonality (Dubois et al., 2014; Ehleringer et al., 1997). The unravelling of environmental and climatic factors is often not fully possible, and interpretation of the paleoclimate proxies therefore should be done within a larger framework based on knowledge about the depositional environment, organic matter sources, ecological and environmental parameters. These can be gained from sedimentological analysis, and other non-organic proxies like pollen analysis or carbonate-based paleothermometry and paleohydrology, described elsewhere in this volume.

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## Further Reading

- Schouten et al. (2013) provide an extensive review of the sources and application of GDGTs. Although much newer research has been published since, this is a good starting point.
- Eglinton and Eglinton (2008) give a good general review of the use of lipid biomarkers used as paleoclimate proxies, with a number of case studies.
- Belt (2019) provides a recent review of the salient features of IP<sub>25</sub>, IP<sub>SO25</sub> and related biomarkers as proxy for past sea ice, together with aspects that are currently less well understood and potentially provide direction for future research.
- Davtian et al. (2023) recently published a new method that provides robust and repeatable analysis of MAs, irrespective of the sample matrix, applicable to a wide concentration range.
- Separation of branched GDGT isomers is shown in De Jonge et al. (2014), while one of the latest calibrations for soils, based on Bayesian-statistics, can be found in Dearing Crampton-Flood et al., (2020).
- Examples of the use of hydrogen isotopes in paleohydrology: Sachs et al. (2009), Smittenberg et al. (2011), Muschitiello et al. (2015), Kjellman et al. (2022).

- More details of the confounding factors that may influence the application of the TEX<sub>86</sub> proxy can be read in a recent review of Inglis and Tierney (2020). More insights about Anhydrosugars as tracers in the Earth system can be read in Suciu et al. (2019).
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- For lake calibrations of the U<sup>K</sup> proxy, including see references in Longo et al. (2016)).

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\*This is a major update of J.P. Sachs, K. Pahnke, R. Smittenberg, Z. Zhang, PALEOCEANOGRAPHY, BIOLOGICAL PROXIES | Biomarker Indicators of Past Climate, Editor(s): Scott A. Elias, Cary J. Mock, Encyclopedia of Quaternary Science (Second Edition), Elsevier, 2013, Pages 775–782, ISBN 9780444536426, <https://doi.org/10.1016/B978-0-444-53643-3.00280-6>.