Biomarker Indicators of Past Climate

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Introduction

Molecular fossils, 'biomarkers', add to the growing arsenal of climate indicators scientists are using to reconstruct climate history and understand what causes climate to change. Advances in analytical chemistry and instrumentation over the last two decades have expanded the analytical window in which organic geochemists can routinely work. Precise and highly sensitive mass spectrometers, that can determine molecular structures and isotope ratios are now coupled to gas and liquid chromatographs, that can separate the complex mixtures of organic constituents found in geologic samples into pure components.

As more geoscientists become facile with these new techniques there are likely to be tremendous advances in organic geochemical climate proxies. Here we review some of the more promising and novel biomarker proxies now being used in paleoclimate research. The article is organized around the climate parameters of most interest to paleoceanographers and paleoclimatologists: temperature and salinity.

Biomarker Proxies of Temperature

The reconstruction of ocean and lake surface temperatures is central to understanding past climatic changes. Impressive advances have been made in organic geochemical approaches to temperature reconstructions. Here we focus on the three most promising and widely applied organic geochemical temperature proxies: $U^{k'}_{37}$, Tex_{86}, and C_{25} HBI.

Alkenone Paleothermometry

The discovery of temperature-controlled unsaturation patterns in long-chain ketones, alkenones (nC_{37}–nC_{39} methyl and ethyl ketones), in the mid-1980's (Brassell et al., 1986), and their identification as biomolecules unique to prymnesiophyte algae, principally the coccolithophorid Emiliania huxleyi (Volkman et al., 1980; Marlowe et al., 1984), was a watershed event in paleoclimate research. For the first time paleoceanographers had a tool for reconstructing sea-surface temperatures (SST) that was unambiguously derived from organisms living in the uppermost part of the ocean where sunlight can penetrate, that was not influenced by salinity (as, for instance, oxygen isotope ratios in planktonic foraminifera are), and that was not corrupted by the chemical composition of ocean bottom water. Thus, a sizeable advantage of the alkenone paleotemperature technique over other temperature proxies such as Mg/Ca ratios in planktonic foraminifera or faunal (foraminifera, radiolarian, 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 Ocean).

The original definition of the unsaturation ratio (U^k_{37} index) includes the tetra-unsaturated C_{37} alkenone (Brassell et al., 1986), which was later omitted due to its insignificant contribution ($U^{k'}_{37} = C_{37:2}/(C_{37:2} + C_{37:3})$). Prahl et al. (1988) were the first to publish a quantitative calibration of the $U^{k'}_{37}$-index to growth temperature ($U^{k'}_{37} = 0.034T + 0.039$, $r^2 = 0.994$) based on cultured E. huxleyi, and initial field measurements showed that this calibration reproduced SSTs in the northeast Pacific Ocean (Prahl and Wakeham, 1987). This calibration has since been widely used for the reconstruction of past SSTs in most parts of the world's oceans (Sachs and Lehman, 1999). Extensive alkenone analyses on a global set of core-top samples and correlation of the $U^{k'}_{37}$ index to overlying mean annual SSTs ($U^{k'}_{37} = 0.033T + 0.069$, $r^2 = 0.981$) have further confirmed the culture-derived relation of Prahl et al. (1998) (Müller et al., 1998) and demonstrated its applicability for most oceanic areas (**Figure 1**). Though slight differences exist in the $U^{k'}_{37}$-temperature dependence for different coccolithophorid species and genetic variations, past changes in species composition do not compromise paleo-SST reconstructions.

Most studies, including the core-top $U^{k'}_{37}$-SST calibration of Müller et al. (1998), assume that $U^{k'}_{37}$ values reflect temperatures at the sea surface. However, alkenone concentrations in sediment traps indicate that the depth of maximum production varies regionally and can even change between different seasons. Alkenone-producing haptophytes show highest abundance in the surface mixed-layer North Atlantic (Conte et al., 2001) and in the northwest Pacific (Hamanaka et al., 2000). Similarly, Ohkouchi et al. (1999) concluded from a sediment transect in the Pacific, that alkenones are produced in the surface mixed layer at high and low latitudes, while thermocline production prevails at mid-latitudes. In the gyre region of the subtropical North

Global core-top calibration (60° S-60° N)
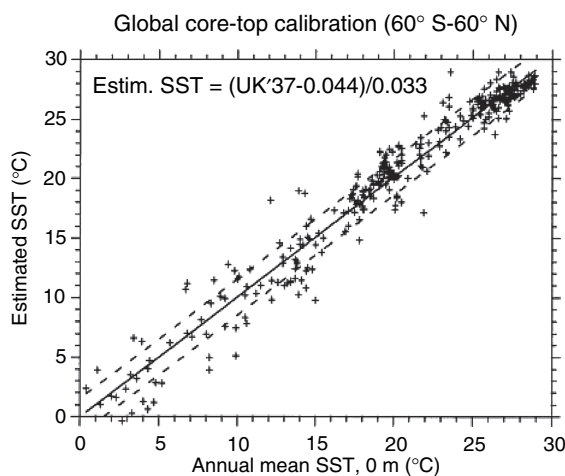
Figure 1 Global core-top calibration of $U^{k'}_{37}$-derived SST *versus* overlying mean annual SST from Müller *et al.* (1998) (SST = $U^{k'}_{37}$ − 0.044)/0.033, r^2 = 0.958).

Pacific, haptophyte growth is linked to the depth of the chlorophyll maximum that can vary in depth throughout the year (Prahl *et al.*, 2005). Alkenone production shows a strong seasonal cycle in most areas (Conte *et al.*, 2001; Prahl *et al.*, 2005), but the unsaturation ratio preserved in the sediments predominantly reflects mean annual temperatures (Müller and Fischer, 2001; Prahl *et al.*, 2005). In contrast, a strong summer to fall bias in $U^{k'}_{37}$-SST estimates is observed in the Subantarctic and Antarctic Pacific and Indian Oceans (Sikes and Volkman, 1993), suggesting that in some areas regional $U^{k'}_{37}$-SST calibrations better reproduce sea surface temperatures.

p0035   Alkenones have been found in sediments as old as the early Cretaceous (Brassell *et al.*, 2004) and used to reconstruct SSTs on time-scales ranging from $10–10^6$ years.

p0040   Despite substantial alkenone loss both in the water column and in the sediment after deposition (Müller and Fischer, 2001), several studies indicate stability of the $U^{k'}_{37}$-index over geologic time and therefore no effect on paleo-SST reconstructions (Müller and Fischer, 2001). However, contrasting studies indicate differential removal of $C_{37:3}$ (Rontani *et al.*, 2005), which can have implications for alkenone paleothermometry, depending on the conditions under which degradation occurred (e.g., anoxic, oxic, denitrifying). Other environmental factors potentially affecting the unsaturation ratio of alkenones include light limitation (causing a bias toward warmer SSTs) and nutrient limitation (bias towards lower SSTs) (Prahl *et al.*, 2003).

Alkenones are analyzed from the total lipid extract   p0045 of sediment samples or 'cleaned' fractions (using a number of successive steps including' for example, hydrolysis and silica column chromatography Villanueva *et al.*, (1997) by gas chromatography (GC) typically coupled to a flame ionization detector (FID). Quantification of di- and tri-unsaturated alkenones by peak integration allows the determination of the $U^{k'}_{37}$-index for SST estimation.

The TEX$_{86}$ Paleotemperature Proxy   s0020

Throughout the 1990s, it was increasingly recognized   p0050 that a certain group of archaea, the kingdom of life previously thought to exist only in extreme environments, were also living in oceans and that these planktonic archaea were actually making up a large part of the pelagic biomass (Karner 2001). It was already known that the membrane lipids of archaea are built up by either glycerol-dialkyl-diethers or glycerol-dialkyl-glycerol-tetraethers (GDGT's) (De Rosa and Gambacorta, 1988), and indeed these lipids were found in large abundance in ocean sediments (Schouten *et al.*, 2000). The advancement of HPLC-MS techniques allowed easy analysis and isolation of intact GDGT's (Hopmans *et al.*, 2000), which led to the structural identification of the crenarchaeota-specific GDGT crenarchaeol (Sinninghe Damsté *et al.*, 2002), and paved the way for discovery of a temperature dependence on DGDT structure during biosynthesis.

Schouten *et al* (2002) found that the extent of   p0055 cyclization in the isoprenoid alkyl chains correlated with the sea surface temperature (**Figure 2**), and in analogy to the $U^{k'}_{37}$ temperature proxy, the Texel (Netherlands) based Tetra-Ether indeX with 86 carbon atoms, Tex$_{86}$, was proposed (**Figure 3**). The original 14 surface sediments used to establish this new proxy was extended to 32 in 2004 (Wuchter, 2004) and is still expanding (**Figure 3**). The current precision of the Tex$_{86}$ temperature proxy is ~0.3 °C, similar to that of the alkenone method (Schouten, pers. comm.).

Non-hyperthermophylic crenarcheota have also   p0060 been discovered in lakes, and the Tex$_{86}$ proxy has been used in lacustrine settings (Powers *et al.*, 2004). In such settings care must be taken to ensure that the sedimentary GDGTs derived primarily from aquatic archaea, as opposed to from soils and peats. This distinction can be made by measuring the so-called Branched-to-Isoprenoidal Tetraether (BIT)-index (Hopmans *et al.*, 2004).

The GDGTs used in the TEX$_{86}$ temperature proxy   p0065 have been found in sediments as old as 112 Ma and used to reconstruct temperatures on sediments as old
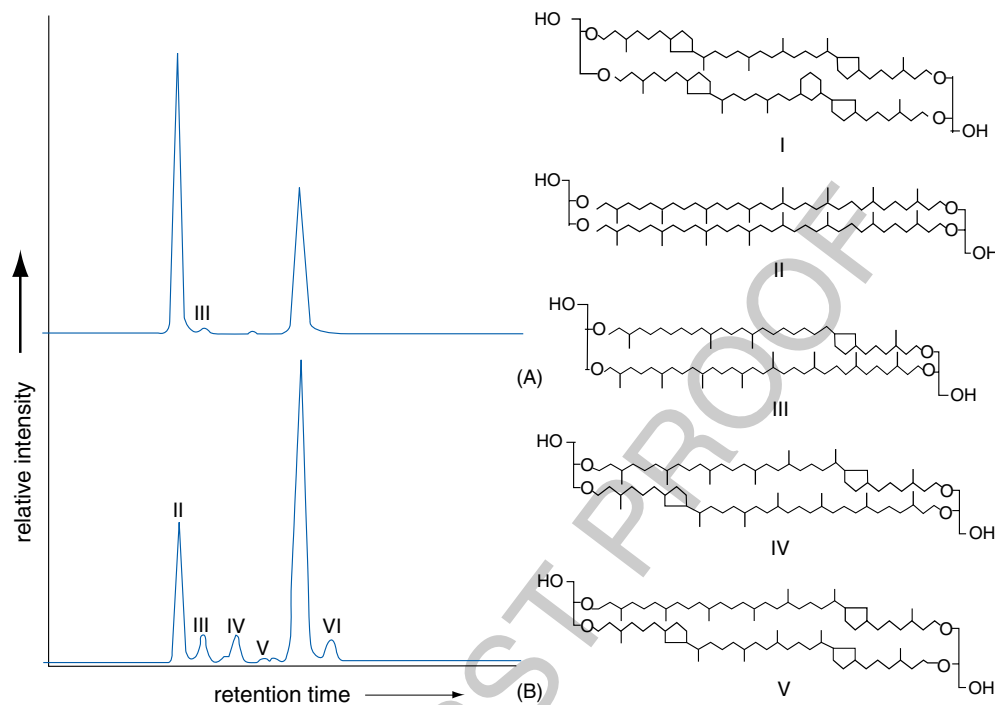
Figure 2 HPLC-MS base peak chromatograms and GDGT structures from Schouten et al. (2004). GDGT distributions in surface sediments from (a) Halley Bay Station, Antarctica and (b) the Arabian Sea. Structure I is crenarchaeol, II-V are GDGTs with increasing amounts of pentacyclic rings. VI is a regio-isomer of crenarchaeol. The Tex$_{86}$ index is calculated by dividing the quantity of each isomers according to: $Tex_{86} = ([IV] + [V] + [VI])/([III] + [IV] + [V] + [VI])$.
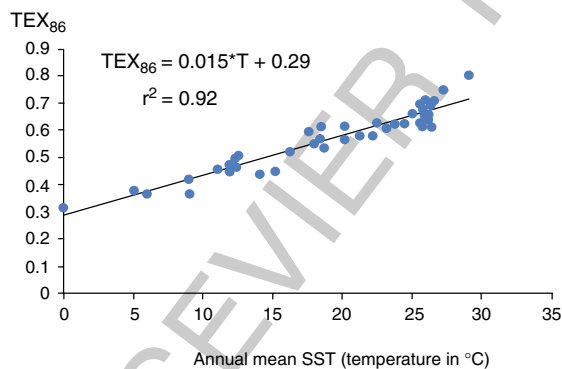
Figure 3 Updated temperature calibration of TEX$_{86}$ from core-top sediments spanning annual mean SSTs from $0°–30°C$ from Wuchter *et al.*, (2004).

as the Cretaceous (Schouten *et al.*, 2003). With ancient sediments, care must be taken to ensure their geological immaturity because high temperatures and pressures can alter GDGT molecular structures (Schouten *et al.*, 2004). The Tex$_{86}$ proxy has expanded the effective age range for biomarker SST reconstructions (> 110 Ma) significantly beyond that of alkenones (*ca*. 6 Ma). Because this proxy is just four years old, and there are few laboratories that

have the instrumentation and expertise to measure GDGT's, published paleotemperature records using TEX$_{86}$ are scarce, limited primarily to the cretaceous (Schouten *et al.*, 2003; Jenkyns *et al.*, 2004) and the late Quaternary (Powers *et al.*, 2005).

The HBI Temperature Proxy

The recent discovery of highly branched isoprenoid (HBI) alkenes in phytoplankton with numbers of unsaturations that co-vary with temperature represents a promising new paleotemperature proxy (Rowland *et al.*, 2001a) (**Figure 4**).

C$_{25}$ and C$_{30}$ HBI alkenes are ubiquitous compo- nents in recent sediments that appear to originate from four genera of marine diatoms (Class Bacillariphyceae), namely: *Rhizosolenia, Haslea, Navicula* and *Pleurosigma* (Grossi *et al.*, 2004). Several C$_{25}$ HBI alkenes (C$_{25:1}$ to C$_{25:6}$) have been characterized in cultures of *Haslea* and *Pleurosigma* (Wraige *et al.*, 1997). C$_{30}$ HBI alkenes have been detected in cultures of *Rhizosolenia sp*. (Rowland *et al.*, 2001b).

The structure and distribution of C$_{25}$ HBI alkenes, termed Haslenes because of their first discovery in *Halsea*, were shown to vary with environmental
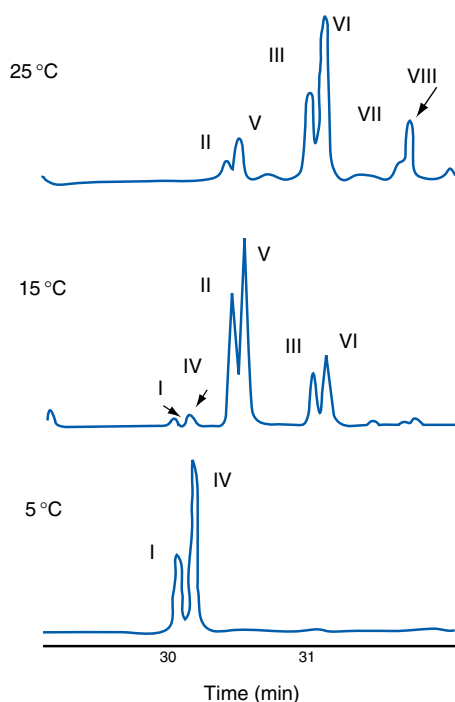
f0020 **Figure 4** Partial gas chromatography illustrating the distributions of C_{25} HBIs in diatom *Haslea ostrearia* cultures grown at 5 (27 days), 15 (7 days) and 25 °C (10 days). Roman letters I, II, III and IV indicate the number of double bonds in the C_{25} HBIs (after Rowland *et al.*, (2001)).

conditions (Wraige *et al.*, 1997). In particular, temperature appeared to strongly influence haslene distributions, while salinity had no appreciable effect (Wraige *et al.*, 1998).

p0085 Rowland *et al.* (2001a) batch-cultured *Haslea ostrearia* over a range of temperatures, keeping salinity and irradiance constant, and demonstrated a strong temperature dependance on haslene unsaturations (**Figure 4**). At 5 °C, only di-unsaturated haslene was produced, while at 15 °C tri-unsaturated haslene predominated. At 25 °C, tetra-unsaturated haslene predominated, accompanied by smaller quantities of pentaenes (Rowland *et al.*, 2001a).

p0090 Similarly, culture studies with the diatom *Rhizosolenia setigera* revealed an increase in the degree of unsaturation of both haslene and C_{30} HBI alkenes as temperatures increased from 18° to 25 °C (Rowland *et al.*, 2001b).

p0095 Although this proxy has not yet been applied in paleotemperature reconstructions, it has great promise as an SST proxy in the high (> 25 °C) and low (< 10 °C) temperature range where the alkenone paleothermometer is not precise (because the concentration of either the di- or tri-unsaturated compounds is exceedingly small, making calculation of the $U^{k'}_{37}$

unsaturation ratio imprecise), in settings where coccolithophorids are not abundant, such as in the Southern Ocean, and as an independent measure of SST when accuracy is important.

Biomarker Proxies of Salinity s0030

p0100 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 that are tightly linked to climate. Recent developments in organic geochemistry has produced two promising paleosalinity proxies: the relative abundance of tetra-unsaturated C_{37} methyl ketones (alkenones), and the hydrogen isotopic composition (D/H) of organic compounds produced by phytoplankton and plants.

The %C37:4 Paleosalinity Proxy s0035

p0105 The $C_{37:4}$ alkenone is produced only primarily in cold regions (surface water temperatures < 10 °C) (Rosell-Melé *et al.*, 1994) and it is not well preserved in sediments compared to the di- and tri-unsaturated alkenones. It is therefore most often excluded from alkenone paleotemperature reconstructions (see **Section 2.1** above, on alkenone paleothermometry). However, in polar regions, the inclusion of $C_{37:4}$ into the unsaturation ratio (U^{k37}) can in some cases increase its correlation with sea surface temperature (Bendle and Rosell-Melé, 2004).

p0110 At concentrations of %$C_{37:4}$ (relative abundance of $C_{37:4}$ to the total abundance of $C_{37:2}$, $C_{37:3}$, and $C_{37:4}$, as percentage) > 5%, the temperature relation breaks down (Rosell-Melé *et al.*, 2002; Sicre *et al.*, 2002), however, and a correlation with salinity has instead been suggested in the Nordic Seas and the North Atlantic (Rosell-Melé *et al.*, 2002; Sicre *et al.*, 2002) (**Table 1**). A %$C_{37:4}$-salinity dependence was also observed in the low-salinity waters of the Sea of Okhotsk (Seki, 2005), the Bering Strait (Harada *et al.*, 2003) and the Baltic Sea (Blanz *et al.*, 2005) (**Table 1**), leading to the suggestion that low salinities may impose stress on the alkenone-producing haptophytes, causing them to increase production of $C_{37:4}$ (Harada *et al.*, 2003).

p0115 The established %$C_{37:4}$-salinity relations, however, vary significantly between oceanic regions (**Table 1**), and no correlation with salinity was found in particulate and surface sediment samples from the Nordic Seas (Bendle *et al.*, 2005) or the Atlantic, Pacific and Indian sectors of the Southern Ocean (Sikes and Sicre, 2002). The applicability of %$C_{37:4}$ as a salinity proxy may thus be restricted to certain cold, low-salinity marine environments.

t0005 **Table 1** %$C_{37:4}$-Salinity relationships in different ocean basins

Ocean Basin	Linear Regression	R^2	Reference
North Atlantic and Nordic Seas	%$C_{37:4} = 146.9(\pm 10.6) - 4.1S\,(\pm 0.3)$	0.69	(Rosell-Melé et al., 2002)
North Atlantic	%$C_{37:4} = 48.1S + 1691$	0.78	(Sicre et al., 2002)
Bering Strait	%$C_{37:4} = 11.7S + 397.6$	0.76	(Harada et al., 2003)

Further studies will need to establish whether this is due to a possible contribution to $C_{37:4}$ production from other environmental factors. Nevertheless, the importance of establishing past salinity variations justifies this further work.

s0040 **The Lipid δD Paleosalinity Proxy**

p0120 The hydrogen isotopic (δD) composition of specific organic compounds reflects the δD value of environmental water (Sessions et al., 1999; Sauer et al., 2001; Sachse et al., 2004; Englebrecht and Sachs, 2005). Precipitation is strongly influenced by environmental variables, mainly temperature and amount of precipitation. The former dominates at high latitudes, while the latter is most important in the tropics, leading to increasing isotopic depletion at lower temperatures and high precipitation amount (Dansgaard, 1964). Since most hydrogen in lipids is bound to carbon and therefore non-exchangeable, the δD value of sedimentary biomarkers contains important information on past hydrological conditions and climate.

p0125 δD values in alkenones have been shown in culture and field studies to closely record the isotopic composition of the water in which they were produced (Englebrecht and Sachs, 2005). Additional D/H fractionation of alkenones occurs with increasing salinity (Schouten et al., 2005). Alkenone δD values are

f0025 **Figure 5** Hydrogen isotopic composition of C_{37} (solid symbol and line) and C_{38} (open symbol, dashed line) alkenones from cultured *Emiliania huxleyi* versus hydrogen isotopic composition of the culture medium (Englebrecht and Sachs, 2005).

depleted relative to that of the water by some $-193‰$ (Englebrecht and Sachs, 2005), reflecting the initial deuterium depletion of the primary photosynthate of $-171‰$ (Yakir and DeNiro, 1990) and further fractionation during alkenone biosynthesis (**Figure 5**).

p0130 Sauer et al. (2001) analyzed δD values of algal sterols from marine sediments and suggested that they reliably reflect the water hydrogen isotopic composition with a fractionation factor of $-201 \pm 10‰$.

p0135 Zhang and Sachs (subm.) cultured five species of freshwater green algae, including three strains of *Botryococcus braunii* (two A Race, one B Race), *Eudorina unicocca* and *Volvox aureus*, under controlled conditions in media containing different concentrations of deuterium. The hydrogen isotopic ratios of lipids in the algae, including alkadienes, botryococcenes, heptadecenes, fatty acids, and phytadiene, were shown to closely track water δD values. Correlation coefficients (R^2) in excess of 0.99 for all lipids in all species demonstrate that lipid δD values can be used to determine water δD values with a high degree of confidence.

p0140 All of these studies suggest that D/H ratios in sedimentary lipids will be a powerful new tool for reconstructing past hydrologic variations and salinity in lakes and oceans.

p0145 Lipid biomarkers are extracted from the sediment and the lipids chosen for compound-specific δD analysis are purified (Englebrecht and Sachs, 2005). D/H ratios are measured using an isotope-ratio-monitoring gas chromatograph-mass spectrometer system (irmGCMS) with combustion interface that converts all H to H_2 (Sessions et al., 1999). D/H is determined with reference to coinjected standards and calibrated against the VSMOW Vienna Standard Mean Ocean Water (VSMOW) scale. Values are reported as deviations from the VSMOW standard in per mil (δD = [(D/Hsample)/(D/Hstandard) − 1]* 1000).
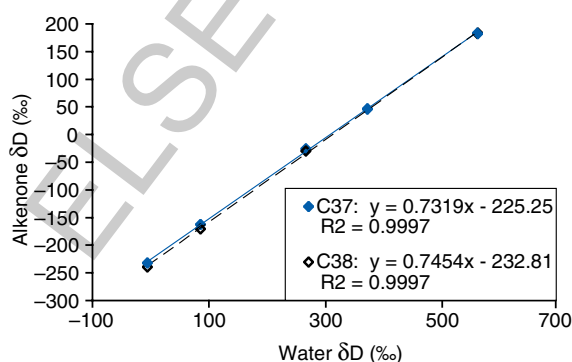
Summary s0045

p0150 Tremendous progress has been made in the last decade developing and applying organic geochemical indicators of past climate. A small set of the most significant of these advances has been reviewed here.

Advances in analytical chemistry in the coming years ought to make all of the temperature and hydrologic proxies described here available to a broad range of geochemists. The different biological, physical, and chemical underpinnings of these proxies, as compared to the inorganic paleoclimate proxies widely used today, such as the oxygen and carbon isotopes and trace metal ratios in foraminiferal shells, make them complementary. In some climatic or depositional environments, one or another of the temperature or hydrologic proxies may work best, while in other settings it ought to be possible to apply two or more indicators of the same climate parameter within a single sediment core. As a result, increasingly robust reconstructions of climate are certain to emerge.

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