

Figure 2: Ternary diagram showing the relative distribution of the three main types of GDGTs (drawn in Fig. 1) in different sedimentary environments. Lakes plotting in the red boxed area have potential for application of the TEX_{86} proxy. See text for further explanation. Adapted from Blaga et al. (2009).

constructed MATs from surface sediments seem to be lower than measured MATs. An explanation for this mismatch could be the production of branched GDGTs in situ in lake sediments, which are in fact aquatic soils. Recently, this possibility has indirectly been shown for sediments from a fjord (Peterse et al., 2009) and two tropical lakes (Tierney and Russel, 2009;

Sinninghe Damsté et al., 2009) by a different distribution of branched GDGTs in these sediments than in soils surrounding the fjord and lakes. This indicates that estimated MATs based on branched GDGT distributions in lake sediments with high in situ GDGT production might be biased towards lake bottom water temperatures.

Conclusions

Both organic geochemical paleothermometers, TEX_{86} and MBT-CBT, are promising new proxies for reconstructing LST and MAT, respectively, from the GDGT lipid distribution in lake sediments. Care should be taken, however, where these proxies are applied: TEX_{86} seems mainly applicable in larger lakes that receive little soil OM input and do not contain large communities of methane-producing Archaea, whereas MBT-CBT seems to be most useful in lakes that do receive a large influx of soil OM relative to in situ production of branched GDGTs in the lake sediments.

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Ancient DNA in lake sediment records

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Ancient DNA in lake sediments offers a novel window into past aquatic ecosystems and the environmental changes that triggered past species successions.

The geological record offers our best opportunity for understanding how biological systems function over long timescales and under varying paleoenvironmental conditions. Understanding these ecosystem responses to change is critical for biologists trying to understand how organisms interact and adapt to environmental changes, and for geologists seeking to use these biology-geology relationships in order to reconstruct past climate conditions from sediment records. For example, enumeration of microscopic fossilizing protists, such as diatoms, has become a standard paleoecological approach in the fields of paleoclimatology and paleolimnology. However, the identification

of morphological remains is not always straightforward, as many taxa lack diagnostic features preserved upon fossilization. Lipid-based records can be particularly valuable for species that do not leave diagnostic features in the sedimentary record. Nevertheless, the interpretation of these molecular stratigraphic records is often complicated by the limited specificity of many lipid biomarkers.

There is thus a need to find new biomarkers with greater source-specificity that can be used to complement and enhance interpretations based on existing methods. The field of molecular biology offers a most promising approach/technique that is just starting to gain wider

utility: The use of ancient DNA preserved in the sedimentary record (i.e., fossil DNA or *fDNA*) to reconstruct past ecosystems. Fossil DNA has been successfully employed to study the succession of species as a result of environmental changes in terrestrial (e.g., Willerslev et al., 2007), marine (Boere et al., 2009; Coolen et al., 2007; 2009; Coolen and Overmann, 2007; Manske et al., 2008), and lacustrine settings (Bissett et al., 2005; Coolen et al., 2004; 2008; Coolen and Overmann, 1998; D'Andrea et al., 2006; Epp et al., 2009). A major advantage of this molecular paleoecological approach is that ancient species can be studied, including those with-

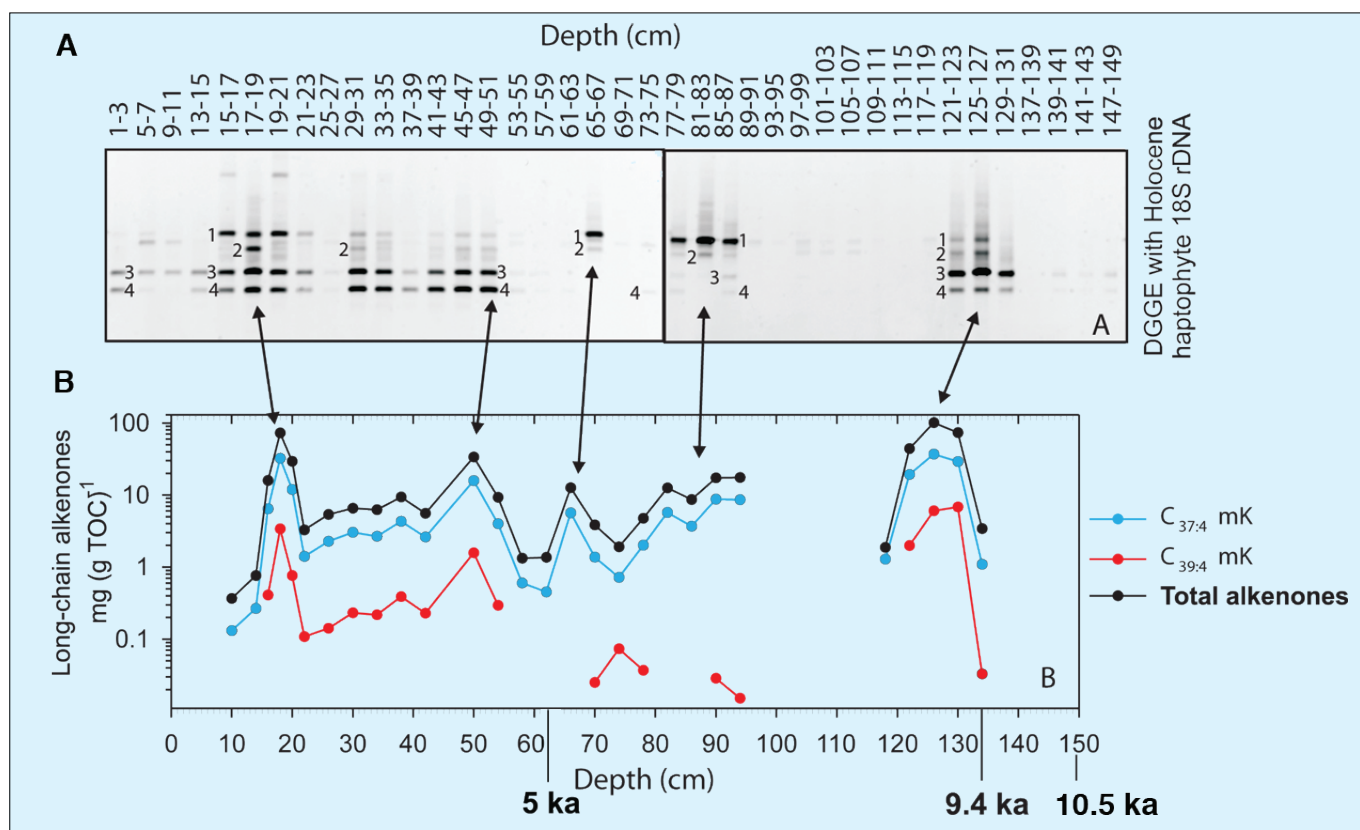


Figure 1: Stratigraphy of biomarkers of photosynthetic haptophyte algae (18S ribosomal DNA, alkenones [mK, methyl ketone]) recovered from the Holocene sediments of Ace Lake (figure modified from Coolen et al., 2004). (A) Denaturing gradient gel electrophoresis (DGGE) gels with PCR-amplified 18S rDNA of Holocene haptophytes. DGGE bands numbered as 1 to 4 were excised from the gel and subsequently sequenced for phylogenetic analysis (Coolen et al., 2004). All sequences were related to non-calcifying *Isochrysis* spp. (data shown in Coolen et al., 2004). (B) Quantity of total alkenones produced by *Isochrysis* spp. (black line), the most predominant alkenone (i.e., the 4 times unsaturated C37:4mK; blue line), and C39:4 mK (red line) [mg (g⁻¹ TOC)]. TOC = Total Organic Carbon. Comparable profiles were found for other alkenones that were produced by the *Isochrysis*-related haptophytes (see Coolen et al., 2004). The oldest analyzed sediment layer was deposited at 10.5 ka BP when the lake was freshwater, but the first haptophytes appeared at the onset of meromictic conditions when the lake became a marine basin and salinity reached 10 psu at 9.4 ka BP. The marine basin became an isolated saline lake at ~5 ka BP. Parallel examination of the alkenones and haptophyte sequences, especially from sediments deeper than 65 cm, revealed that the less common C39:4 mK was not biosynthesized by phylotype 1. The species-specific identification of alkenone sources is important for the calibration of alkenone-based SST reconstructions. See Coolen et al. (2004) for further details.

out diagnostic features retained in the fossil record.

Study sites and analytical approaches for fDNA studies

Most fDNA surveys have been conducted on Holocene lake sediments that were deposited under cold and/or anoxic conditions. In particular, permanently stratified (meromictic) lakes with anoxic bottom waters and undisturbed laminated sediments provide excellent preservation conditions for fDNA. The strong vertical stratification of (microbial) species in meromictic environments makes it easier to identify species that are and were indigenous to the surface waters.

However, despite excellent preservation conditions, the degradation of ancient DNA to shorter fragments occurs within the first several thousands of years after deposition (Coolen and Overmann, 1998). Therefore, only short, less than 500 base pair (bp)-long fDNA fragments should be analyzed using molecular methods that involve polymerase chain reaction (PCR; a technique used to amplify DNA across several orders of magnitude to yield billions of copies of a particular DNA sequence of interest). Furthermore, the use of group-

specific oligonucleotides (i.e., primers, which serve as a starting point for PCR) instead of universal primers significantly lowers the detection limit of fDNA during PCR. Identification of the ancient species composition most commonly involves the separation of PCR-amplified fDNA by denaturing gradient gel electrophoresis (DGGE) and subsequent phylogenetic analysis of sequenced DGGE bands (e.g., Boere et al., 2009; Coolen et al., 2004; 2007; 2008; 2009; Manske et al., 2008). DGGE is a fast and inexpensive way to analyze 200 to 500-bp-long PCR-amplified fDNA. We recently started to perform parallel phylogenetic surveys of reverse transcribed and PCR-amplified gene transcripts from the environmental samples (Coolen and Shtereva, 2009). Species that express genes in deep anoxic waters and sediment samples at the time of sampling are considered to be metabolically active and indigenous to those samples, and are therefore excluded as proxies for past environmental conditions of the surface waters. In order to further ground-truth fDNA data, we always perform a parallel analysis of the more traditional (morphological) proxies (Boere et al., 2009).

fDNA in Antarctic lakes: Protists and photosynthetic bacteria

Ace Lake (Vestfold Hills, Antarctica) was originally a freshwater lake. Due to sea level changes resulting from Holocene deglaciation, the lake became a meromictic saline fjord system with sulfur-rich bottom waters at 9.4 ka BP, and then a saline, meromictic lake about 5 ka BP (Cromer et al., 2005; Coolen et al., 2006). We expected these major hydrologic changes to have a strong impact on the ancient pelagic communities of the lake. The onset of photic zone anoxia at 9.4 ka BP was verified from the presence of fDNA and carotenoids of anoxygenic photosynthetic green sulfur bacteria, which still inhabit the sulfidic chemocline of Ace Lake today (Coolen et al., 2006). Furthermore, this freshwater/fjord transition was marked by a shift in pelagic cyanobacteria to a predominance of a *Synechococcus* strain (Coolen et al., 2008) that is still abundant in Ace Lake and which requires a minimum salinity of 10 psu for growth. The passing of a critical salinity threshold also resulted in the occurrence of non-calcifying alkenone-biosynthesizing haptophytes related to coastal *Isochrysis* species (Coolen et al., 2004; Fig. 1). Alkenones are of great in-

terest to paleoceanographers because of the strong empirical relationship between the degree of unsaturation in alkenones and growth temperature, which forms the basis for their use as a molecular proxy of past sea surface temperatures (SST) (e.g., Brassell et al., 1986). Based on the phylogenetic information inferred from *f*DNA, it should be possible to calibrate the reconstructed alkenone-based Holocene SST in eastern Antarctica once modern cultivars related to the Holocene haptophytes become available. Thus, the *f*DNA analysis served to identify past (morphologically non-fossilizing) species with clearly defined paleoenvironmental growth requirements, as well as biological sources of fossil lipid biomarkers.

***f*DNA in Antarctic lakes: Ancient copepods**

The lack of diagnostic features is not restricted to unicellular aquatic organisms but can also be the case for metazoans. For example, the best preserved copepod remains present in lake sediments are eggs, which often have few if any distinguishing features. Therefore, the *f*DNA approach was developed to track changes in ancient copepod diversity in lakes (Bissett et al., 2005). This approach allowed the characterization of copepod species

in sediments from three fresh to brackish Antarctic lakes as old as 10 ka BP. In most cases, the fossil species found matched those of extant lake populations, but analysis of early- to mid-Holocene sediments from one lake revealed a species that is not known to exist today (Gibson and Bayly, 2007). It was furthermore shown that it is possible to recover copepod DNA from lake sediments that underwent long-term refrigeration (4°C) or preservation in polyethyleneglycol (Bissett et al., 2005).

Potential of this new approach

It is obvious that *f*DNA can be used to: (a) study the succession of a large variety of ancient species with defined environmental requirements, including those that lack diagnostic features, (b) identify biological precursors of (lipid) biomarkers, and (c) verify paleoenvironmental information (i.e., alkenone SST) inferred from (lipid) biomarkers. However, the extent to which *f*DNA is preserved and the factors/conditions that control the preservation (survival) of DNA remain largely unknown. These and other issues must be addressed and resolved before *f*DNA techniques can be broadly applied, especially in non-polar lakes. In addition, many group-specific PCR/DGGE runs are required to cover the total diversity of ancient species. We there-

fore recently explored the use of parallel tag-encoded amplicon pyrosequencing (Sogin et al., 2006) as a molecular paleoecological tool. Price reduction and easier and faster computing of the gigantic sequencing datasets should eventually make pyrosequencing the standard method for *f*DNA studies, thereby replacing currently used molecular methods.

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Tracking the effects of “aquatic osteoporosis” using paleolimnology

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Recent but widespread observations of aqueous calcium (Ca) declines in softwater lakes/ponds have spurred research on Ca-rich crustacean zooplankton as paleoindicators of the ecological consequences of Ca declines attributable to acidification, forestry, and other environmental stressors.

Emergence of calcium decline as an ecological stressor?

Understanding the ecological consequences of acid deposition has been an environmental topic of interest for many years. Recently, the focus of study in affected areas has shifted from an examination of impacts of pH decline to an evaluation of the effectiveness of current emission controls and the detection of recovery. In softwater regions, chemical recovery in lakes has generally been slower than anticipated (Stoddard et al., 1999; Jeffries et al., 2003). A common explanation for this muted response has been the depletion of base cations (principally Ca) from watershed soils, and a subsequent decline in lakewater Ca concentrations that

counteract the reduction in sulfate inputs (Kirchner and Lydersen, 1995; Likens et al., 1996). Moreover, it is now clear that many ecosystems are being subjected to the effects of multiple stressors, and some of these might exacerbate Ca decline. Given the lack of reliable long-term monitoring data, paleolimnological approaches are being used extensively in the study of a wide variety of lake management issues (Smol, 2008). The mechanisms driving Ca depletion in soils are thought to be a site-specific combination of accelerated release due to acid rain (Stoddard et al., 1999), and forest regrowth following biomass harvesting (Watmough et al., 2003). Reduced atmospheric Ca inputs may also contribute to depleted soil Ca pools. In the

softwater lakes and ponds of the Canadian Shield in North America, declines in Ca concentrations of up to 40% below 1980s levels have been observed (Jeziorski et al., 2008a). As Ca is an essential nutrient, there has been growing concern that Ca levels may be approaching concentrations low enough to constitute an environmental stressor in their own right. This is especially the case for those aquatic invertebrates that have relatively high Ca demands due to their use of Ca biominerals as structural elements in their exoskeleton.

Although there is a general understanding of the mechanisms driving Ca decline, we are faced with a widespread lack of baseline or pre-impact reference conditions, because the long-term causes

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