

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.

References

- Bissett, A., Gibson, J.A.E., Jarman, S.N., Swadling, K.M. and Cromer, L., 2005: Isolation, amplification, and identification of ancient copepod DNA from lake sediments, *Limnology and Oceanography: Methods*, **3**: 533-542.
- Coolen, M.J.L., Muyzer, G., Rijpstra, W.I.C., Schouten, S., Volkman, J.K. and Sinninghe Damsté, J.S., 2004: Combined DNA and lipid analyses of sediments reveal changes in Holocene haptophyte and diatom populations in an Antarctic lake, *Earth and Planetary Science Letters*, **223**: 225-239.
- Coolen, M.J.L., Muyzer, G., Schouten, S., Volkman, J.K. and Sinninghe Damsté, J.S., 2006: Sulfur and methane cycling during the Holocene in Ace Lake (Antarctica) revealed by lipid and DNA stratigraphy. In: Neretin, L.N. (ed), *Past and Present Marine Water Column Anoxia*, NATO Science Series: IV-Earth and Environmental Sciences, Springer, 41-65.
- Coolen, M.J.L. and Overmann, J., 1998: Analysis of subfossil molecular remains of purple sulfur bacteria in a lake sediment, *Applied and Environmental Microbiology*, **64**: 4513-4521.
- Coolen, M.J.L., Talbot, H.M., Abbas, B.A., Ward, C., Schouten, S., Volkman, J.K. and Sinninghe Damsté, J.S., 2008: Sources for sedimentary bacteriohopanepolyols as revealed by 16S rDNA stratigraphy, *Environmental Microbiology*, **10**: 1783-1803.

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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

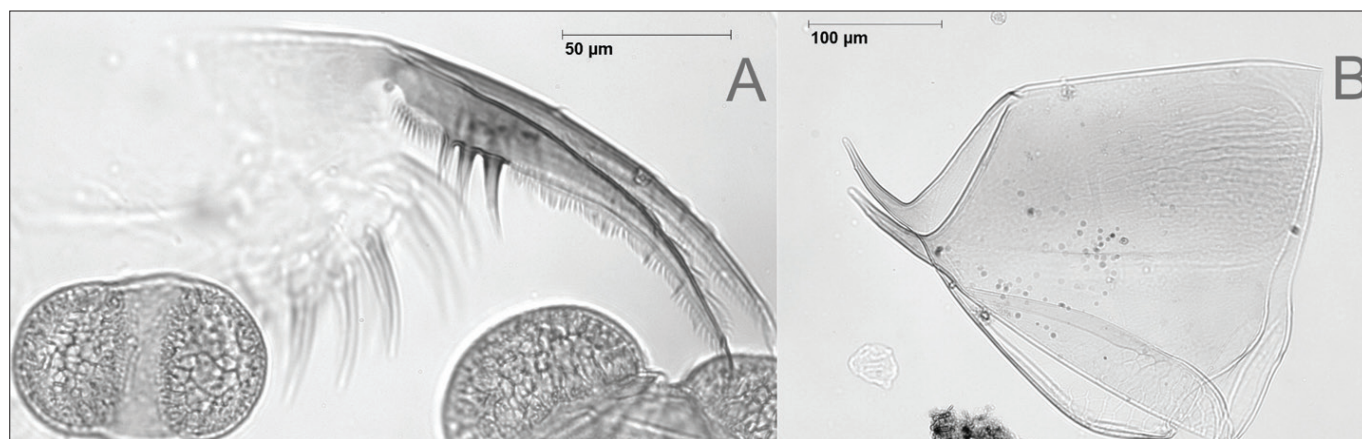


Figure 1: Micrographs of fossil water flea body parts. **A**) *Daphnia* post-abdominal claw, with two pine pollen grains. **B**) *Bosmina* carapace (Photographs: A. DeSellas).

of measured Ca declines pre-date the longest environmental records in North America (Likens et al., 1996). Therefore, we must employ indirect methods to address questions regarding the onset and extent of Ca decline. One such approach is geochemical modeling, with predictions suggesting that an acid-induced release of Ca from watershed soils has led to artificially elevated lakewater Ca levels, and that current declines are a return from this elevated state (Watmough and Aherne, 2008). However, questions remain, including how current Ca levels compare to historical levels, what minimum levels will eventually be reached, and what will be the ecological implications of falling Ca concentrations? These are all questions that are well suited to paleolimnological

investigations, provided that an appropriate indicator is available.

Recent methodological developments

The crustacean zooplankton, especially many cladoceran taxa (water fleas), have long been studied in an acidification context, but several of their features also make them important paleoindicators of environmental Ca decline. They leave exoskeletal remains that preserve well in lake sediments (Fig. 1), and their survival depends on lakewater Ca levels above taxon-specific survival thresholds, given a life strategy of repeated molts and subsequent regeneration of a Ca-rich exoskeleton (Alstad et al., 1999). There is clear differentiation in the Ca requirements of different cladoc-

eran taxa, with differences of up to 20-fold in the Ca content among crustacean zooplankton species. In particular, Ca-rich daphniids have much higher needs than non-daphniid crustacean species such as bosminids, *Holopedium* and calanoid and cyclopoid copepods (Waervagen et al., 2002; Jeziorski and Yan, 2006). Recent syntheses of laboratory and field work have identified that a Ca concentration of 1.5 mg/L acts as a performance threshold for the most common crustacean zooplankton found in Canadian Shield lakes (*Daphnia pulex*; Ashforth and Yan, 2008; Cairns and Yan, 2009), where many water bodies are nearing (or have already crossed) this threshold value (Jeziorski et al., 2008a). An initial attempt to use the differences in Ca content in daphniid resting eggs (ephippia) as the basis for a paleoindicator of Ca change failed (Jeziorski et al., 2008b), apparently because of the previously unknown capacity of daphniids to withdraw Ca from ephippia. Instead, more conventional assemblage approaches are required and have proved to be more successful, as described below (Jeziorski et al., 2008a).

Findings to date

Recent investigations of softwater lakes that are nearing or have recently fallen below the 1.5 mg/L threshold in eastern North America have recorded declines in the relative abundance of Ca-rich daphniid sedimentary remains (post-abdominal claws; Fig. 1A) in dated sediment cores, relative to other preserved crustacean zooplankton (Fig. 2; Jeziorski et al., 2008a). These declines could not be readily attributed to changes in chemical variables other than reduced Ca availability, because other changes over this time period should have favored larger Ca-rich daphniid species over their smaller Ca-poor competitors. This trend of a relative decrease in daphniid remains since pre-industrial times was also recorded in lakes with current Ca concentrations up to 2.0

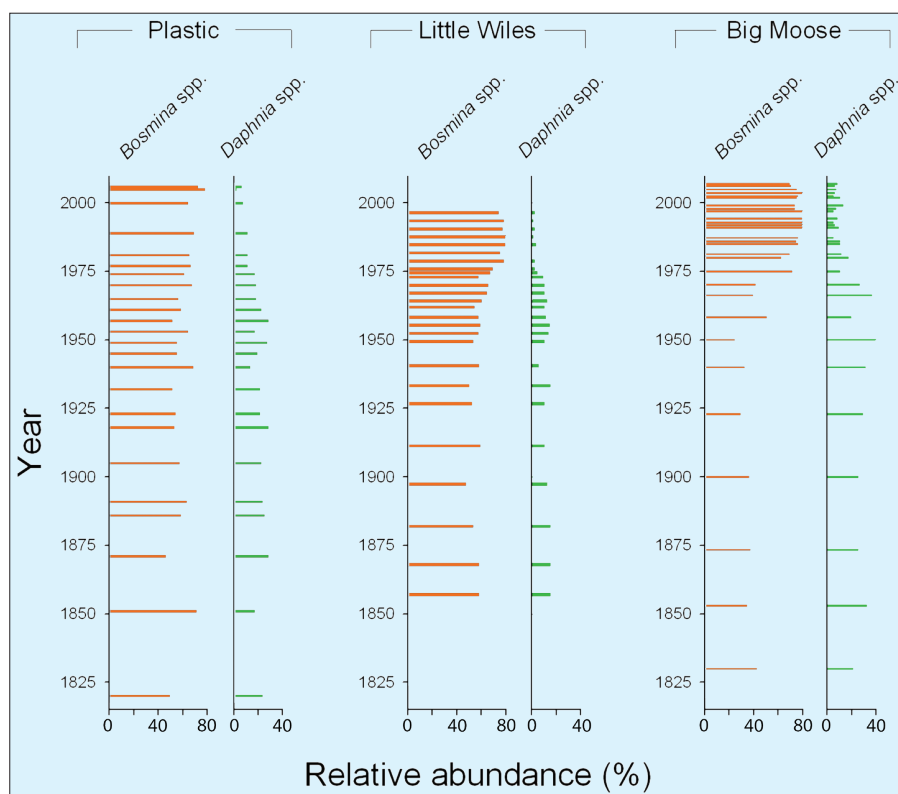


Figure 2: Sedimentary profiles of changes in the relative abundance of crustacean zooplankton remains for three softwater lakes in eastern North America (Plastic Lake, Ontario, Canada; Little Wiles Lake, Nova Scotia, Canada; Big Moose Lake, New York, USA). Similar trends of declining sedimentary abundances of Ca-rich daphniids (green) relative to Ca-poor bosminids (orange) are common across all three regions (from Jeziorski et al., 2008a. Reprinted with permission from AAAS).

mg/L (Jeziorski et al., 2008a), suggesting that the threshold measured under natural conditions may actually be higher than the 1.5 mg/L threshold identified in laboratory studies. The number of lakes at or nearing this threshold is considerable and increasing; an examination of changes in Ca concentrations for a set of 770 lakes covering a broad geographical area in Ontario (Canada) found that 35% of lakes currently have <1.5 mg/L of Ca, while 62% are already below 2.0 mg/L, reflecting substantial increases in percentages since the 1980s (Jeziorski et al., 2008a).

Future research avenues

We are continuing to examine the use of crustacean zooplankton as indicators of past Ca conditions across a wide spectrum of Canadian Shield lakes. For example, to better interpret changes observed in sediment profiles, more information is

required regarding specific laboratory-determined Ca thresholds for taxa other than *Daphnia pulex* to further refine our current separation of taxa into Ca-rich vs. Ca-poor categories. Additionally, further examinations of the potential indirect impacts of Ca decline are warranted, such as the differential uptake of Ca and Sr, and/or isotopic differences as Ca concentrations fall (Peters et al., 2008). Finally, Ca decline has been largely attributed to either acid deposition, biomass harvesting or some combination of both, yet similar declines have been observed in regions where the effects of these stressors have been minimal, and so other environmental changes, such as decreases in atmospheric deposition of particulate dust (Hedin et al., 1994) may also be contributing to the observed Ca declines. Given our limited current understanding of both the biological consequences of reduced Ca availability, as well

as its geographic extent, further investigations are clearly warranted.

Note

The term "aquatic osteoporosis" was coined by M. Turner, a co-author on Jeziorski et al., 2008a.

References

- Ashforth, D. and Yan, N.D., 2008: The interactive effects of calcium concentration and temperature on the survival and reproduction of *Daphnia pulex* at high and low food concentrations, *Limnology and Oceanography*, **53**: 420-432.
- Cairns, A. and Yan, N., 2009: A review of the influence of low ambient calcium concentrations on freshwater daphniids, gammarids and crayfish, *Environmental Reviews*, **17**: 67-79.
- Jeziorski, A. et al., 2008a: The widespread threat of calcium decline in fresh waters, *Science*, **322**: 1374-1377.
- Likens, G.E., Driscoll, C.T. and Buso, D.C., 1996: Long-term effects of acid rain: Response and recovery of a forest ecosystem, *Science*, **272**: 244-246.
- Smol, J.P., 2008: *Pollution of Lakes and Rivers: A Paleoenvironmental Perspective*, Second Edition, Blackwell Publishing, Malden.

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Calibration-in-time: Transforming biogeochemical lake sediment proxies into quantitative climate variables

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Lake sediment records are underrepresented in comprehensive quantitative high-resolution (annual, sub-decadal) multi-proxy climate reconstructions for the past 1 ka due, in part, to the problems associated with calibration of biogeochemical lake sediment proxies. Here we present a case study and highlight five methodological issues that are important to establish quantitative paleoclimate records from biogeochemical proxies in non-varved lake sediments.

Comprehensive quantitative annually resolved multi-proxy climate reconstructions for the past ca. 1 ka are arguably among the most spectacular and widely recognized highlights of current paleoclimate research (see PAGES Focus 2: Regional Climate Dynamics). In this context, lake sediment archives are surprisingly underrepresented in the data series used for such purposes. Indeed, the number of well-calibrated long time series of quantitatively reconstructed climate variables at (near) annual resolution derived from lake sediments is limited; most of the reconstructions are descriptive, and samples have variable temporal resolution or are discontinuous; with the exception of the few annually laminated lake sediments. In contrast to biota-based transfer functions (e.g., diatoms, pollen, chironomids; e.g., Birks, 1998), bio-geochemical sediment proxies can be transformed into quantitative climate variables only through a calibration-in-time approach: a time series of sediment proxies regressed against a series of meteorological observations.

Recently, we started to explore possibilities to produce millennial-long, highly resolved (near-annual) quantitative climate reconstructions from biogeochemical data using a calibration-in-time approach in non-varved lake sediments (von Gunten et al., 2009a). The question was "can a methodology be developed to produce time series of adequate quality for regional climate reconstructions?" Yes, and the result is shown in Figure 2.

The statistical procedure per se is known from tree-ring research (e.g., Cook et al., 1994; Esper et al., 2005) and has been applied in quantitative climate reconstructions from annually laminated lake sediments (e.g., Trachsel et al., 2008). But would a calibration-in-time also work in non-varved sediments? Evidently, two things are needed: a) an extremely accurate high-resolution sediment chronology with smallest uncertainties for the calibration period (which typically covers ca. 100 years; von Gunten et al., 2009b; Fig. 1); and b) sediments corresponding to the calibration period must be sampled at highest possible resolution (e.g., near-annual; von Gunten et al., 2009a; Fig. 2). We consider

the following five methodological issues to be highly critical for the success of this approach:

1) Rigorous testing of calibration chronology

While radiometric techniques (mostly ²¹⁰Pb) are widely used to date young sediments, little attention is paid to the choice and evaluation of the numerical model used to convert activity profiles into sediment ages. Although well documented (e.g., Appleby, 2008), it is often ignored that the three established models (Constant Flux Constant Sedimentation, CFCS; Constant Initial Concentration, CIC; Constant Rate of Supply, CRS) may yield very different chronologies for the same profile (Fig. 1A). A fourth model, the inductive Sediment Isotope Tomography (SIT) model is poorly established in the paleolimnological literature. SIT uses a forward modeling approach and calculates, in contrast to CIC and CRS, sediment ages without a-priori model assumptions (Carroll and Lerche, 2003). Our systematic comparison of all the models in two different lakes in Chile (Fig. 1; von Gunten et al., 2009b) shows

Full Reference List:

- Alstad, N.E.W., Skardal, L. and Hessen, D.O., 1999: The effect of calcium concentration on the calcification of *Daphnia magna*, *Limnology and Oceanography*, **44**: 2011-2017.
- Ashforth, D. and Yan, N.D., 2008: The interactive effects of calcium concentration and temperature on the survival and reproduction of *Daphnia pulex* at high and low food concentrations, *Limnology and Oceanography*, **53**: 420-432.
- Cairns, A. and Yan, N., 2009: A review of the influence of low ambient calcium concentrations on freshwater daphniids, gammarids and crayfish, *Environmental Reviews*, **17**: 67-79.
- Hedin, L.O., Granat, L., Likens, G.E., Buishand, T.A., Galloway, J.N., Butler, T.J. and Rodhe, H., 1994: Steep declines in atmospheric base cations in regions of Europe and North America, *Nature*, **367**: 351-354.
- Jeffries, D.S. et al., 2003: Assessing the recovery of lakes in southeastern Canada from the effects of acidic deposition, *Ambio*, **32**:176-182.
- Jeziorski, A. and Yan, N.D., 2006: Species identity and aqueous calcium concentrations as determinants of calcium concentrations of freshwater crustacean zooplankton, *Canadian Journal of Fisheries and Aquatic Sciences*, **63**: 1007-1013.
- Jeziorski, A. et al., 2008a: The widespread threat of calcium decline in fresh waters, *Science*, **322**: 1374-1377.
- Jeziorski, A., Paterson, A.M., Yan, N.D. and Smol, J.P., 2008b: Calcium levels in *Daphnia ephippia* cannot provide a useful paleolimnological indicator of historical lakewater Ca concentrations, *Journal of Paleolimnology*, **39**: 421-425.
- Kirchner, J.W. and Lydersen, E., 1995: Base cation depletion and potential long-term acidification of Norwegian catchments, *Environmental Science & Technology*, **29**: 1953-1960.
- Likens, G.E., Driscoll, C.T. and Buso, D.C., 1996: Long-term effects of acid rain: Response and recovery of a forest ecosystem, *Science*, **272**: 244-246.
- Peters, S.C., Lockwood, R., Williamson, C.E. and Saros, J.E., 2008: Using elemental ratios of calcium and strontium to track calcium availability in the freshwater zooplankton *Daphnia pulicaria*, *Journal of Geophysical Research*, **113**: G04023, doi:10.1029/2008JG000782.
- Smol, J.P., 2008: *Pollution of Lakes and Rivers: A Paleoenvironmental Perspective*, Second Edition, Blackwell Publishing, Malden.
- Stoddard, J.L. et al., 1999: Regional trends in aquatic recovery from acidification in North America and Europe, *Nature*, **401**: 575-578.
- Wærvågen, S.B., Rukke, N.A. and Hessen, D.O., 2002: Calcium content of crustacean zooplankton and its potential role in species distribution, *Freshwater Biology*, **47**: 1866-1878.
- Watmough, S.A., Aherne, J. and Dillon, P.J., 2003: Potential impact of forest harvesting on lake chemistry in south-central Ontario at current levels of acid deposition, *Canadian Journal of Fisheries and Aquatic Sciences*, **60**: 1095-1103.
- Watmough, S.A. and Aherne, J., 2008: Estimating calcium weathering rates and future lake calcium concentrations in the Muskoka-Haliburton region of Ontario, *Canadian Journal of Fisheries and Aquatic Sciences*, **65**: 821-833.