

DEVELOPMENT AND APPLICATION OF ORGANIC BIOMARKERS TO
CONTINENTAL PALEOCLIMATE PROBLEMS IN THE INTERIOR OF
NORTH AMERICA

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13. Toney, J.L. and Anderson, R.S., 2006, A postglacial paleoecological record from the San Juan Mountains of Colorado: fire, climate and vegetation history. *The Holocene* **16**(4), 505-517.
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Selected Abstract Publications

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Fawcett, P.J., Werne, J.P., Anderson, R.S., Heikoop, J.M., Brown, E.T., Berke, M.A., Smith, S., Goff, F.E., Hurley, L.L., Cisneros-Dozal, L.M., Schouten, S., Sinninghe Damsté, J.S., Huang, Y., Toney, J.L., Fessenden, J.E., WoldeGabriel, G.W., Geissman, J.W., and Allen, C.D., (2009) Mid-Pleistocene Orbital and Millennial Scale Climate Change in a 200 ky lacustrine sediment core from SW North America. *Eos Trans. AGU*, 90(52), Fall Meet. Suppl., Abstract PP13F-11

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Gremillion, P., D. Rodbell, K. Hollocher, and J. Toney, 2004. Paleolimnological evidence of water quality responses to extreme hydrologic events. 24th Annual

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Anderson, R.S., Allen, C.D., Bair, A.N., Jass, R.B., and Toney, J.L. Holocene Forest
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George, ND may hold the key to tracking historical climate change.

Dedicated to my brilliant & lovely daughter:

Ms. Megan Hazel Toney

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CHAPTER 1

INTRODUCTION

JAIME L. TONEY

BROWN UNIVERSITY – *Department of Geological Sciences*

1. INTRODUCTION

1.1 Holocene, continental paleoclimate

Today, more than any preceding time, understanding the response of terrestrial ecosystems to changes in climate is imperative due to the extremes to which we, as humans, are pushing the global climate system.

Understanding the response of terrestrial ecosystems can be challenging, because for many ecosystems we have altered the landscape, and no natural analogues exist. One way to see how ecosystems respond to natural climate change is to reconstruct changes in environmental and ecological parameters in the past. Holocene records provide a unique opportunity, because with the right archive (i.e., laminated lake sediments, tree rings, speleothems), high-resolution records can track both abrupt and high frequency changes in climate and ecosystems. Understanding Holocene, continental climate change poses multiple challenges though, because climate changes are often abrupt, spatially diverse, and the response of the terrestrial system is not always proportional to the forcing. On Holocene timescales, often the terrestrial response is not directly attributable to a specific forcing such as insolation, but likely a response to variations internal to the climate system, such as coupled ocean-atmosphere modes over various timescales (e.g., interannual, decadal, multidecadal). Generating quantitative, high-resolution records of environmental change by developing new biomarkers or using existing

biomarkers for new regions can capture both high and low frequency terrestrial responses, as well as, abrupt responses that can then be linked to changes in mean climate state and abrupt climate events.

In the past five years I have pioneered multiple biomarkers (i.e. alkenone unsaturation, compound specific H-isotopes, polycyclic aromatic hydrocarbons) for use in specific terrestrial environments. These new biomarker techniques can be used on their own or in a complimentary approach with more traditional proxies and provide new climate and environmental information. The records generated from these proxies can be directly compared to suspected climate drivers, such as: solar radiation, atmospheric composition, ocean circulation and variability in the mode of climate (e.g. El Niño/Southern Oscillation and the Arctic Oscillation) to provide links between the climate forcing and the climate response. These links lead to new testable theories regarding the mechanisms of climate change.

Specifically, my research has focused on developing new organic biomarkers and new applications for existing organic biomarkers to create quantitative reconstructions of temperature (i.e. alkenone unsaturation ratio) and growing season precipitation (i.e. H-isotopes of leaf waxes) for lakes of the Northern Great Plains, and wildfire (i.e. distribution of polycyclic aromatic

hydrocarbons) in the southwestern United States where these records do not exist or extant proxies are confounded by other factors and provide limited information regarding links to global climate system.

Developing new organic biomarkers requires strategic planning to determine not only for what regions they can provide the new and pertinent information, but also what are the limitations both spatially and temporally given the range of environmental controls. I have successfully pioneered this type of research in the semi-arid grasslands of the U.S. by following two main steps: 1) Field work and reconnaissance to understand the production, distribution and deposition of biomarkers in the modern environment; and 2) Calibration of the biomarker either in the laboratory, the field, or with existing instrumental data.

In this thesis I present the development of the long-chain alkenone proxy for spring temperature and H-isotopes of leaf waxes for growing season precipitation in the Northern Great Plains (NGP), North America. Currently no quantitative temperature records exist beyond the instrumental period for the region, and in-lake conservative ions reconstruct annual precipitation minus evaporation budgets, but integrate the effects of precipitation and evaporation. I also present the development of a new biomarker that shows promise for detecting local and regional wildfire events in the sedimentary

records and may help with the regional interpretation of fire history. Finally, the closing chapter addresses the need for inspiring upcoming generations of scientists and bridging the gap between scientists and the general public by presenting an example of how primary teachers can use inquiry-based investigations centered on a local problem to engage students and teach content associated with state and national science standards. The closing chapter comes out of an outreach opportunity that I engaged in for two years through the National Science Foundation's Graduate STEM Fellows in K-12 Education program.

1.2 Dissertation outline

1.2.1 Development of a paleotemperature proxy from lacustrine alkenones

In this thesis, in collaboration with a number of experts in different fields, I undertake extensive environmental, enrichment culture, genomic, and isolation culture analyses to resolve the numerous questions that hinder the progress of using long-chain alkenones as a lacustrine paleotemperature proxy. The results of these analyses show that from an organic geochemist's perspective with a goal to apply the alkenone proxy to paleo-reconstructions, it is no longer 'good enough' to report lipid profiles from a survey of lakes without delving deeper into the analyses of the ecology, physiology, and taxonomy of the producing organisms. Similarly, the conclusions that can be drawn from reporting only operational taxonomic units (OTUs) derived from

lake sediments and associated lipid profiles provides limited information about the lake system and does not provide enough information to adequately apply the proxy to deeper time scales. While all of these approaches individually provide relevant information about the system, ultimately the combination of these approaches with taxonomy and culture manipulation is necessary before we can adequately interpret paleo-records.

In this thesis, I present a cohesive series of studies that were necessary to understanding the Lake George haptophytes and alkenone profiles. An initial survey of lake surface sediment in the region provided insight on the environmental controls on the presence and absence of long-chain alkenones (Chapter 2). This survey identified key sites for paleo-reconstructions and allowed us to focus on Lake George, ND for continued water column monitoring and resulted in the development of an *in situ* calibration of alkenone unsaturation to temperature (Chapter 2). I extended this survey farther north into the prairie lakes of Canada to confirm the environmental controls on alkenone presence (Chapter 3).

From the environmental samples collected from Lake George, our target site, I began enrichment cultures from various parts of the lake system (i.e., water column, shallow sediments, deep sediments). In the enrichment cultures, we used information from the survey to guide culture conditions and were able to

separate two distinct haptophyte phylotypes that were previously reported from core-top sediments (Theroux et al. 2010)(Chapter 4). Through collaboration with haptophyte taxonomist, Robert Andersen, we were able to isolate the haptophyte algae responsible for producing the dominant two lipid profiles in Lake George from the enrichment cultures, and learn that this algae has at least five different life stages and makes different alkenone profiles associated with each stage (Chapter 5). Finally, knowing that there is a wealth of information that we still do not understand about lacustrine haptophytes, even ones that are assigned places on the phylogenetic tree, I have analyzed the lipid profile from a culture collection of *Pseudoisochrysis paradoxa*, which is the closest genetically-related haptophyte to the one that we have isolated from Lake George and report officially for the first time¹ the lipid profile associated with *Pseudoisochrysis paradoxa* (Chapter 6).

Using what we now know about haptophytes and long-chain alkenone production in Lake George, ND, I have reconstructed a quantitative temperature record for the last 9,400 years that provides new insights into how spring temperature change in the Northern Great Plains is linked with climate in the tropical western Pacific Ocean and the Atlantic Ocean (Chapter 7).

¹*Pseudoisochrysis paradoxa* was reported to produce long-chain alkenones in an undergraduate thesis by Farmer (1993) in John Volkman's research group, but the results were never formally published, and the original data has not yet been located (Volkman, *personal communication*).

1.2.2 Development and application of H-isotopes of terrestrial leaf waxes (n-acids)

In Chapter 8, I show that the H-isotopes from terrestrial leaf waxes (δD_{WAX}), in this case C_{24} -acids, track major droughts in the overlapping instrumental record and can be applied to the down-core sediments at Lake George, ND to reconstruct growing season precipitation. This study has important implications, including that Dust Bowl-level droughts were not common throughout the Holocene and that droughts in the Northern Great Plains are connected to changes in meridional overturning circulation in the Atlantic ocean and are likely driven on shorter timescales by teleconnections between the El Niño/Southern Oscillation region, mid-continent atmospheric circulation, and changes in the Atlantic hurricane formation region.

1.2.3 Assessing the use of polycyclic aromatic hydrocarbons as a proxy for wildfires

Understanding wildfires and biomass burning are exceedingly important in the context global climate change, because they have direct effects on global carbon storage, atmospheric chemistry (input of CO_2 , CH_4 , NO_x), and ecosystem diversity (Gill et al. 1995; van der Werf et al. 2004). Traditional methods for reconstructing fire events (e.g., sedimentary charcoal analysis and tree ring fire scars) have limitations, for example, secondary deposition

or degradation of charcoal can lead to ambiguous results (Whitlock and Larsen, 2001), while tree-ring fire scar analysis requires a large population of trees and is limited temporally due to tree mortality. In Chapter 9 we assess if polycyclic aromatic hydrocarbons (PAHs), can be used either alone or complimentary to the above methods to resolve the above issues and detect additional fire parameters, such as fire intensity, which cannot be detected by these other methods.

1.2.4 Science in Education and Outreach

I have been fortunate to participate in science outreach in the local inner city elementary schools in Providence, RI. Education and outreach by research scientists is important, not only because it is now required as a broader impacts consideration by large funding agencies, such as the National Science Foundation, but because with advent of science blogs and information a Google search away, we need to communicate to the public the rigor that goes into good science. The public needs to trust and respect the results that come from the scientific community in order for the results to impact policy decisions, for example, in the case of global climate change and anthropogenic warming. One way to facilitate this trust is communicating to a broad range of individuals at a level that makes science accessible. Chapter 10 presents the results of collaboration with local teachers to bring new and exciting

science to elementary students, while also incorporating the fundamental and mandated science concepts.

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CHAPTER 2

CLIMATIC AND ENVIRONMENTAL CONTROLS ON THE OCCURRENCE AND DISTRIBUTIONS OF LONG-CHAIN ALKENONES IN LAKES OF THE INTERIOR UNITED STATES

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ABSTRACT

Long chain alkenones (LCA) are temperature-sensitive lipids with great potential for quantitative reconstruction of past continental climate. We conducted the first survey for alkenone biomarkers from 55 different lakes in the Northern Great Plains and Nebraska Sand Hills of the United States. Among those surveyed, we found 13 lakes that contain LCAs in the surface sediments. The highest concentrations of alkenones in sediments are found in cold (mean annual air temperature $\sim 11^{\circ}\text{C}$ vs. 17°C in our warmest sites), brackish to mesosaline (salinity = 8.5 to 9.7 g / L), and alkaline (pH = 8.4 to 9.0) lakes with high concentrations of sodium and sulfate. The dynamics of stratification and nutrient availability also appear to play a role in LCA abundance, as early spring mixing promotes a bloom of alkenone-producing haptophytes. Four of the alkenone-containing sites contain the $\text{C}_{37:4}$ alkenone; however, we discovered an unprecedented lacustrine alkenone distribution in a cluster of lakes, with a total absence of $\text{C}_{37:4}$ alkenone. We attribute this unusual composition to a different haptophyte species and show that the sulfate:carbonate ratio may control the occurrence of these two distinct populations. We created a new *in-situ* temperature calibration for lacustrine sites that contain $\text{C}_{37:4}$ using a water-column calibration from Lake George, ND and show that U_{37}^K is linearly correlated to lake water temperature ($R^2=0.74$), but $U_{37}^{K'}$ is not. A number of lakes contain an unidentified compound series that elutes close to the LCAs, highlighting the importance of

routine GC-MS examination prior to using lacustrine LCAs for paleotemperature reconstructions.

Keywords: haptophytes, alkenones, phylogenetics, U_{37}^K

1. INTRODUCTION

Long-chain alkenones (LCAs), with carbon number ranging from C₃₆ to C₃₉, are a class of organic biomarkers well established for reconstruction of past sea surface temperatures (SSTs) (Brassell et al., 1986, Prahl and Wakeham 1987) and surface ocean productivity (Moreno et al., 2004; Prahl et al., 2005). These biomarkers are produced by cosmopolitan haptophyte algae (*Emiliani huxleyi* and *Gephyrocapsa oceanica*) in the ocean and can be preserved in sediments for millions of years (Volkman et al., 1980; Rontani et al., 1997; Sonzogni et al., 1997). The degree of unsaturation of C₃₇ alkenones (U_{37}^K or $U_{37}^{K'}$ index) has been shown to be primarily a function of temperature in both core-top and culture experiments (Prahl et al., 1988; Marlowe et al., 1990; Rosell-Melé et al., 1995). Since its establishment, the alkenone unsaturation index has become an indispensable tool for paleoceanographic research (Müller et al., 1998; Lawrence and Herbert 2005).

LCAs also have been reported in lakes (Cranwell, 1985; Zink et al., 2001; D'Andrea and Huang 2005; Chu et al., 2005; D'Andrea et al., 2006, Pearson et al., 2008) but they have not received significant attention by the continental paleoclimate community, primarily because (1) their occurrence is assumed to be rare; and (2) the identity of the algal species that produce lacustrine LCAs are not well-known and may be diverse (Coolen et al., 2004), thus potentially complicating interpretations of the unsaturation index as a function of

temperature. Yet, there is a major need for new, quantitative paleo-temperature proxies for continental sites, because the most common extant proxies either have geographically limited applicability (e.g., oxygen and hydrogen isotope ratios in ice cores) or are influenced by multiple climate or non-climate factors (e.g., speleothem $\delta^{18}\text{O}$ is affected by temperature, precipitation, source water evaporation, water vapor trajectory, etc. and chironomids are potentially influenced by salinity, temperature, hydrology, and nutrients (Porinchu and MacDonald, 2003)). This is particularly important in the Northern Great Plains, where in-lake proxies for drought are well developed (i.e. diatom- and ostracod-inferred salinity), but no temperature proxy exists for before the instrumental period. New techniques are being developed contemporaneously with the LCA temperature proxy, such as the distributions of glycerol dialkyl glycerol tetraethers (GDGTs) from bacteria (Weijers et al., 2007; Peterse et al., 2009) and archaea (Schouten et al., 2002). The TEX₈₆ proxy has been used to reconstruct SST successfully (Schouten et al., 2002, Kim et al., 2008, Rueda et al., 2009), but appears to be only applicable in small number of large lakes (Powers et al., 2004; Tierney et al., 2008; Sinninghe Damasté et al., 2009). The MBT and CBT ratios based on GDGTs from soil bacteria (Weijers et al., 2007) are correlated to soil temperature and pH, but their applicability in lakes is complicated by in-lake production of GDGTs with different distributions (Tierney and Russell, 2009). Therefore, the LCA temperature proxy, if fully developed, will be an

important complement to existing tools for continental temperature reconstruction.

For LCAs to become a useful continental temperature proxy, the key environmental conditions controlling their occurrence and distribution need to be determined. To date, LCAs have been found in saline (D'Andrea and Huang, 2005; Liu et al., 2006; Pearson et al., 2008), and also freshwater lakes (Zink et al., 2001). Pearson et al., (2008) find that sodium sulfate-dominance is associated with the occurrence of LCAs in Spanish lakes, but LCAs also are found in carbonate-dominated lakes in Tibet and Greenland (Chu et al., 2005; D'Andrea and Huang, 2005). Thus, the factors that affect the presence or absence of alkenones are uncertain, and additional lake surveys are needed to understand the occurrence of alkenones in lakes.

Previous studies demonstrate a relationship between growth temperature and degree of unsaturation of sedimentary lacustrine LCAs (Zink et al., 2001; Chu et al., 2005; Liu et al., 2006; Pearson et al., 2008), but there are significant uncertainties in the alkenone-temperature calibration, presumably because individual lakes contain unique (although closely related) haptophyte species (Coolen et al., 2004; D'Andrea et al., 2006). Cultivation of lacustrine algae from individual lakes is one approach for establishing an appropriate calibration. This has proven successful when the haptophyte strain can be

determined, for example Sun et al.,(2007) isolated a strain of *C. lamellosa* from Lake Xiarinur and create a culture-based calibration using the $U_{37}^{K'}$ index. However, isolating and laboratory-cultivation alkenone-producing alga from other lakes has been challenging (Chu et al.,2005; Pearson et al.,2008). Thus, often calibrations for lake alkenones have been established using core-top sediments from transects of lake sites (e.g. Zink et al.,2001). This surface-sediment calibration approach makes implicit assumptions that all lakes contain the same haptophyte species or that different species have an equivalent relationship to temperature. Whether such assumptions are valid for all lakes requires additional testing. Theissen et al.,(2005), on the other hand, used the alkenone unsaturation index from downcore samples as an indicator of relative temperature change without a specific calibration. The relative temperature change is of limited use in paleoclimatology, especially for regions that do not have tree ring, speleothem or other high-resolution datasets, such as the Great Plains, US.

The main objectives of the present study are to survey a large number ($n = 55$) of lakes in the North American interior, including the Northern Great Plains (NGP- ND, SD, and MT) and Nebraska Sand Hills (NE Sand Hills) (Fig. 1) for LCAs and to explore the feasibility of a seasonal temperature reconstruction from the time alkenone-producers bloom in this region. We also provide water-chemistry data and other geochemical and physical

information from the lakes in order to constrain the environmental conditions that favor the occurrence of LCA-producing haptophytes. To date, LCAs have only been reported from one North American lake, Brush Lake in eastern Montana (Zink et al.,2001). We hypothesize that given alkenone-producers have been found from numerous saline lakes elsewhere (D'Andrea and Huang, 2005; Liu et al.,2006; Pearson et al.,2008) that LCAs are regionally widespread among saline lakes and common in the arid and semiarid regions of central North America. Rather than conducting a core-top calibration, we develop an *in-situ* calibration of the alkenone unsaturation index against water temperature using seasonal water-column filters, taking advantage of the water column temperature variation in a single stratified lake (Lake George, ND) where haptophytes occur.

2. METHODS

2.1. Field and Sampling Methods

The sediments from the NGP lakes ($n = 19$) and NE Sand Hills ($n = 34$) were collected from the top 0-1cm of a gravity surface corer (Fig. 1). Water samples for the analysis of major cations and anions were collected from the surface (0.5 m) of the NGP lakes with a Van Dorn water sampler. Samples from the NE Sand Hills were part of a previous study on lake water chemistry (Bennett et al., 2007), and chemical measurements are not available for all lakes. Field measurement of surface water temperature, conductivity, salinity, pH, and dissolved oxygen were made in July 2007 with a YSI hydrolab. In the laboratory sediment samples were frozen, freeze-dried, and processed for alkenone analysis (see below). Mean annual air temperature (MAAT) data were obtained for each site from the closest weather station (from weather.com). Water samples were collected from Lake George from May through July 2008 using a Van Dorn water sampler at the surface, 5 m, and 10 m depth. One liter of water was filtered using a water vacuum filtration unit with combusted (550°C) Whatman GF/F 0.7 μm , 47mm glass filters. The available physical data and water column chemistry for all lakes are summarized in Table 1.

2.2. Analytical Methods

Water samples were measured for major cation and anion concentrations at the University of Nebraska – Water Sciences Laboratory using atomic absorption spectrophotometry for cations and ion chromatography for anions. Each of the 55 freeze-dried sediments (>2 g) and 39 filter samples were homogenized and extracted with dichloromethane (DCM):methanol (MeOH) (9:1, v/v) via an Accelerated Solvent Extractor ASE200 (Dionex). C₃₆ *n*-alkane standard was added, and the total lipid extracts (TLEs) were run on a GC-FID for detection and quantification of alkenones using the internal standard. An Agilent DB-1 GC column (60m × 320µm × 0.10 mm) was used with the following temperature program: an initial temperature of 40°C (hold 1 min), ramp 30°C/min to 290°C (hold 1 min), ramp 5°C/min to 300°C (hold 0 min) then 2°C/min to 325°C, GC temperature program (hold 10 min). The same temperature program was used on the GC- MS, and samples were run to confirm the identity of the alkenones using the known ion chromatograms and by comparison of mass spectral data with published data and GC retention times (de Leeuw et al.,1980, Marlowe et al.,1984). The final GC program described here is the result of many permutations of GC parameters, including the switch from helium as the carrier gas to hydrogen. As such we are able to resolve LCAs with >4µg per sample (i.e. grams of dry sediment or liter of water). Alkenone standards of known temperature calibration are run on the GC-MS to ensure analytical precision (< 0.1°C).

Samples that contained peaks in the alkenone retention time region in the TLE phase were further separated into neutral and acid fractions by elution through a LC-NH₂SPE column using DCM:isopropyl alcohol (2:1, v/v), followed by ether with 4% acetic acid (v/v) (Huang et al.,2002). The neutral fraction was separated into the aliphatic (*n*-hexane), alkenone (DCM), alcohol (ethyl acetate:*n*-hexane 1:3, v/v), and polar (MeOH) fractions using silica gel column chromatography. In order to remove alkenoates, alkenone fractions were then saponified in 5% KOH in MeOH with 3% water and heated overnight at 75°C, acidified and extracted using *n*-hexane and rerun on the GC-FID.

A subsample from Hackberry Lake (ID 34, Table 1), NE that contains an unknown compound series (base peak *m/z* 152) was hydrogenated by adding 5 ml of 10% *n*-hexane in ethyl acetate, 20 µl of acetic acid, and a small amount of platinum oxide catalyst. Hydrogen gas was bubbled into the solution for 2 hours. The catalyst was filtered from solution, and the sample analyzed by GC-MS.

2.3. Potential problems with alkenone identifications in lake sediments

Alkenones generally display characteristic distribution of peak clusters of $C_{37:4}$, $C_{37:3}$ and $C_{37:2}$. These molecular distributions based on similar retention times in the GC-FID are commonly taken as evidence of the presence of alkenones. However, in 7 alkenone-containing lakes (see asterisks in Table 2) as well as 12 of the NE Sand Hill lakes that do not contain alkenones, we found a series of compounds that display similar looking molecular distributions and that elute closely with alkenones in the GC-FID. Due to the similar GC retention times on the non-polar GC columns commonly used for alkenone analysis, these compounds that are not alkenones, can be easily mistaken for alkenones without examination by GC-MS.

The GC retention time and mass spectra for this structurally unknown series of compounds are shown in Fig. 2a-d. The C_{36} compound with molecular weight ($m/z = 516$) elutes slightly after (0.01 min) the $C_{37:3}$ alkenone. Its mass spectrum shows a distinct m/z 152 base peak. The $C_{36:1}$ compound has the molecular weight of 514 and is the one double-bond unsaturated form of the C_{36} compound, as demonstrated by hydrogenation of C_{36} (Fig. 2b).

Amazingly, the distributions of C_{36} and $C_{36:1}$ closely mimic the alkenone distributions (Fig. 2d). The mass chromatogram of m/z 152 reveals a full series of compounds with m/z 152 as the base peak mass, and molecular

weight ranging from 348 to 544 (Fig. 2a). The 516 and 514 mass compounds elute in the $C_{37:4}$ and $C_{37:3}$ LCA range, while the 542 and 544 molecular ions elute with the $C_{39:3}$ and $C_{39:2}$ LCAs (Fig. 2d). Different GC programs were tested to separate the compound from the alkenone peak; however, the retention time difference between these compounds and alkenones is within a hundredth of a minute. Therefore, the m/z 152 series of compounds are virtually impossible to distinguish from alkenones without GC-MS analysis. Hydrogenation of $C_{36:1}$ leads to the formation of $C_{36:0}$, which cannot be further hydrogenated using H_2/PtO_2 , suggesting the presence of two carbon rings in these compounds. Full structure elucidation of this compound series is beyond the scope of this paper. The discovery of this compound shows the importance of running all alkenone samples on the GC-MS to ensure the proper identification and quantification.

3. RESULTS

3.1. LCA occurrence and chain length distribution

Of the 55 lake surface sediments examined in this study, 13 (~25%) contain detectable concentrations of LCAs (Table 2). LCA concentrations from 11 of the sites show well-resolved alkenone peaks in the GC-FID traces and are sufficiently high for accurate quantification. GC-MS analyses reveal that the $C_{37:4}$, $C_{37:3}$, $C_{37:2}$ and $C_{38:3}$ alkenones are present at Skoal Lake and $C_{37:3}$ and $C_{37:2}$ are present at Coldwater Lake. However, alkenone concentrations are

very low, and peaks are poorly resolved in these two lakes. LCAs of chain lengths 37, 38, 39 and di-, tri-, and tetra-unsaturation were accurately quantified from the other 11 sites and are displayed as histograms in Fig. 3, a-k. The alkenones from 8 sites (Fig 3 d-k) show a complete lack of $C_{37:4}$ alkenone and contain $C_{37:3}$ as the dominant LCA.

3.2. Environmental data

In our data set, major cation chemistry is dominated by sodium for seven of the nine lakes that contain alkenones and measured water chemistry (we lack cation data from Freshwater and Meng Lakes). The remaining two lakes (Wickson and Coldwater) are respectively potassium or magnesium dominant, with sodium as the next major cation. LCAs were found only in sites with a pH range of 8.4 to 9.4. Lakes with pH > 9.4 did not contain alkenones. Sites with the $C_{37:4}$ alkenone all have pH below 9.0.

3.3. LCA concentration

C_{37} alkenone concentrations range from 1.17 to 670 $\mu\text{g } C_{37} \text{ g}^{-1}$ of dry sediment (Table 2). The highest concentrations of alkenones occur in lakes in the northeastern section of our sampling area (e.g. Lake George, ND, Moon Lake, ND, and Medicine Lake, SD).

3.4. Seasonal production of LCAs

Seasonal sampling of water-column filtrates from Lake George from the timing of ice-off through the early fall in 2008 (May 16 through September 3) show that alkenones are produced in the water column in early spring, shortly after ice out. Over these 16 weeks, alkenones were present in the water column for the first 66 days (May 16 through July 21). At 5 m depth, alkenone concentration in lake water decreased logarithmically over the sampling season, while stratification index increased exponentially (Fig. 4 a, b). All filters contain C_{37:4}, C_{37:3}, C_{37:2}, C_{38:4et}, C_{38:3et,me}, C_{38:2et,me}, C_{39:4}, and C_{39:3} LCAs. These LCAs are resolved and quantifiable in samples from May through July 9. After July 9, the concentrations fall below 4 µg / L water, and the C₃₇ LCAs are not resolvable. Water samples collected through the ice in February 2009 from 0 m, 5 m, 10 m, and 13 m (max water depth at site 13.5 m) were sampled for LCAs, but none were detected.

4. Discussion

4.1. Environmental Influences on LCA occurrence, concentration and chain length distribution

4.1.1 Groundwater controls on LCA distribution

A first order control on the composition of LCAs in the NGP versus those in the NE Sand Hills appears to be the geochemistry of the groundwater sources feeding the lakes. Eastern SD and ND are underlain by the Upper

Cretaceous aquifer that has low (3000 mg / L) to moderate total dissolved solids (up to 10,000 mg / L) (Whitehead, 1996). The shale unit confining this aquifer, the Pierre Shale, contributes to its dissolved mineral content, including abundant sulfate due to the oxidation of pyrite. This aquifer is a major water source for the lakes with the C_{37:4} alkenones. In contrast, the NE Sand Hill lakes are within the High Plains aquifer, which is composed of Holocene eolian sands overlying Pleistocene and late-Tertiary alluvial deposits and is mostly bicarbonate dominated (Bleed, 1998), as are the Sand Hill lakes that are fed by this aquifer.

4.1.2 Carbonate and Sulfate content on LCA distribution

Six of the eight LCA-containing lakes in the NE Sandhills are carbonate-dominated, whereas, one (Freshwater Lake) is sulfate-dominated. Anion chemistry is not available for Meng Lake. All five LCA-containing lakes in the NGPs are sulfate-dominated, and four of the five lakes contain C_{37:4} (Table 1). The anion chemistry supports the geographic distribution of LCAs with one exception in the NE Sandhills. While the absolute concentration of sulfate and carbonate are variable in these sites (Table 2), the sulfate:carbonate ratio (SCR) is significantly different ($p < 0.05$) for the alkenone-containing NE Sandhill sites (SCR=0.01 to 2.59) that lack C_{37:4} versus the alkenone-containing NGP sites (SCR=15.17 to 117.06) that contains abundant C_{37:4} with the exception of Coldwater Lake. This suggests

that the SCR is an important factor in determining the presence of $C_{37:4}$, which may be due to the presence of different haptophyte species, or the geochemistry may control $C_{37:4}$ production. If this separation is due to different taxa, then SCR may influence the competition between different haptophyte species. We speculate that lacustrine haptophytes may use similar adaptive advantages as marine haptophytes such as the adaptive advantage to use of sulfur under low-nitrogen conditions. Whereas most marine algae use GBT (glycine betaine – $(CH_3)_3N^+CH_2COO^-$) as an osmoregulator, some haptophytes can use DMSP (β -dimethylsulphoniopropionate – $(CH_2)_2S^+(CH_2)_2COO^-$), substituting sulfur for nitrogen under low nitrogen conditions (Andreae, 1986). Likewise, low nitrogen availability (shown in Salm et al., 2009) and high sulfate concentrations (Table 2) in the NGP sites may make them more suitable for haptophytes that use DMSP. Alternatively, high sulfate may influence species composition by favoring haptophyte species that can use ammonium rather than nitrate, similar to a phenomenon that has been observed in culture experiments with saline-lake diatoms (Saros and Fritz, 2002).

In our data set, major cation chemistry is dominated by sodium for seven of the nine lakes that contain alkenones. The cation distribution is independent from geographic distribution, but suggests that elevated sodium may be a

critical for alkenone production, although enhanced potassium concentration does not appear to interfere with LCA producing haptophytes.

Brush Lake, MT is an interesting site in the most northward and westward extent of our study region. Brush Lake is within the Williston Basin and is fed by a lower Tertiary freshwater aquifer. Although Zink et al.,(2001) found alkenones in a Younger Dryas section of the Brush Lake sediment core and low concentrations in modern sediments (ca. 2001), the lake surface sediments collected in 2007 in this study do not contain alkenones. The late-Glacial and early Holocene sediments at Brush Lake, MT contain LCAs with C_{37:4} dominance, which suggest that the source water for the lake had different relative contributions from groundwater sources than today or that Cretaceous shales from glacial deposits added sulfate to the lake. Donovan (1994) shows that Brush Lake today receives groundwater from two sources, the eastern Grenora Channel and the western Clear Lake aquifer, which have very different SO₄ concentrations; Grenora Channel (SO₄ = ~1140 mg L⁻¹) and Clear Lake aquifer (SO₄ <200 mg L⁻¹). In addition, the early Holocene hydrology in this region was much different from today due to the retreat of the Laurentide Ice Sheet and groundwater in shallow glacial deposits which had high concentrations of SO₄ from gypsum dissolution (Hendry et al.,1986). We suggest that either scenario may have created more favorable geochemical conditions for alkenone-producing haptophytes in the early

Holocene. In terms of the modern lake, Brush Lake is fresher today than two decades ago (conductivity decreased from 6450 to 1330 $\mu\text{S} / \text{cm}$ between 1991 to 2004) (Fritz, unpublished data) and during the same period it switched from sulfate-dominated to a carbonate-dominated composition. Today, Brush Lake has the lowest SCR ratio of the NGP alkenone-containing sites (Table 2). Any of the seasonal changes in Brush Lake, including the freshening of the lake and associated changes in dominant ions, stratification, or nutrient regime may have produced an unsuitable habitat for the alkenone-producing algae that once inhabited in the lake.

4.1.3 LCA relationship to pH

All alkenone-containing lakes in the study region are alkaline (Table 1), with pH ranging from 8.0 to 10.3. However, alkenones were found only in sites with a pH range between 8.4 and 9.4. Sites with the $\text{C}_{37:4}$ alkenone all have pH below 9.0. We also report that surface sediments collected from Mono Lake, CA, which has a pH > 10, has no alkenones despite having similar sodium (60mM) and sulfate levels (120 mM) to our LCA-containing lakes. The effect of pH on haptophytes, if any, remains unknown.

4.1.4 LCA Occurrence and Salinity

Sites in which alkenones were detected cover a range of salinity (0.1 to 49.5 g L^{-1}) (Table 1), all but one site with alkenones in the sediments have salinity >

1 g L⁻¹. Thus, we suggest that elevated salinity is an important control on alkenone production. This is consistent with unpublished data from 33 fresh lakes in the eastern US (lakes in Huang et al.,2002) and 32 freshwater lakes from the southwestern US (lakes in Hou et al.,2008) that show no detectable alkenones in modern sediments.

4.1.5 Alkenone concentration

Lake George, ND and Medicine Lake, SD have alkenone concentrations that are an order of magnitude higher than any of the other lakes (Table 3). The characteristics that differentiate Lake George and Medicine Lake from other regional lakes with lower alkenone concentrations are their greater depths, seasonal stratification, and high sulfate concentration. These two lakes, as well as nearby Moon Lake and Skoal Lake, are the only lakes that contain the C_{37:4} alkenone, suggesting the presence of a different haptophyte species. One explanation for the higher alkenone concentrations in the two lakes that stratify is the presence of a physical refuge for haptophyte cells during times of the year when conditions are unsuitable for population growth. Similarly stratification and anoxic bottom waters may help preserve alkenones. In shallower lakes, physical mixing and biological interactions may reduce the number of viable cells that over-winter and germinate to produce the spring bloom and may cause degradation to sedimentary alkenones. Nutrient availability and high sulfate concentration also may play a role in the high

alkenone concentrations in Lake George and Medicine Lake. During spring, overall primary production in the NGP sites commonly is both nitrogen and phosphorus limited, and NO_3 concentrations are relatively low, even in the absence of lake water stratification (Salm et al 2009). The occurrence of haptophytes in spring suggests that the alkenone producers may be adapted to low nutrient conditions (i.e. S for N replacement in osmoregulatory compounds and ammonium – nitrate substitution). Another adaptation to low nutrient conditions may be the reduction of phosphorus demand during times of low availability by substituting non-phosphorus lipids for phospholipids, as has been observed recently in marine phytoplankton (Van Mooy, et al.,2009).

Another feature that separates the sites with the highest concentrations of LCAs, is their location in the northeastern section of our sampling area. This region is coincidentally the coldest part of our study region. The negative relationship between temperature and alkenone abundance has been found in both marine (Sikes et al.,1997; Volkman et al.,1998) and lacustrine (Cranwell, 1985; Zink et al.,2001) environments. For example, D'Andrea and Huang (2005) reported the highest alkenone concentrations in sediments in a set of Greenland lakes, with MAAT of -31°C .

4.2 LCAs in Core Top Sediments as a Salinity and Temperature

Proxy

4.2.1. *C_{37:4} Proportion as a Proxy for Salinity*

The most distinctive feature of the C₃₇ alkenone distribution among the study lakes is the presence or absence of the tetra-unsaturated ketone (Fig. 3). All previously published data have shown that lacustrine alkenones contain C_{37:4} and several studies suggest that the relative abundance of C_{37:4} among C₃₇ alkenones may be a function of salinity, with higher percentage of C_{37:4} in lower salinity lakes (Liu et al.,2006; Chu et al.,2005). The four North American lakes that contain C_{37:4} all have salinity higher than 2.74 g L⁻¹, but the nine lakes that lack C_{37:4} have a wide range of salinities (0.1 to 49.5 g L⁻¹) (Table 1). Our data suggest that there is no clear relationship between salinity and C_{37:4} presence or absence. Thiel et al.,(1997) suggest that the presence of C_{37:4} could be used as a facies marker for lacustrine or select marine settings, however, here we show for the first time that not all lacustrine settings contain the C_{37:4} alkenone.

4.2.2 *Unsaturation indices and haptophyte taxonomy in Core Top Sediments*

U_{37}^K , $U_{37}^{K'}$, $U_{3738}^{K'}$, U_{38}^K , and $U_{38}^{K'}$ indices (defined in Table 2) describe the degree of LCA unsaturation and were calculated for LCAs in the surface sediments of the NGP and NE Sand Hill lakes (Table 2). The U_{37}^K index calibration to temperature has traditionally been used in alkenone lake studies (D'Andrea

and Huang 2005; Zink et al.,2001); however, in a recent study, Pearson et al.,(2008) use the U_{38}^K index for lakes with low C_{37} concentrations. Pearson et al.,(2008) suggest that predominance of C_{38} LCAs may be related to alkalinity or salinity. There is no correlation between U_{38}^K and salinity at our sites. Similarly, Pearson et al.,(2008) hypothesize that the dominance of $C_{38:3}$ could be an indicator of alkaline, evaporitic or saline settings; however, at our sites regressions were run for all LCA chain lengths vs. all environmental parameters for core top LCAs. The $C_{38:3}$ alkenone shows no significant relationship to temperature ($r^2 = 0.35$), salinity ($r^2 = 0.0003$), pH ($r^2 = 0.45$), or alkalinity ($r^2 = 0.075$).

4.2.3 $C_{37}:C_{38}$ ratio and Haptophyte taxonmomy

We do find that the $C_{37}:C_{38}$ ratio is highest (4.6 ± 1.8) in the lakes with $C_{37:4}$ and C_{38} LCAs are dominant in those that do not contain $C_{37:4}$ ($C_{37}:C_{38} = 2.4 \pm 2.9$) (Fig. 5). In these two groups of lakes, NE Sandhills vs. NGP, the $C_{37}:C_{38}$ ratio is significantly different ($p < 0.01$). This ratio has been used in other studies to infer haptophyte taxonomy (Pearson et al.,2008). Based on previously determined $C_{37}:C_{38}$ ratios, our NGP sites group closely to *Isochrysis galbana* and *Chrysotila lamellosa* (Marlowe et al.,1984; Rontani et al.,2004), while our NE Sandhill sites are more similar to *Emiliana Huxleyi* (Marlowe et al.,1984) or Chinese Lakes (Sun et al.,2004; Chu et al.,2005). This taxonomic separation is also supported by the LCA distribution, because

published lacustrine alkenone distributions are characterized by high abundances of the $C_{37:4}$, such as those in English (Cranwell, 1985), Asian (Li et al., 1996, Chu et al., 2005), Antarctic (Volkman et al., 1988) and German (Zink et al., 2001) lake sediments, as well as Pliocene, Oligocene, and Eocene samples (Marlowe et al., 1984, 1990). The alkenone distributions for lakes that lack $C_{37:4}$ are most similar to those reported from the North Atlantic (Rosell-Melé et al., 1994; Brassell et al., 1986), North Sea (Volkman et al., 1980), the Mediterranean (Cacho et al., 1999), and other marine sites. Interestingly, Pearson et al., (2008) report the dominance of $C_{37:3}$ over $C_{37:4}$ in Spanish lakes.

4.2.4 Unsaturation indices and temperature

The U_{37}^K , $U_{37}^{K'}$, $U_{37:38}^K$, U_{38}^K , and $U_{38}^{K'}$ indices were all regressed against a variety of environmental temperatures from the lake sites, including: July lake temperature at 0.5 m depth, MAAT, mean summer air temperature (AT), mean fall AT, and mean winter AT. Regressions against these variables showed no significant correlation. Instead, the two geographic groups consistently clustered showing the difference between the north and south regions, where presumed taxonomic differences in haptophytes preclude the use of these indices for calibration to climate parameters from our core top sediments. Whether or not such a taxonomic difference could itself be related to environmental temperature is not yet known. Because no correlation was

found between $U_{37}^{K'}$, $U_{37:38}^K$, U_{38}^K , or $U_{38}^{K'}$ and temperature, temperature may not be the primary environmental factor affecting these indices. Instead these indices may respond to light, nutrients, or other environmental factors independent from $C_{37:4}$.

4.2.5 Potential degradation of LCAs

While we acknowledge studies that suggest there may be differential degradation of LCAs dependent on the degree of saturation (Gong and Hollander, 1999; Rontani et al., 2005), there are several lines of evidence to suggest that differences between NGP and NE Sandhill sites are taxonomic differences (i.e. the geographic separation, $C_{37}:C_{38}$ ratio, SCR). Degradation is an important question to test in order to the use LCAs as a temperature proxy and should be considered when choosing a site for reconstruction. For example, Gong and Hollander (1999) show that less saturated chains degrade preferentially over more saturated chains in oxic sediments, Teece et al., (1998) show that no chain length alteration occurs during anaerobic degradation. As such, a site should be chosen that is thought to have remained anoxic for the length of the reconstruction. As Teece et al., (1998) report and is seen in high-sulfate lakes in Greenland (D'Andrea and Huang, 2005), alkenones tend to preserve better under sulfate-reducing conditions. These are important considerations for proxy reconstruction and guide our

future plans for a reconstruction from Lake George, ND (60 m maximum depth; 12,210 mg L⁻¹ SO₄).

4.2.6 Comparison of Core Top LCAs to a known calibration

The only other lacustrine alkenone calibration to U_{37}^K (Zink et al., 2001) found a significant relationship between U_{37}^K and summer surface water temperature in German lake sites ($r^2=0.67$). The lack of correlation to July surface water temperature in our sites could be a function of several factors. For example, (1) alkenone production may not occur during mid-summer in our region - based on *in situ* water column filtration in the NGP lakes, alkenones were not present in July of 2007; (2) even if present, the alkenone producers may not inhabit the uppermost waters (0.5 meters water depth) – Zink et al., (2001) suggested that haptophyte habitat may be deeper in the water column; (3) summer surface water temperature is a point sample that can change significantly over short time periods, whereas the sediment record presumably captures a more integrated period of time.

If we plot the U_{37}^K values of the three NGP lakes that contain C_{37:4} versus MAAT, the relationship fits well along the calibration previously determined for German lakes (Zink et al., 2001) (Fig. 6). While the slopes of the surface water temperature- U_{37}^K relationship are similar in German lakes and the oceans (Müller et al., 1998), there is a large difference between the values of

U_{37}^K in these two measurements at a given temperature. Moreover, the NE Sand Hills lakes occupy a third space (Fig. 6) that does not agree with either the lacustrine or marine array. This is further evidence that the NE Sand Hills sites may contain a new and unique haptophyte species. Sedimentary rDNA samples have been collected and are being analyzed at the Marine Biology Lab, Woods Hole for more conclusive taxonomic distinctions. It is not clear why our $C_{37:4}$ plotted versus MAAT falls along the calibration for German Lakes of surface water temperature- U_{37}^K . Here we suggest that lack of information on lacustrine haptophyte habitat limits our ability to precisely define what specific environmental temperature these organisms record in different lakes.

4.3 Single Site LCA Proxy calibration

4.3.1 Seasonal Water Sampling and Haptophyte Ecology

Much remains unknown about production of alkenones in lakes. Here we focus field studies on the lake in our study region with the highest concentrations of sedimentary alkenones to understand when and in what part of the water column the alkenones are produced. Seasonal sampling of water-column filtrates from Lake George, ND beginning at ice-off through the early fall in 2008 (May 16 through September 3) indicates that the haptophyte bloom at the surface begins in early spring, shortly after ice out. Over these 16 weeks, alkenones were present in the water column for the

first 66 days (May 16 through July 21). LCAs are in the highest concentrations initially at 10 m, then 5 m, then 0 m suggesting that they ascend through the water column over the course of ~20 days (Fig. 4a). Alkenone concentrations in lake water at 5 m depth decreased logarithmically, while stratification index increased exponentially (Fig. 4 b,c). LCA concentrations decreased as stratification index intensified, and as the epilimnion waned. This observed sequence suggests that the LCA producers may have a winter resting stage, similar to haptophyte taxa *Prymnesium* (Billard, 1994), at depth during ice-cover and subsequently move toward the surface when triggered by a critical environmental parameter. Water column samples collected at multiple depths in February 2009 contain no LCAs and suggest the organisms are not present in the water column. Culture experiments have previously suggested that the movement of Chrysophytes toward the surface is influenced by light (Holen, 1999). Similarly, the movement of the alkenone-producers to the surface in spring may be triggered by solar radiation, while cool water and a relative increase in nutrients associated with water-column mixing promote the bloom. Culture samples from Lake George collected in the spring of 2009, contain mobile algae with 2 unequal flagella that we suspect are haptophytes. Cultures also contain colonizing green algae and have been sub-sampled to isolate a monoculture. Unfortunately, so far we have failed to find alkenones in laboratory cultures incubated from Lake George water.

4.3.2 Unsaturation Indices from In-situ Water Column Samples from Lake George, ND: a new approach in calibrating alkenones to temperature in lakes

Due to the presumed difference in algal species between the NGP sites and the NE Sand Hill sites, development of a core-top U_{37}^K - temperature calibration in interior US lakes is not possible. Culture samples were collected in June of 2009, after the 2008 monitoring season and enough data was collected to predict the time of their presence in the water column. A culture calibration is forthcoming. Here we use a novel approach to such a calibration for sites containing $C_{37:4}$ LCA using water column filters collected at 0 m, 5 m, and 10 m depth from May 16, 2008 through July 21, 2008 and additional sampling in June, 2009 from Lake George, ND. During this time, water temperatures range from 2 to 22°C (Table 2). The U_{37}^K index has a significant relationship to lake water temperature ($r^2 = 0.75$) (Fig. 7a); whereas the $U_{37}^{K'}$ shows no correlation ($r^2 = 0.14$) to lake water temperature. This suggests that $C_{37:4}$ responds much more strongly than $C_{37:3}$ or $C_{37:2}$, but the proportion of $C_{37:3}$ to $C_{37:2}$ still has a small response, otherwise the index would not work. The $U_{37}^{K'}$ shows about one quarter of the variance (0.004) than the U_{37}^K index (0.016) across the filter data, and $C_{37:4}$ ranges between 46 to 81% of the C_{37} homologues. Not surprisingly, due to the predominance of $C_{37:4}$ alkenone, the proportion of $C_{37:4}$ is also linearly correlated to temperature ($r^2 = 0.73$) (Fig. 7b). Scatter about the linear calibration may be

the result of alkenones produced close to the lake surface that subsequently rain down, or deeper alkenones brought up via wind mixing.

This Lake George *in-situ* U_{37}^K calibration predicts consistently higher temperature values than the calibration of Zink et al.,(2001), although the slopes are similar (Fig. 7c). The main difference between these calibrations is that the Lake George calibration documents the LCA ratios from presumably live organisms and compares them directly with the temperature at which they are growing. This technique is similar to that of Goni et al (2004) where the U_{37}^K of LCAs in sediment traps in the Cariaco Basin correlates to seasonal changes in SSTs. The Zink et al.,(2001) calibration compares alkenones from core top sediments, which may combine multiple years and different growth depths into a single sample to a single summer surface lake-water temperature. By using *in-situ* measurements, we remove the complicating factors of taphonomy and alkenone transport to the sediments, growth depth, and seasonal bias from our calibration. We suggest that this new calibration is the best for the $C_{37:4}$ -containing lakes in the NGPs.

5. CONCLUSIONS

The discovery of 13 new alkenone sites from the interior US provide new understanding regarding lacustrine alkenone presence, abundance, and distribution. We show that: (1) The highest concentrations of alkenones are

found in deep, cold lakes with high sodium and sulfate concentrations and a pH range 8.4 to 9.0; (2) A potentially new lacustrine haptophyte species, as suggested by a relatively low $C_{37}:C_{38}$ ratio and the absence of $C_{37:4}$ LCA exists in the lakes of the interior US. The haptophyte taxa in NGP sites and NE sandhill sites are strongly separated according to the SCR of the lake water; (3) Alkenone-producers bloom in the early spring in Lake George, probably triggered by increased solar radiation. The bloom is promoted by cool water temperatures and increased nutrient concentrations due to lake overturning; (4) A new alkenone unsaturation to temperature calibration is derived from water column filtration from Lake George. It is the first time that such a approach is used to derived LCA temperature calibration; (5) We demonstrate that the $U_{37}^{K'}$ in our study sited is not sensitive to temperature; and (6) Alkenone samples must be run on the GC-MS to ensure the correct identification of compounds due to an unknown compound series (key ion m/z 152).

Our study provides an improved understanding on the environmental conditions promoting the occurrence of alkenones in lakes. The environmental parameters that apparently most strongly affect the LCA distribution are salinity, SCR and temperature. For the first time we show that the SCR of lake water strongly influences the presence of $C_{37:4}$. This ratio should be assessed for marine and lacustrine sites from which LCAs are

reported. The presence of the C_{37:4}, is geographically restricted to sites in the northeastern portion of our study area. These sites generally have cooler temperatures and a dominance of sodium and sulfate ions. The addition of new sites, from a larger geographic and environmental range will help to further define the conditions in which LCAs are produced and will further aid in interpreting LCAs in the paleoclimate record.

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Table 1. Compiled environmental, physical and chemical data for lakes presented in this study. (Note: lakes denoted with an * contain alkenones in the core-top sediments)

ID #	LCA	Lake	State	Lat.	Long.	Sample Water Depth (m)	Conductivity (uS/cm)	pH	DO (%) 0.5m	July Lake T	Salinity (g / L)	Dominant anion	Dominant Cation
<i>NE Sandhills Lakes</i>													
1		Box Butte	NE	42.46	103.10	4.61	322	8.69	8.59	18.15	0.15 x		x
2		Chadron	NE	42.45	103.00	4.32	301	8.96	8.18	20.96	0.11 x		x
3		Champion	NE	40.28	101.45	1.30	392	8.66	6.80	23.66	0.19 x		x
4		Cub Creek S	NE	40.24	97.05	2.87	166	8.08	7.10	22.44	0.07 x		x
5		Cochran	NE	41.46	103.32	x	1444	9.27	6.48	20.62	0.77 x		x
6		Cozad	NE	42.22	99.44	x	348	9.06	6.84	19.33	0.17 x		x
7		Hayes Center	NE	40.35	100.56	1.86	472	8.77	8.23	23.60	0.24 x		x
8		Isham Dam	NE	42.55	102.47	4.46	767	9.32	7.40	20.74	0.39 x		x
9		Kilpatrick	NE	42.05	103.17	1.90	475	9.41	7.20	20.60	0.24 x		x
10	*	Meng	NE	42.96	103.59	1.07	9847	9.65	7.63	18.27	5.57 x		x
11		Merritt	NE	41.56	103.56	20.03	180	8.66	9.98	18.63	x x		x
12		Minatare	NE	41.55	103.30	5.87	722	8.65	7.62	18.22	0.35 x		x
13		Norman	NE	42.86	103.54	0.77	477	9.19	8.28	16.37	0.27 x		x
14		Ogallala	NE	41.21	101.65	10.56	752	8.53	8.87	16.79	0.46 x		x
15		Wellfleet	NE	40.45	100.44	2.50	363	8.70	7.83	23.24	0.18 x		x
16		Whitney	NE	42.47	103.18	2.42	390	8.77	6.76	18.64	0.19 x		x
17		CP Johnson	NE	42.68	103.52	3.87	304	8.58	8.46	18.79	0.11 x		x
18		Two Mile	NE	42.40	101.16	x	235	10.34	9.37	18.34	0.11 CO3		Na(Ca)
19	*	Big Alkali	NE	42.38	100.36	2.11	544	8.76	9.08	19.84	0.26 CO3		Na
20		Border Lake	NE	41.79	102.54	0.90	31621	10.02	6.77	17.44	0.20 CO3(SO4)		Na
21		Dewey Lake	NE	42.33	100.39	1.65	339	8.59	8.97	18.64	0.15 CO3		Ca
22	*	Dry Lake	NE	41.40	101.17	0.96	3475	9.25	8.12	19.93	2.22 CO3		Na
23	*	Freshwater Lake	NE	42.22	102.60	1.07	41285	9.39	7.41	21.77	49.45 SO4		x
24	*	Goose Lake	NE	42.42	101.19	0.91	4008	8.92	9.16	17.27	4.06 CO3		Na
25		Jesse Lake	NE	42.11	102.64	0.53	61412	9.85	6.04	18.33	64.91 CO3(SO4)		x
26		Jesse Road	NE	42.10	102.60	0.68	28978	9.51	6.74	19.93	25.16 CO3(SO4)		x
27	*	Palmer	NE	42.09	102.59	0.85	10469	9.36	8.37	20.28	8.63 CO3(SO4)		x
28		Pony Lake	NE	42.29	100.31	1.18	283	9.07	9.53	19.46	0.14 CO3		Na
29		Roundup Lake	NE	41.75	102.41	1.01	1145	8.70	8.87	18.39	0.75 CO3		Na
30	*	Sand Beach	NE	41.68	101.36	2.13	2653	9.17	8.72	20.52	1.68 CO3		Na
31		Swan Lake	NE	42.14	100.46	0.74	180	9.38	9.02	20.62	0.08 CO3		Ca
32		Three Claim	NE	41.78	102.50	x	3001	8.82	8.36	15.53	3.39 CO3		x
33	*	Wickson Lake	NE	42.16	102.59	0.84	19169	9.15	7.53	20.31	5.50 CO3(SO4)		K(Na)
34		Hackberry	NE	41.44	102.26	x	578	x	x	x	0.29 CO3		Na(Mg)(K)
35		West Long	NE	42.32	100.43	1.23	321	8.94	9.30	19.25	0.17 CO3		x
<i>Northern Great Plains Lakes</i>													
36		Albert	SD	44.53	97.13	2.30	1995	8.46	0.89	31.05	0.89 SO4		Mg(Ca)
37		Alkali	ND	48.58	103.60	<0.5	28608	x	18.07	23.97	18.07 x		x
38		Alkaline	ND	46.66	99.56	2.90	4263	8.93	2.31	24.08	2.31 x		Mg(Na)
39		Bitter	SD	45.27	97.27	5.20	3209	8.83	1.70	24.25	1.70 SO4(CO3)		Mg(Na)
40		Brush	MT	48.60	104.11	15.50	5547	9.00	3.23	21.56	3.23 SO4		Na
41		Carlson	ND	47.92	101.47	4.50	1181	9.15	0.64	21.07	0.64 x		x
42		Clear	MT	48.65	104.15	1.70	7608	9.19	4.58	21.09	4.58 CO3		Na
43	*	Coldwater	ND	46.01	99.07	6.80	2240	8.89	1.12	25.24	1.12 SO4(CO3)		Mg(Na)
44		Enemy Swim	SD	45.44	97.29	5.70	407	8.95	0.20	24.68	0.20 x		x
45	*	George	ND	46.74	99.49	18.80	16584	8.98	9.72	24.90	9.72 SO4		Na
46		Goose	MT	48.84	104.06	<0.5	131807	x	92.20	x	92.20 x		x
47		Isabel	ND	46.82	99.74	2.20	1733	8.84	0.85	26.41	0.85 CO3(SO4)		Na(Mg)
48		Kettle	ND	48.61	103.62	9.70	1624	8.79	0.87	21.86	0.87 x		x
49	*	Medicine	SD	44.98	97.36	8.30	14754	8.37	8.50	25.36	8.50 SO4(CO3)		Na
50	*	Moon	ND	46.86	98.16	8.20	5355	8.98	2.74	27.35	2.74 SO4(CO3)		Na(Mg)
51		Nelson	ND	47.92	101.47	4.00	1650	9.20	0.91	20.84	0.91 SO4		x
52		Rice	ND	48.01	101.53	5.80	1751	8.95	0.93	22.73	0.93 SO4		x
53	*	Skoal	ND	47.92	101.47	1.80	12018	8.90	7.68	20.07	7.68 SO4		x
54		Spiritwood	ND	47.07	98.61	14.00	2050	9.27	0.99	25.66	0.99 SO4(CO3)		Na(Mg)
55		Waubay	SD	45.45	97.38	7.30	2216	8.86	1.13	24.91	1.13 SO4		Mg

* LCAs are present.

Table 2. Sulfate and carbonate anion data and calculated ratios for alkenone-containing lakes. The two groups, NE Sandhills Lake and Northern Great Plains lakes are significantly different ($p < 0.05$) (Data from previously unpublished fieldwork by Fritz.)

Lake	Carbonate (mg/L)	Sulfate (mg/L)	SO ₄ /CO ₃
<i>NE Sandhills Lakes</i>			
Big Alkali	209	3	0.01
Dry Lake	1240	49	0.04
Freshwater Lake	10713	27783	2.59
Goose Lake	1887	84	0.04
Palmer	4618	692	0.15
Sand Beach	1071	10	0.01
Wickson Lake	2127	931	0.44
<i>Northern Great Plains Lakes</i>			
George	346	12210	35.25
Medicine	245	28680	117.06
Moon	73	2541	34.67
Skoal	277	8955	32.34
Coldwater	125.1	1898	15.17
Brush	281.8	2304	8.18

Table 3. Organic geochemical data and calculated alkenone distribution indices for alkenone-containing core top sediments and Lake George, ND water filter samples.

Lake	State	C_{37}^a	UK37' ^b	UK37 ^c	UK38 ^d
		Concentration (ug / g sed)			
Moon Lake	ND	1.93 *	0.18	-0.58	-0.14
Medicine Lake	SD	669.99	0.19	-0.61	-0.12
Lake George	ND	313.95	0.16	-0.64	-0.12
Sand Beach Lake	NE	16.57	0.21	0.21	0.06
Freshwater Lake	NE	3.84 *	0.18	0.18	0.18
Goose Lake	NE	4.49 *	0.18	0.18	0.06
Wickson Lake	NE	37.35	0.15	0.15	0.01
Dry Lake	NE	1.24 *	0.25	0.25	-0.30
Palmer Lake	NE	2.83	0.21	0.21	0.14
Big Alkali Lake	NE	1.17 *	0.14	0.14	0.29
Meng Lake	NE	4.74 *	0.16	0.16	-0.02
Coldwater Lake	ND	1.98 *	x	x	x
Skoal Lake	ND	x	x	x	x
LG ^d 5/16 0cm	ND	111.72	0.15	-0.70	0.19
LG 5/16 5cm	ND	393.59	0.16	-0.71	0.19
LG 5/16 10cm	ND	547.19	0.16	-0.72	0.20
LG 5/27 0cm	ND	291.70	0.15	-0.67	0.20
LG 5/27 5cm	ND	109.61	0.15	-0.69	0.20
LG 5/27 10cm	ND	118.36	0.15	-0.69	0.20
LG 6/03 0cm	ND	96.44	0.15	-0.62	0.21
LG 6/03 5cm	ND	175.16	0.14	-0.63	0.20
LG 6/03 10cm	ND	104.74	0.14	-0.65	0.21
LG 6/10 0cm	ND	72.54	0.15	-0.55	0.22
LG 6/10 5cm	ND	67.20	0.16	-0.52	0.21
LG 6/10 10cm	ND	29.28	0.15	-0.53	0.23
LG 6/20 0cm	ND	26.42	0.20	-0.35	0.20
LG 6/20 5cm	ND	20.12	0.22	-0.38	0.22
LG 6/20 10cm	ND	88.17	0.20	-0.45	0.24
LG 6/30 0cm	ND	20.95	0.16	-0.50	0.22
LG 6/30 5cm	ND	23.07	0.18	-0.49	0.22
LG 6/30 10cm	ND	13.12	0.07	-0.57	0.17
LG 7/09 0cm	ND	9.51	0.15	-0.50	0.20
LG 7/09 5cm	ND	9.78	0.16	-0.44	0.22
LG 7/09 10cm	ND	10.16	0.16	-0.50	0.26
LG 7/21 0cm	ND	2.95	0.00	-0.63	0.20
LG 7/21 5cm	ND	0.00	0.00	-0.53	0.00
LG 7/21 10cm	ND	4.08	0.00	-0.56	0.00

a. C_{37} concentration = 37:4 + 37:3 + 37:2

b. UK37' = 37:2 / (37:2 + 37:3)

c. UK37 = (37:2 - 37:4) / (37:2 + 37:3 + 37:4)

d. UK38 = (38:2 + 37:2) / (38:2 + 38:3 + 37:2 + 37:3)

e. LG = Lake George Filter

*These lakes contain the MS 152 peak, indicating that they are coeluted with another compound.

x signifies that sample concentrations were too low to quantify.

Fig. 1. Map showing the location of investigated lakes sites. Numbers correspond to lakes listed in Table 1. Numbers enclosed by a square represent sites with alkenone presence in the modern lake sediments.

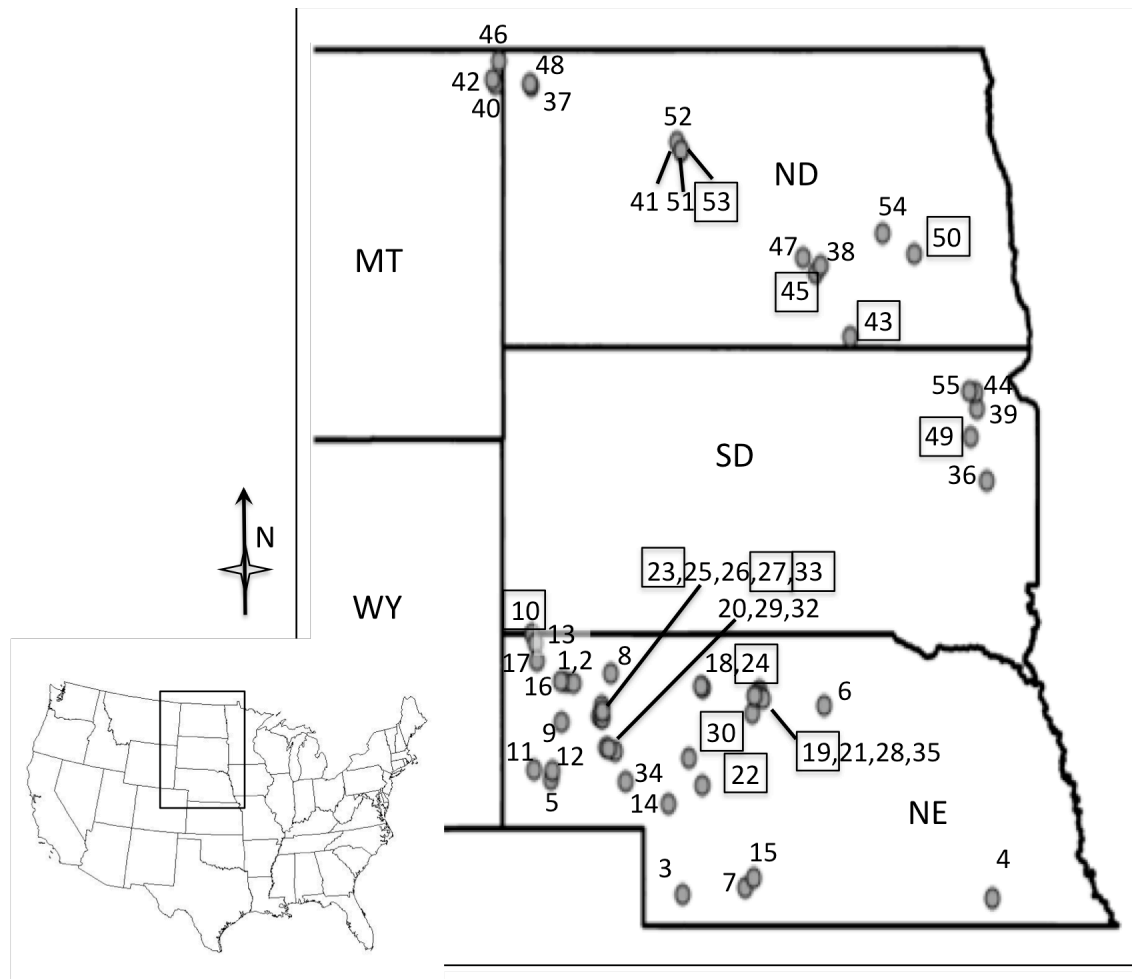


Fig. 2. GC-MS data for a novel compound series found in a number of study sites: (a) Ion chromatogram of the compound series (m/z 152), (b) same as (a) but after hydrogenation, (c) mass spectrum of the $C_{36:0}$ homologue of the compound series, (d) Ion chromatogram showing elution of the unknown compound series (m/z 152) in relation to alkenones (characterized by ions 81 and 95) on a DB-1 GC column.

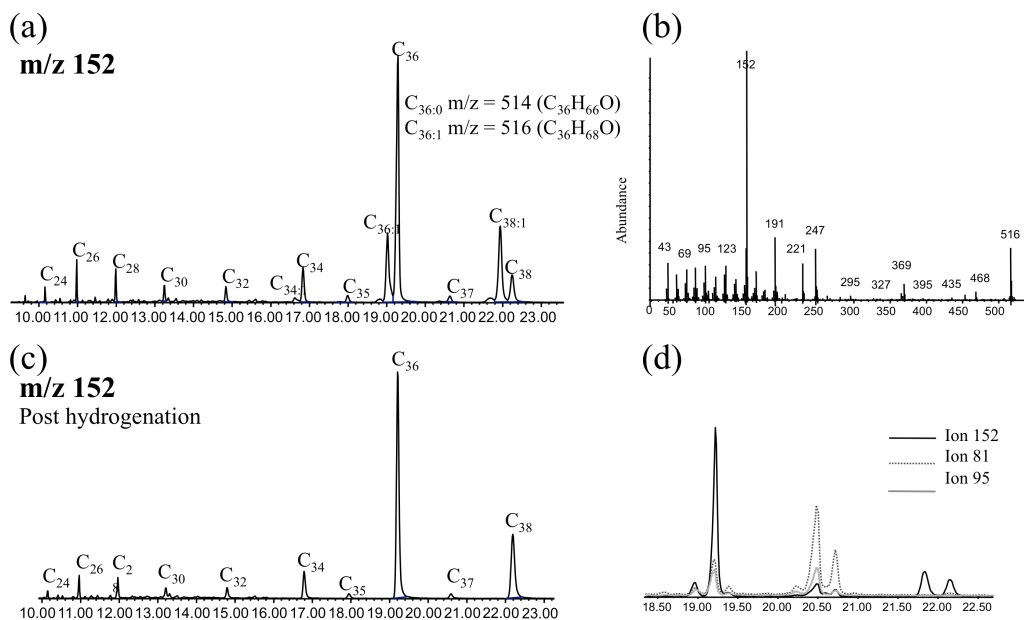


Fig. 3. Histograms showing the relative abundance of long chain methyl and ethyl ketones for lake sites with quantifiable alkenone peaks. Lakes a-c are in the NGP and contain the $C_{37:4}$ alkenone, which is absent from the LCA profiles from the NE lakes shown in d-k. (NGP = Northern Great Plains; MAAT = Mean Annual Air Temperature) (Note: Skoal Lake which contains $C_{37:4}$ and Coldwater Lake are not shown due to poorly resolved peaks.)

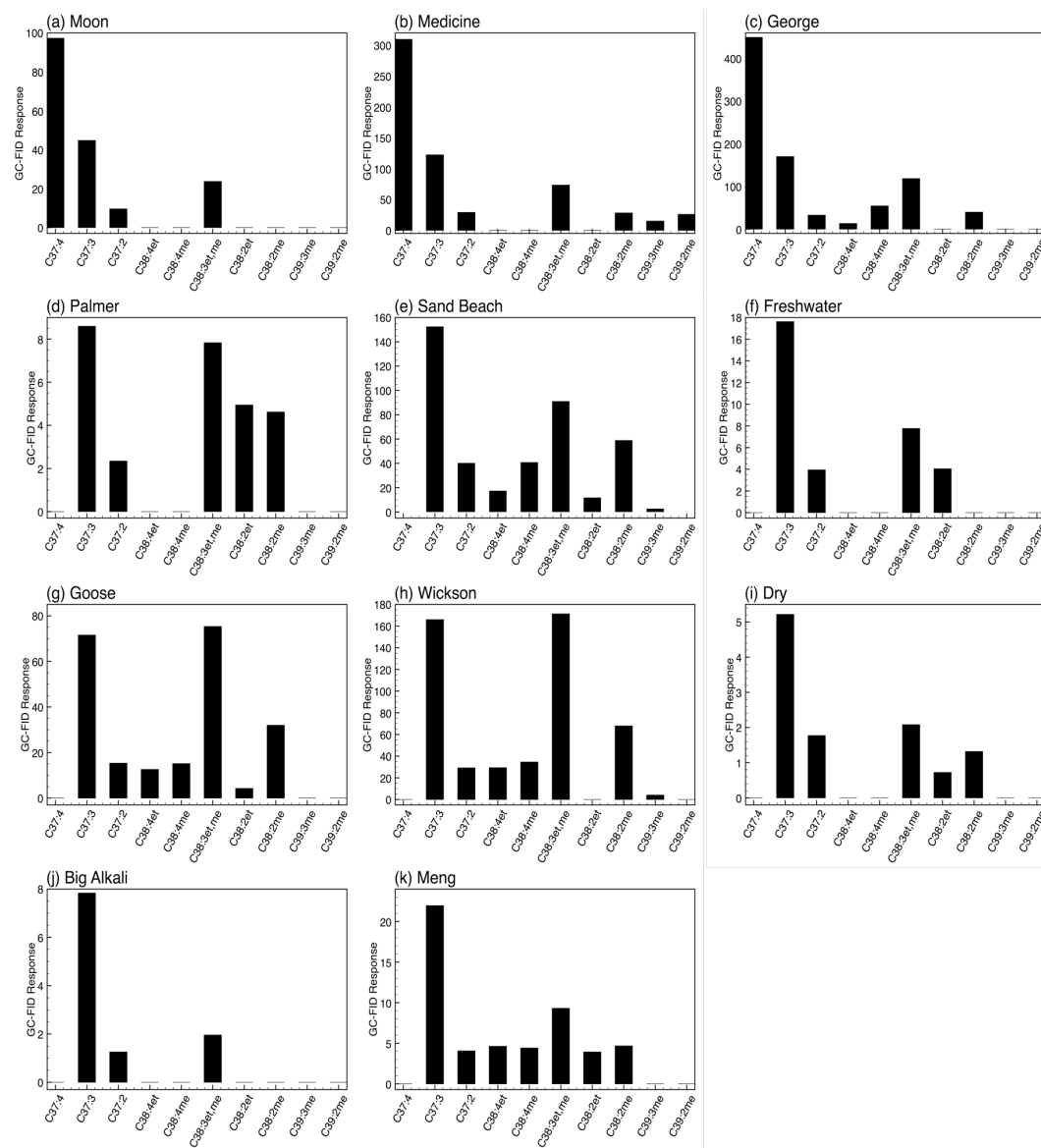


Fig. 4. (a) Alkenone concentration data from water column sampling at 5 m depth from Lake George, ND. Day 1 = May 16, 2008. (b) The stratification index (surface water temperature subtract the temperature at 10 m water depth) for Lake George, ND. (c) Alkenone concentration data from water column sampling at 0 m (diamonds), 5 m (squares) and 10 m (triangles) from Lake George, ND with exponential trend (0 m = gray solid line, 5 m = gray hatched line, 10 m = solid black line).

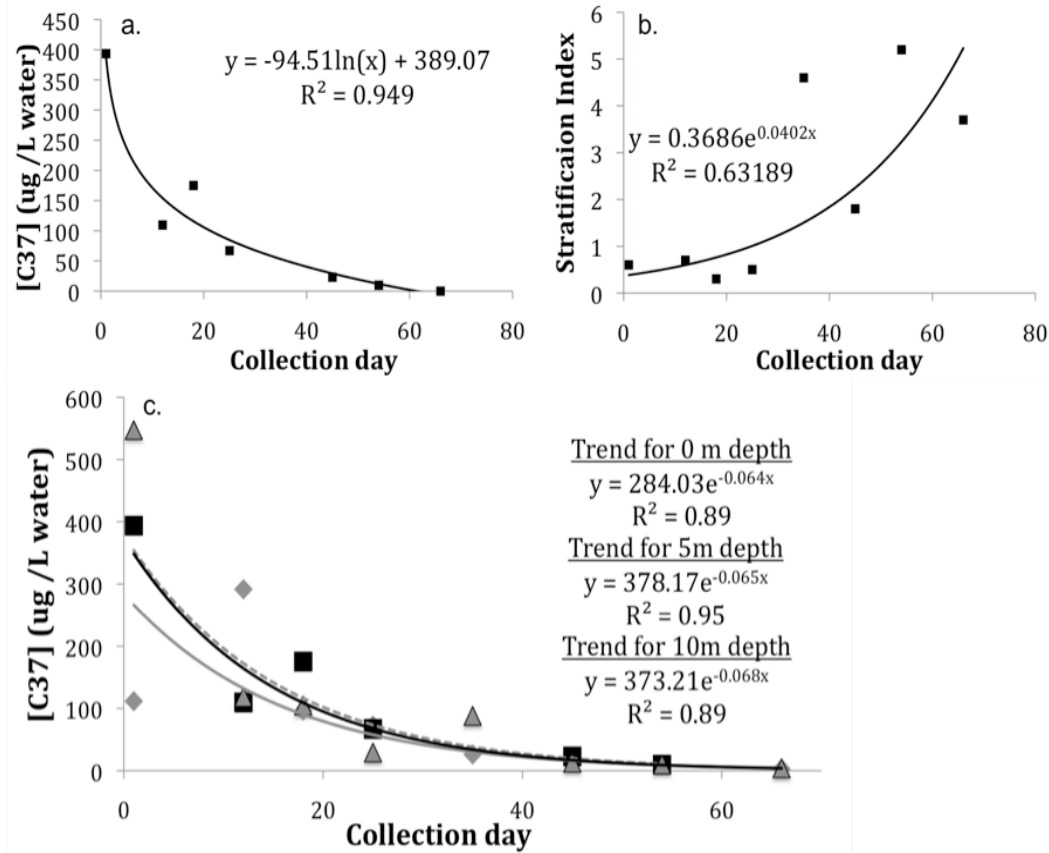


Fig. 5. The $C_{37}:C_{38}$ ratio distribution for lakes that contain the $C_{37:4}$ LCA versus lakes that do not contain $C_{37:4}$.

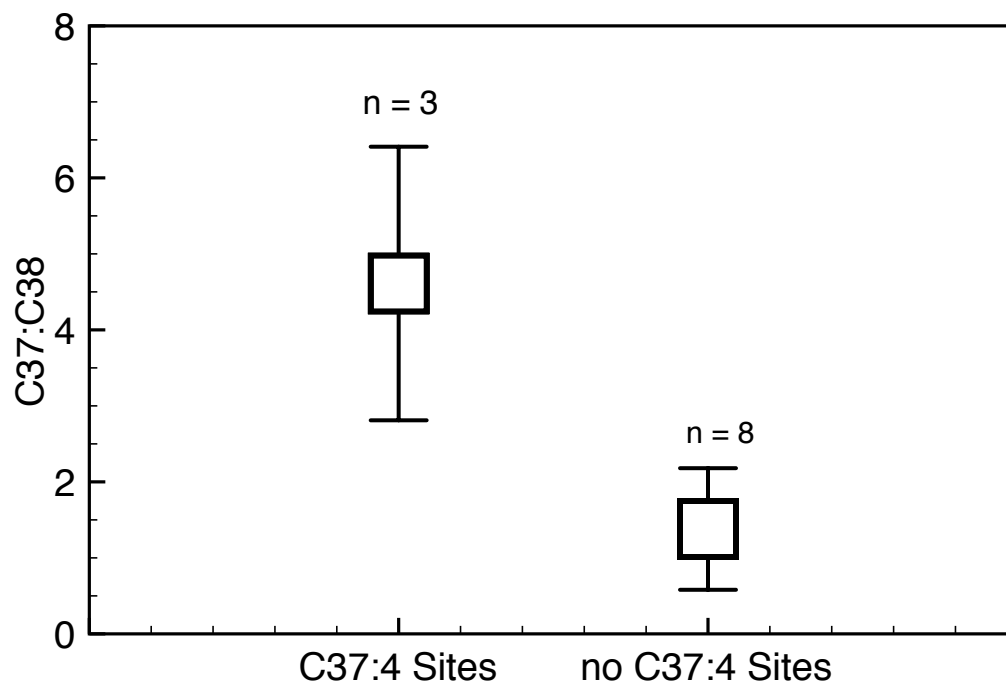


Fig. 6. The previously published Zink et al. (2001) lacustrine LCA U_{37}^K calibration (diamonds) with the NGP core top U_{37}^K (triangles), the NE Sand Hill sites U_{37}^K (X) and the accepted marine linear calibration from Müller et al., 1998. (NGP = Northern Great Plains; MAAT = Mean Annual Air Temperature)

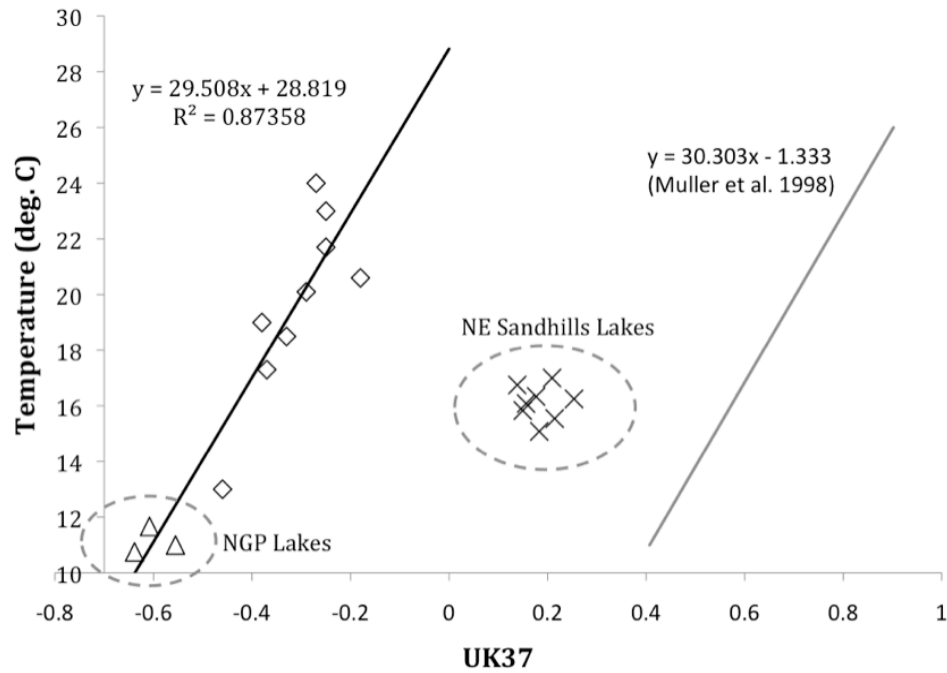
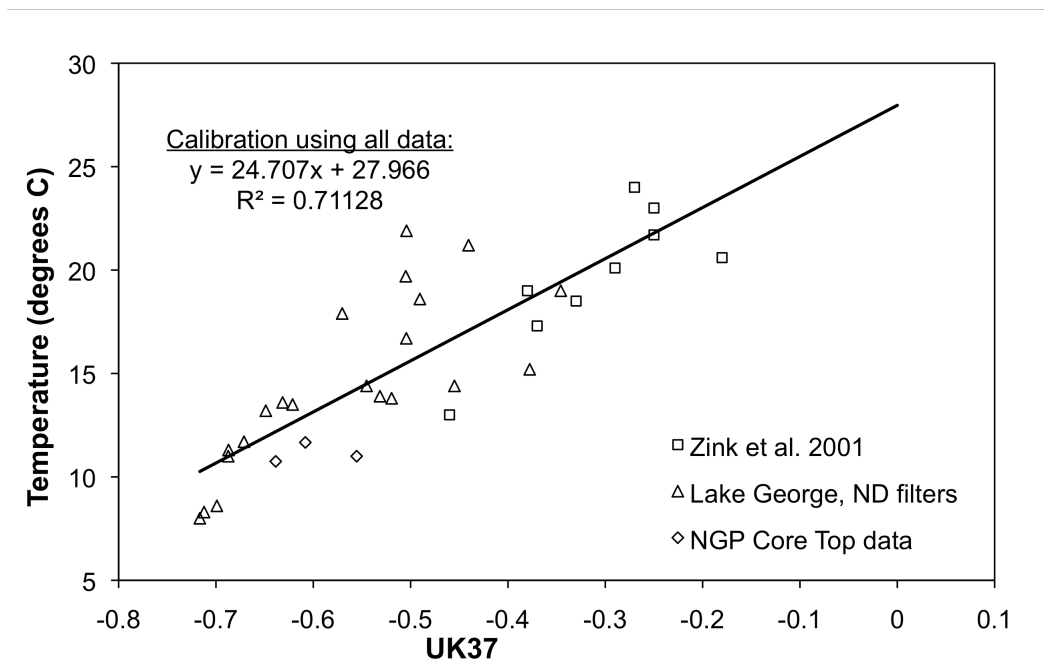
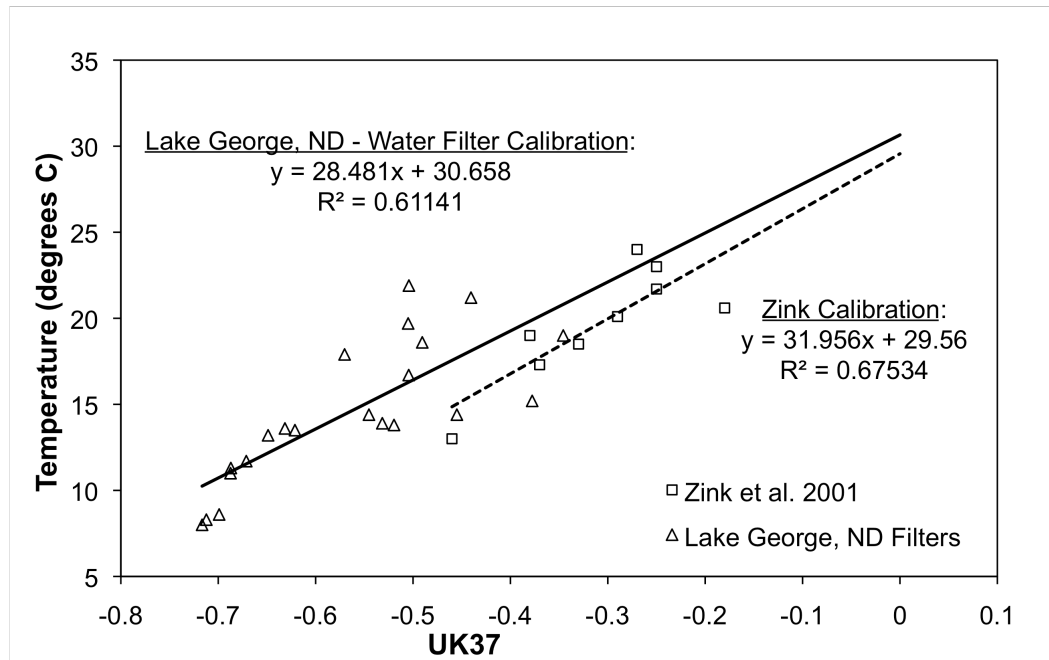


Fig. 7. (a) The lacustrine LCA U_{37}^K calibration from the 2008 Lake George, ND for water column filters with *in-situ* lake water temperature (linear regression $r^2 = 0.74$, $p < 1.4 \times 10^{-8}$), (b) The proportion of $C_{37:4}$ from Lake George, ND water column filters plotted versus *in-situ* lake water temperature (linear regression $r^2 = 0.72$, $p < 4.0 \times 10^{-8}$). (c) The lacustrine LCA U_{37}^K calibration from the 2008 Lake George, ND for water column filters with *in-situ* lake water temperature versus the Zink et al.,(2001) calibration of U_{37}^K with summer surface water temperature.



CHAPTER 3

ALKENONES ARE COMMON IN PRAIRIE LAKES OF INTERIOR CANADA

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In Review:

Organic Geochemistry

ABSTRACT

Long-chain alkenone (LCA) lipids produced by haptophyte algae are ubiquitous in marine environments and are increasingly reported from enhanced conductivity, solute-rich lakes worldwide. Following our survey of lakes in the United States (Toney et al., 2010 doi:10.1016/j.gca.2009.11.021), we examined surface sediments from 19 lakes in central Canada for alkenones and compared our data with lake characteristics. We found that 68% of surveyed lakes contained sedimentary LCAs with C_{37:4me}-dominated profiles. LCA concentrations were highly elevated ($\Sigma C_{37} > 500 \mu\text{g g}^{-1}$ sediment) in sediments of meromictic lakes with permanently anoxic deep waters. Overall, the distribution of LCAs was consistent with a previous survey of lakes in the interior of the United States demonstrating that tetra-unsaturated lipids were most common in sulfate-rich, saline lakes. Alkenone unsaturation indices (U_{37}^K) from different lakes are marginally correlated with spring water temperature ($r^2 = 0.30$, $p = 0.067$), consistent with our previous findings (Toney et al., 2010 doi:10.1016/j.gca.2009.11.021; Theroux et al., 2010 doi:10.1016/j.epsl.2010.10.009) that lakes can harbor different haptophyte species, and individual calibrations for study lakes are critical for paleotemperature reconstructions.

Keywords: alkenones; paleolimnology; haptophyte; paleotemperature; lake; paleoclimate; biomarkers

1. Introduction

The unsaturation ratio of long-chain alkenones (LCAs) has been used as a paleothermometer in marine studies for decades (Brassell et al., 1986; Prah1 and Wakeham, 1987). Since the first report of LCAs in lakes (Cranwell, 1985), LCAs have increasingly been reported in modern saline lakes from around the world, including lakes in China (Li et al., 1996; Wang and Zheng, 1998; Chu et al. 2005; Sun et al., 2007; Liu et al., 2008), Turkey (Thiel et al., 1997), Germany, Austria, Russia (Zink et al. 2001), United States (Zink et al. 2001, Toney et al., 2010), Greenland (D'Andrea and Huang, 2005), Spain (Pearson et al, 2008), and Canada (herein). However, it is clear that the haptophyte species that produce LCAs in lakes are not the same as the ubiquitous marine species, *Geophryocapsa oceanica* and *Emiliani huxleyii*, and can differ from lake to lake (Coolen et al., 2004; D'Andrea et al. 2006; Theroux et al. 2010). Fortunately, although the existence of multiple lacustrine taxa may complicate the development of a universal LCA proxy for past environmental temperatures, Toney et al. (2010) showed that such a calibration could be derived for LCAs using *in situ* techniques at individual lake sites.

Development of LCAs as metrics of past temperatures requires improved information on the occurrence and distribution of LCAs in lakes of different chemical and physical properties around the world. Toney et al. (2010) found

that haptophyte production in central North American lakes is greatest in cool spring season, particularly in lakes with abundant dissolved sodium and sulfate, and elevated $\text{SO}_4 : \text{CO}_3$ ratios (SCR). Since similar lake characteristics are also common in Canadian prairie lakes, we analyzed lipid compositions in sediments from 19 lakes of southern Saskatchewan, Canada (Figure 1). Our study lakes have well-established background data in water chemistry (Pham et al. 2008) and hydrology (Pham et al. 2009), allowing us to further constrain conditions favoring the occurrence of alkenone-producing haptophytes.

2. Methods

2.1. Sites, Sampling, and geochemical analyses

Nineteen lake sediment samples were collected in Saskatchewan, Canada in August of 2008 (Table 1, Figure 1). The study lakes occur in the semi-arid grasslands of the northern Great Plains, in which evaporation exceeds precipitation by approximately two-fold, lakes are sustained by the influx of snowmelt and groundwater, and water chemistry is subject to evaporative concentration during summer (Pham et al. 2008, 2009). All lakes reported here are closed basin lakes and physical, chemical, and hydrologic parameters were previously reported by Pham et al. (2008, 2009). Subsamples (5-cc) of surface sediments collected by Ekman grab sampler (~10 cm) were freeze-dried for organic analyses at 0.1 Pa for 48 h.

2.2. LCA extraction and analysis

Nineteen freeze-dried sediments were homogenized and extracted with dichloromethane (DCM):methanol (MeOH) (9:1, v/v) via an Accelerated Solvent Extractor ASE200 (Dionex). C36 *n*-alkane standard was added, and the total lipid extracts (TLEs) were run on a GC-FID for detection and quantification of LCAs using the internal standard. See Toney et al. (2010) for specific instrumentation parameters. Samples were run on the GC-MS to confirm the identity of the LCAs using the known ion chromatograms and by comparison of mass spectral data with published data and GC retention times (de Leeuw et al., 1980; Marlowe et al., 1984). Lab derived LCA standards of known temperature calibration are run on the GC-MS to ensure analytical precision (<0.1 °C) and prevent instrumental drift.

2.3 Numeric analysis

Statistical relationships between LCA concentrations and indices of lipid unsaturation were determined using least-square regression analysis following appropriate transformation to normalize variance distribution of predictor variables. All predictors were derived from mean values presented in Pham et al. (2008, 2009). Both $U_{37}^{K'}$ and U_{37}^K unsaturation indices were calculated for all lakes following Brassell et al. (1986).

3. RESULTS AND DISCUSSION

3.1. Occurrence and abundances of LCAs and relationship to lake water chemistry

Lake water chemistry has a strong effect on determining the presence of LCAs in lakes. In particular, thirteen of nineteen (68%) lake surface sediment samples contained LCAs (Table 1; Figure 1). These lakes had salinities greater than 1.5 g L^{-1} , elevated $\text{SO}_4 : \text{CO}_3$ ratios (SCRs), and enriched ratios of both H and O isotopes during summer. These findings are consistent with those of Toney et al. (2010) who show that production or preservation of haptophyte $\text{C}_{37:4}$ lipids is greater in lakes with elevated SO_4 content and $\text{SCR} > 1.0$. Overall, LCA distribution was unrelated to lake position (latitude, longitude) or elevation, but concentrations were generally elevated in lakes with sodium and sulfate concentrations greater than 110 mg L^{-1} and 1270 mg L^{-1} , respectively, and elevated SCRs (1205 ± 163). In contrast, lakes without LCAs exhibited both low sulfate concentrations ($< 1260 \text{ mg L}^{-1}$) and low SCR (0.47 ± 0.06). However, high SCR does not guarantee the presence of alkenones: the hyper-saline Arthur Lake ($33,265 \text{ mg L}^{-1}$, $\text{SCR} = 175$) contains no alkenones.

Interestingly, $\log_{10} \text{SCR}$ was highly correlated with both the oxygen stable isotope ratios during summer or fall ($\delta^{18}\text{O}$, $r^2 = 0.50$, $P < 0.001$), or the ratio of $\delta^2\text{H}$ to $\delta^{18}\text{O}$ ($r^2 = 0.48$, $P < 0.001$) presented by Pham et al. (2009), suggesting

that evaporative concentration of lake water influenced anion ratios, and hence LCA occurrences. Finally, extremely elevated LCA concentrations ($\Sigma C_{37} > 500 \mu\text{g g}^{-1}$ dry sediment) were recorded in four lakes (Deadmoose, Lenore, Success, Waldsea) known to exhibit permanent stratification and deepwater anoxia due to chemically-derived meromixis (Table 1; Pham et al. 2009). As noted by Pham et al. (2009) and Pennock et al. (2010), elevated SO_4 content is common in Canadian prairie lakes and ponds with pronounced groundwater contributions to local hydrologic budgets. At present, it is unclear whether groundwater resources directly favor LCA production, or whether influx of groundwater favors the strong chemical stratification (meromixis) and profound deepwater anoxia common in regional lakes deeper than 10-m. Consistent with the latter hypothesis, the highest concentrations of LCAs were recorded in the four lakes known to be meromictic (Pham et al. 2009). Therefore, high SCR ratios and lake stratification with anoxic bottom waters appear to be particularly favorable for the presence of alkenone-producing haptophytes.

3.2. Comparison of lakes in interior Canada and United states: role of cation chemistry

In contrast to Toney et al. (2010), there was little consistent relationship between cation concentration and LCA presence in Canadian prairie lakes (Table 1). For example, LCAs were absent from lakes with both low Na^+ and

Mg²⁺ concentrations (<100 mg L⁻¹) (e.g., Edouard, Kipabishku, Pellitier), and those with greatly elevated cation content (>300 mg L⁻¹) (e.g., Authur, Claire, Wakaw). Such independence of cation composition and LCA presence may arise because regional solute chemistry is highly variable in the Canadian Prairies, even over the scale of <100 km (Last, 1992). Regardless of the precise mechanism, our analysis demonstrates that sodium and magnesium concentrations alone cannot be used to predict LCA presence in the Canadian lakes.

3.3. LCA composition and ratios

The chemical composition of sedimentary LCAs in Canadian lakes was similar to that reported for sedimentary LCAs in high-sulfate, deep lakes in the northern Great Plains of the United States (Toney et al. 2010). Like the northern-most U.S. sites, Canadian sites were characterized by abundant C_{37:4} lipids in a region with cold mean annual air temperatures (~1°C annual, -19°C January), high solute content (brackish to mesosaline), and alkaline water (pH 8.5-10). The C₃₇ LCAs are dominated by the C_{37:4} in all lakes except Success Lake, where C_{37:4} is just under fifty percent of the ΣC₃₇ LCAs (Table 1). Overall, concentrations of C₃₈ lipids were highly correlated with those of C₃₇ compounds ($r^2 = 0.99$, $p < 0.0001$) suggesting a common haptophyte source in most lakes. Consistent with this interpretation, the C₃₇:C₃₈ ratio was uniformly greater than 1.0 and relatively constant among

lakes. $U_{37}^{K'}$ and U_{37}^K indices for all lakes show little variance (0.14 ± 0.03 and 0.56 ± 0.09 , respectively), while the U_{38}^K index exhibited greater variance (0.24 ± 0.14). The indices were statistically uncorrelated to measured variation in any chemical, morphological or hydrological parameter measured by Pham et al. (2008, 2009), although the U_{37}^K index was marginally correlated ($r^2 = 0.30$, $p = 0.067$) with variation in spring temperature of surface waters.

More recent studies of LCAs and haptophyte genomics from the U.S. sites show that two haptophytes with distinct profiles are present in the U.S. sites (Theroux et al. 2010, Chapter 4), with OTU7/Hap-B types exhibiting both $C_{38:3me}$ and $C_{38:3et}$ homologues. The Canadian sites presented here do not contain $C_{38:3me}$, which suggests that LCA production is dominated by the haptophyte dubbed OTU8 by Theroux et al. (2010) (also isolated in enrichment cultures as Hap-A, Chapter 4). Although speculative, we suggest that the OTU7/Hap-B haptophyte may also exist in Canadian sites, since preliminary data suggest that it has similar geochemical tolerances to OTU8/Hap-A (Chapter 4), and recommend that further surveys be conducted in this and adjoining regions of contrasting brine chemistry (Last 1992).

3.4. Relationship of U_{37}^K and temperature

Statistical analysis revealed that only the U_{37}^K unsaturation index exhibited a marginally significant ($r^2 = 0.30$, $p = 0.067$) positive relationship with surface

water temperatures recorded in spring (Figure 3). Furthermore, the slope of the relationship (0.012) was similar to that derived from site-specific calibration of the index in Lake George, North Dakota (0.017). While there is a possibility that the weak correlation is the result of different haptophytes species from lake to lake, the consistency in the lipid profiles and the dominance of $C_{37:4}$ suggest that they are produced by the same species. Toney et al., (Chapter 4) suggest that in lakes with high SCRs, as reported here, the $C_{37:4}$ -producing haptophyte out-competes the haptophyte that produces the dominant $C_{37:3}$ profile. Alternatively, the comparatively weak correlation in this survey relative to site-specific calibrations is expected, because it comes from a single, surface water temperature measurement in the spring and likely reflects the fact that the lacustrine haptophytes set the U_{37}^K value that is recorded in the lake sediments when the bloom migrates upward into the surface waters (Toney et al. 2010). The timing of this is unique for each lake site, because it is controlled by two end members, ice-out and stratification. While ice-out is likely similar for these lakes (Dröscher et al. 2009), stratification is dependent on depth, salinity, volume, and other physical lake parameters that are highly variable among the thirteen lakes (Pham et al. 2008). An exact calibration for temperature for the sites presented here is possible, but additional field investigations are necessary to determine the timing of maximum LCA production in each lake, so that the U_{37}^K index at

that time can be regressed with the appropriate environmental temperature, which has successfully been done for Lake George, ND (Toney et al., 2010).

4. CONCLUSION

Long-chain alkenones are reported from thirteen new lake sites in the Great Plains of Canada. The discovery of LCAs in these sites suggests that paleotemperature reconstructions are possible from this region.

Reconstructions for the region are significant, because it is sensitive to seasonal modes of climate variation due to its proximity to the path of the polar jet stream (Michels et al. 2007). Because the LCA composition in Canadian sites is similar to that from Lake George, ND, it is likely that the temperature calibration from Lake George could be used in these sites.

Future haptophyte culture work from these sites is also important, and could be used to test the relationship between LCA unsaturation and growth temperature, as well as, manipulated to learn more about controls on LCA biosynthesis. Quantitative temperature records from this region are particularly useful in detecting arctic air bursts related to the waning Laurentide Ice Sheet in the early Holocene that have been speculated from other studies (Bryson, 1966; Hu et al. 1999; Dean et al. 2002).

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Table 1. Organic geochemical data and calculated alkenone distributions for core-top sediments of Canadian Lakes.

ID	Lake Name	LCAs	Lat. (deg N)	Long. (deg W)	Z max ^a (m)	Strat. a, g	Dominant Cation	Dominant Anion	SO ₄ :CO ₃ ^a	C ₃₇ conc. (µg g ⁻¹ sed) ^b	C ₃₈ conc. (µg g ⁻¹ sed) ^c	UK37 nd	UK37 ^o	UK38 ^o	C ₃₇ :C ₃₈	% C _{37:4}	Spring Surf. Temp (°C)
<i>Alkenones present</i>																	
1	Antelope	Yes	50.17	108.24	5.2	N	Na	SO ₄	13.6	120.7	48.8	0.20	-0.59	-0.13	2.47	65.9	12
2	Charron	Yes	52.24	104.2	5	N	Mg	SO ₄	10.8	484.8	192.4	0.16	-0.62	-0.16	2.52	67.5	11.3
3	Deadmoose	Yes	52.19	105.1	29.9	T,M	Na	SO ₄	109.0	2357.8	930.3	0.17	-0.68	-0.17	2.53	72.6	0
4	Fishing	Yes	51.5	103.3	19.5	N	Mg	SO ₄	5.1	71.8	58.6	0.10	-0.56	-0.46	1.22	59.9	7.8
5	Humboldt	Yes	52.09	105.6	10	N	Mg	SO ₄	3.5	21.3	11.2	0.13	-0.45	-0.24	1.90	51.7	9.6
6	Lenore	Yes	52.3	104.59	4.3	N	Mg	SO ₄	18.6	1232.6	450.9	0.18	-0.58	-0.10	2.73	64.6	10.4
7	Little Manitou	Yes	51.45	105.3	6	T	Mg	SO ₄	296.7	6.9	3.6	0.10	-0.55	-0.50	1.90	59.3	12.6
8	Rabbit	Yes	52.36	107	4.9	N	Mg	SO ₄	67.4	2.7	1.0	0.18	-0.62	-0.25	2.82	67.8	7.5
9	Redberry	Yes	52.43	107.09	15.5	T	Mg	SO ₄	42.7	199.3	81.7	0.14	-0.71	-0.37	2.44	75.1	3.5
10	Shannon	Yes	52.38	105.26	7.3	N	Mg	SO ₄	9.1	50.6	44.9	0.10	-0.53	-0.37	1.13	57.5	4.6
11	Snakehold	Yes	50.3	108.28	3	x	Na	SO ₄	567.2	3.3	1.7	0.11	-0.45	-0.13	1.90	50.3	
12	Success	Yes	50.29	108.01	20	T,M	Mg	SO ₄	11.4	584.2	428.6	0.14	-0.40	-0.05	1.36	47.5	11.9
13	Waldsea	Yes	52.17	105.12	12.9	T,M	Ca	SO ₄	50.2	5110.3	1950.9	0.14	-0.56	-0.16	2.62	61.6	12.7
<i>Alkenones absent</i>																	
14	Arthur	No	52.34	105.26	4	T,M	Mg	SO ₄	175.1	x	x	x	x	x	x	x	8
15	Clair	No	51.59	104.03	3	N	Na	x	nd	x	x	x	x	x	x	x	11.6
16	Edouard	No	52.23	104.2	5	N	Mg	x	nd	x	x	x	x	x	x	x	12
17	Kipabiskau	No	52.34	104.12	8	N	Mg	x	nd	x	x	x	x	x	x	x	10.2
18	Peltier	No	49.59	107.56	8.5	N	Na	CO ₃	0.5	x	x	x	x	x	x	x	
19	Wakaw	No	52.4	105.35	10	N	Na	CO ₃	0.4	x	x	x	x	x	x	x	4.8

a. Data used to calculate the SO₄:CO₃ are from Pham et al. (2008)

b. C₃₇ concentration = 37.4 + 37.3 + 37.2

c. C₃₈ concentration = 38.4 + 38.3 + 38.2

d. UK37 = 37.2 / (37.2 + 37.3)

e. UK37 = (37.2 - 37.4) / (37.2 + 37.3 + 37.4)

f. UK38 = (38.2 + 37.2) / (38.2 + 38.3 + 37.2 + 37.3)

g. Stratification - thermal (T), none (N), meromictic (M)

Figure 1. Site map showing lakes that contain alkenones in the surface sediment (gray circles) and those that do not contain alkenones (white circles). The map shows sites within the U.S. from Toney et al., 2010) and new sites reported here in Saskatchewan, Canada.

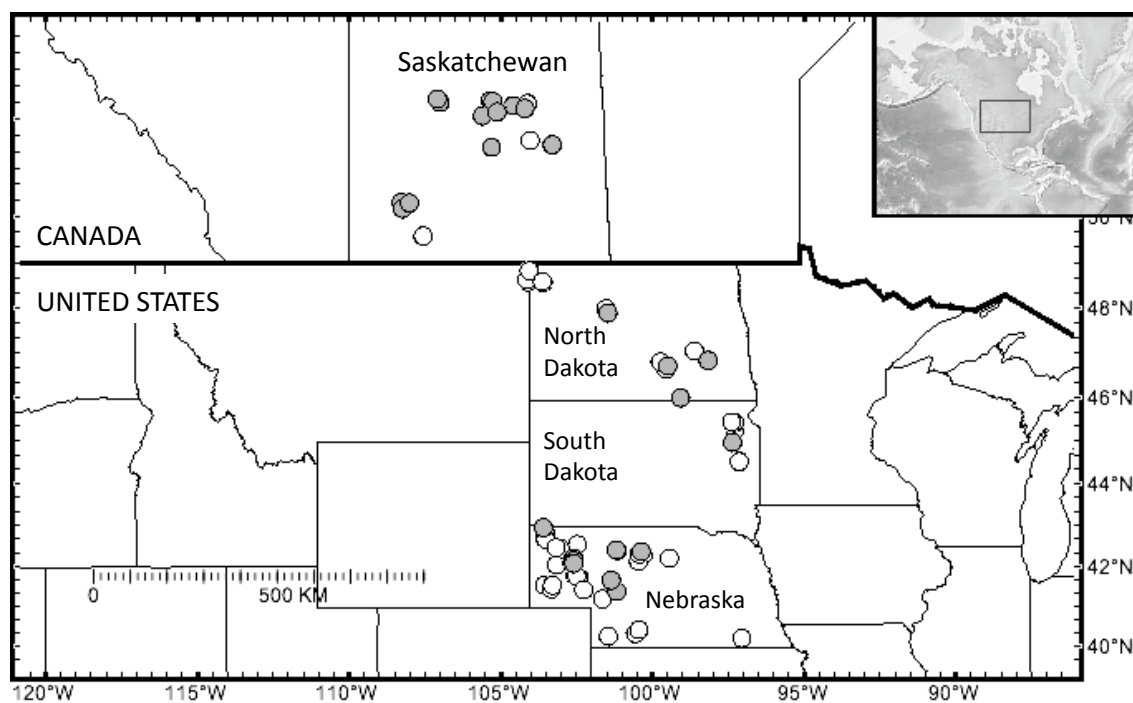


Figure 2., A.) Signature sedimentary alkenone profile for Canadian lakes; (B.) signature alkenone profile for isolated and cultured haptophyte, Hap-A (Toney et al. 2010), also reported as OTU8 (Theroux et al. 2010); (C.) signature alkenone profile for isolated and cultured haptophyte, Hap-B (Toney et al. 2010), also reported as OTU7 (Theroux et al. 2010).

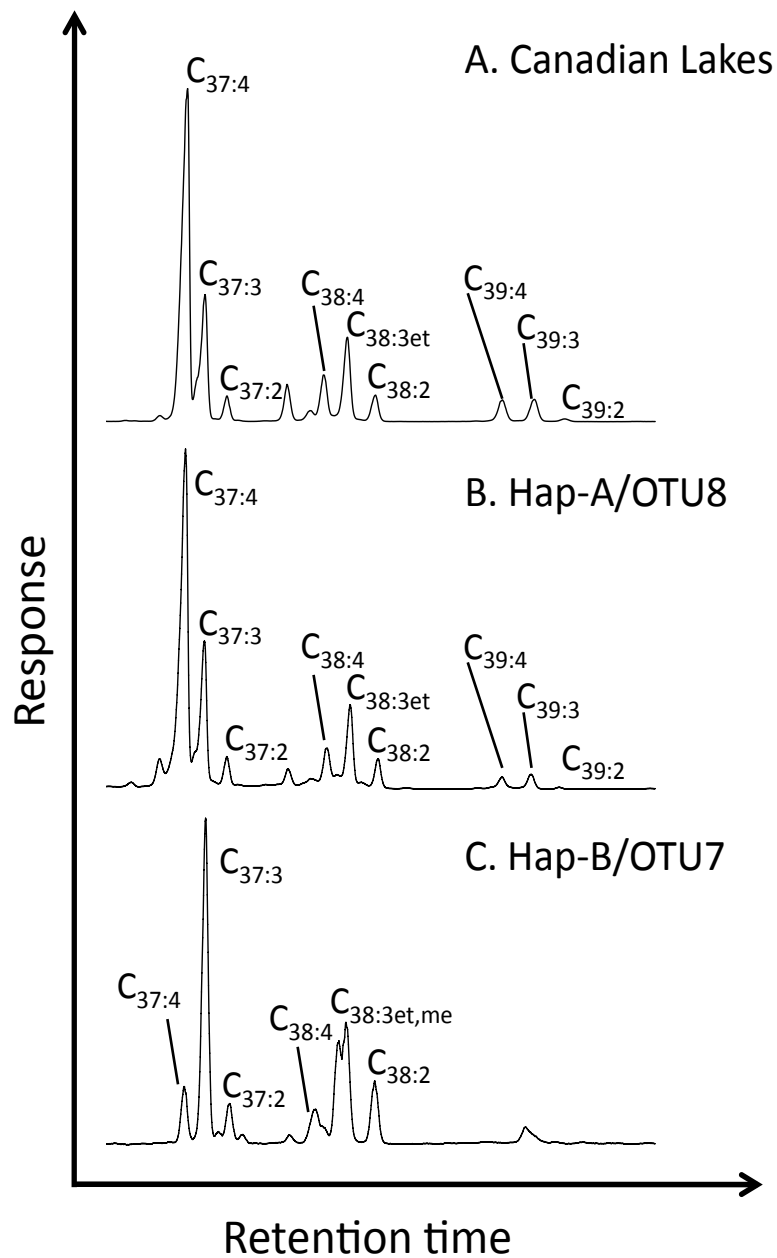
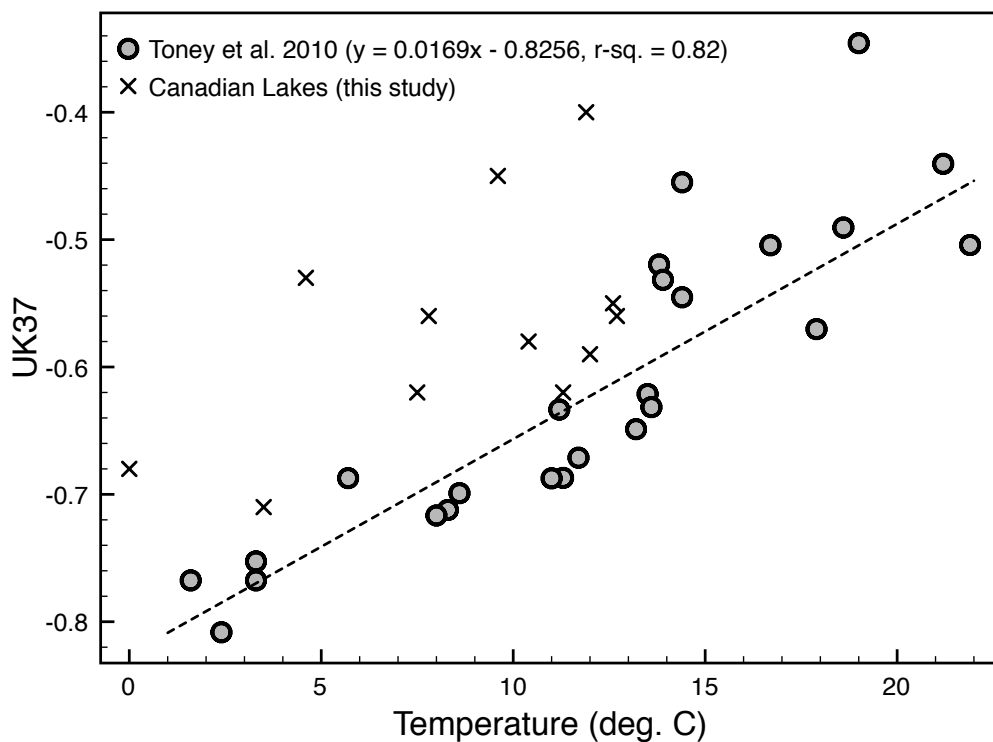


Figure 3. The lacustrine alkenone U_{37}^K from Canadian lake core-top sediments (x) plotted versus a single lake surface water temperature in Spring 2004, compared with the *in-situ* U_{37}^K calibration from Lake George, ND (circles).



CHAPTER 4

CULTURING OF THE FIRST LACUSTRINE 37:4 PREDOMINANT HAPTOPHYTE: GEOCHEMICAL, BIOCHEMICAL, AND GENETIC IMPLICATIONS

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ABSTRACT

Lacustrine long-chain alkenones (LCAs) are potential biomarkers for quantitative paleotemperature reconstructions from lacustrine environments. However, progress in this area has been severely hindered by the lack of culture studies of haptophytes that exemplify alkenone distributions in lake sediments: the predominance of the C_{37:4} LCA. Here we report the first enrichment culturing of a novel haptophyte phylotype (Hap-A) from Lake George, ND that produces predominantly the C_{37:4} LCA. We found that Hap-A can only be cultured from its resting phase in the deep sediments, rather than water column samples. In contrast, enrichment cultures of near surface water yield a different haptophyte phylotype (Hap-B), closely related to *Chrysotila lamellosa* and *Pseudoisochrysis paradoxa*, which does not display C_{37:4} LCA predominance (similar cultures have been reported previously). LCA profiles from sediments resemble that of Hap-A enrichments, suggesting that Hap-A is the dominant alkenone producer of the sedimentary LCAs. In enrichment cultures, excess lighting appears to be crucial for triggering blooms of Hap-A. Both U_{37}^K and U_{38}^K indices show a linear relationship with temperature for Hap-A in culture, but the relationship appears to be dependent on the growth stage. Based on 18S SSU rRNA gene analyses, several lakes from the Northern Great Plains, as well as Pyramid Lake, NV and Tso Ur, Tibetan Plateau, China contain the same two haptophyte phylotypes. The Great Plains lakes show the Hap-A-type LCA distribution,

whereas Pyramid and Tso Ur show Hap-B type distribution. Waters of the Great Plain lakes are dominated by sulfate, whereas those Pyramid and Tso Ur are dominated by carbonate, suggesting sulfate to carbonate ratio may be the determining factor for the competitiveness of the Hap-A and Hap-B phylotypes in the natural settings.

1. INTRODUCTION

Sedimentary long-chain alkenones (LCAs) produced by haptophyte algae have been widely and successfully used for sea surface temperature reconstructions for decades (Brassell et al., 1986; Prahl and Wakeham, 1987). Recent studies revealed that LCAs are also very common in lakes, especially saline lakes, worldwide (Cranwell, 1985; Zink et al., 2001; Chu et al., 2005; D'Andrea and Huang, 2005; Pearson et al., 2008; Toney et al., 2010; Theroux et al., 2010). However, the use of lacustrine LCAs as a paleotemperature proxy is less straightforward than for ocean systems because different species of haptophytes may reside in different lakes and may require different temperature calibrations (Coolen et al., 2004; D'Andrea and Huang, 2005; Theroux et al., 2010). Extensive sampling of the water column at different depths and seasons from the study lakes offers one viable solution to the calibration problem (Toney et al., 2010). However, the ultimate solution to understanding the response of lacustrine haptophyte algae to environmental parameters (e.g., temperature) is to culture the organism in question, as has been extensively carried out for ocean haptophytes, *Emiliana huxleyi* and *Gephyrocapsa oceanica* (e.g., Volkman et al., 1980; Volkman et al., 1995).

One distinctive feature of LCA distributions in lake sediments is the exceptionally high abundance of tetra-unsaturated C₃₇ alkenone (C_{37:4}) in many lakes (Cranwell, 1985; Volkman et al., 1988; Thiel et al., 1997; Wang

and Zheng, 1998; Zink et al., 2001; Sun et al., 2007; Chu et al., 2005; D'Andrea and Huang, 2005; Toney et al., 2010). However, to date, no laboratory has been able to capture and culture the lacustrine alkenone-synthesizers that produce such LCA signature profiles. Reported enrichments from lakes have found species closely related to *Chrysotila lamellosa* (e.g, Sun et al., 2007), which do not produce predominantly C_{37:4} LCA (Sun et al., 2007, Rontani et al., 2004). A recent environmental molecular survey of lake surface sediments (Theroux et al., 2010) further highlights the problem: multiple species of haptophyte algae are present in lakes worldwide and *Chrysotila lamellosa* represents only one type of haptophyte. Many lakes show overlapping distributions of species, which are not necessarily defined by biogeography. Although different haptophyte DNA phylotypes do not necessarily denote different temperature sensitivities, the results point to a major gap in our understanding of lacustrine haptophyte species. The mismatch between the cultured lacustrine haptophytes and the sedimentary LCA signatures, and the lack of cultures for lake species that produce the C_{37:4}-dominant profile, represent major barriers to the application of alkenone proxy for paleotemperature reconstructions in lake sediments.

We present in this paper the first successful enrichment of a lacustrine haptophyte, referred to as Hap-A, that produces predominantly C_{37:4} alkenone from Lake George, ND. We used unusual conditions to maintain

this organism, which may account for previous unsuccessful attempts to establish cultures. In addition to Hap-A, we also maintain enrichments of Hap-B that are closely related to *Chrysotila lamellosa*. We used 18S rRNA gene analyses to determine the phylogenetic placement of our Lake George haptophyte species and Fluorescence *In Situ* Hybridization (FISH) experiments to visualize haptophytes in our enrichments. We discuss the implications of these findings with respect to modern LCA production and application to paleotemperature reconstructions.

2. METHODS

2.1. Field and sampling methods

Water and sediment samples were collected from Lake George, North Dakota (46.74°N, 99.49°W) in June of 2009 with a Van Dorn water sampler and an Ekman grab sampler. Two 1-liter water samples were collected at 5-m, 6-m and 10-m depths, while sediment samples were collected from the near-shore, oxic environment at 5-m depth and from the deep basin of the lake at 44-m depth (See Table 1). One 1-L water sample from each depth was filtered using a vacuum filtration unit with combusted (550°C) GF/F 0.7µm, 47 mm glass filters. Additional water was collected in June of 2010 for the second set of experiments. Twelve liters were collected from the surface water and filtered with a 0.2µm Micropore (Pall Corporation, Michigan) filter to use as stock water and 1-L was collected at 5-m depth, unfiltered.

2.2. Enrichment Methods

2.2.1. *Experiment 1: producing LCAs in enrichments*

Six enrichments (A, B, C, D, E, and F) were started on June 9, 2009 in 2-L Erlenmeyer culture flasks in a growth chamber at 20°C during light and 18°C during dark, 68% humidity, and a 12-hr:12-hr, light:dark cycle using different initial starting materials (details in Table 1). These growth chamber conditions were maintained until February 15, 2010. After this date, light intensity was increased so that the surface of the enrichment water received $200\mu\text{mol m}^{-2} \text{ s}^{-1}$ (previous light settings were $\sim 100\mu\text{mol m}^{-2} \text{ s}^{-1}$). Enrichments A, B, and D were started from 1-L unfiltered lake water with added f/2 nutrient medium (NaH_2PO_4 – 1ml/1-L, vitamin – 0.5ml/1-L, trace metal – 1ml/1-L, NaNO_3 1ml/1-L; to prevent diatom blooms Si was not added) (MKF220L-CCMP) (Guillard, 1975), while enrichment C and E were started from 30-cc of sediment in 1-L of lake water filtered at $0.7\mu\text{m}$ and inoculated into f/2 nutrient medium. For enrichment F, 50-ml of unfiltered lake water was added to seawater from Boothbay Harbor, Maine that was brought down to Lake George salinity 9.7 g L^{-3} with distilled water and inoculated with f/2 nutrient medium. 15-ml subsamples were collected from the enrichments on June 24, July 15, August 15, and November 15 of 2009 and on April 18, 2010. Growth chamber temperature was increased to 25°C on April 19, 2010 and enrichments were subsampled again on May 2, 2010.

2.2.2. Experiment 2: testing temperature dependence of LCAs in enrichments

To test the effect of growth temperature (4°C, 10°C, and 20°C) on the alkenone unsaturation index, twelve liters (12 L) of Lake George water were filtered through a 0.2µm Millipore (Pall Corporation, Michigan) filter into an autoclaved storage container. One hundred milliliters was sent to the Colorado Plateau Stable Isotope Laboratory for analysis of major anion and cation water chemistry. Three control enrichments were set up in 2-L Erlenmeyer flasks that contained 1-L filtered (0.2 µm) lake water plus f/2 medium. Three, unfiltered water and three, deep-sediment enrichments were set up by adding 50-ml of unfiltered lake water or 30-cc of deep sediment to 1 L of 0.2 µm-filtered Lake George water and f/2 medium. Culture flasks were placed in three separate growth chambers (4°C, 10°C, and 20°C with 12hr light:dark cycle, light intensity of at the top of the enrichment water surface was 200µmol m⁻² s⁻¹) so that each chamber had a control, unfiltered, and deep sediment treatment. Enrichments were started on June 28, 2010. Every ~15-days (July 13, July 27, August 10, August 25) a 10-ml subsample of water was collected in a 15-ml centrifuge tube to test for alkenones with care taken not to disturb any sediment at the bottom of the culture flask. On August 25, samples of the agitated water and sediment were collected to analyze both the living and sedimentary organics. Centrifuge tubes were stored in a freezer, freeze-dried and then run for organic analysis (see Section 2.4).

2.3. 18S rRNA Gene Analyses

2.3.1. DNA extraction

Subsamples were collected for DNA analyses from enrichments A-F in April, 2010. Fifteen milliliters of sample were filtered onto a 0.2µm Sterivex (Millipore, Billerica, MA) filter, flooded with lysis buffer (Qiagen, City, State), and kept frozen at -20°C until processing. Samples were extracted using the Gentra Puregene Tissue Kit (Qiagen, Valencia, CA 158667) according to the manufacturer's instructions. Total extracted genomic DNA was quantified using a NanoDrop nucleic acid spectrophotometer (Thermo Scientific, Wilmington, DE).

2.3.2. DNA amplification and sequencing

Genomic DNA was amplified using haptophyte specific primers (Simon et al., 2000; Coolen et al., 2004) targeting 18S rRNA coding regions. Forward and reverse primers correspond to *Escherichia coli* 16S rRNA positions 429 and 887, respectively. Polymerase chain reactions (PCRs) were performed on an Eppendorf Gradient Thermocycler (Eppendorf, Hamburg, Germany) with the following conditions after D'Andrea et al., (2006): 4 min initial denaturing at 96 °C, 35 cycles of denaturing for 30 s at 94 °C, followed by 40 s primer annealing at 55 °C and primer extension 40 s at 72 °C, with a final extension

of 10 min at 72 °C. PCR products were purified using the Purelink PCR purification kit (Invitrogen, Carlsbad, CA). Cloning was performed using the Invitrogen TOP10 cloning kit with electro-competent cells. The protocol followed the manufacturer's instructions. Ten clones were picked for each sample. Plasmid DNA was isolated using a RevPrep Orbit robotic template preparation instrument (Genomic Solutions, Ann Arbor, MI), and prepared templates were sequenced on an ABI 3730XL (Applied Biosystems, Foster City, CA) capillary sequencer using the BigDye protocol with universal M13 forward and reverse primers according to the manufacturer's instructions. All sequencing was performed at the Marine Biological Laboratory W. M. Keck Ecological and Evolutionary Genetics Facility.

2.3.3. Bioinformatics and phylogenetic reconstructions

A bioinformatics pipeline using the programs phred, cross-match, and phrap, translated chromatograms into base-calls and associated quality scores, removed vector sequences and assembled forward and reverse reads into full-length sequences for each of the cloned PCR amplicons (Ewing and Green, 1998; Ewing et al., 1998). Only sequences greater than 400 bp and with a complete forward and reverse primer were retained. Base-calls were verified and sequences were manually edited with the program Consed (Gordon et al., 1998) for chromatogram viewing. Assembled sequences were aligned using the ARB software program v. 07.07.11 (Ludwig et al., 2004) against the

October 2008 Silva 96 Ref database (Pruesse et al., 2007) using the FastAligner option followed by manual adjustment. Sequences were aligned with reference haptophyte sequences and subjected to a Bayesian analysis after Theroux et al., (2010).

2.3.4. Fluorescence *In Situ* Hybridization

Subsamples (5-ml) were collected from each enrichment during week 4 of the temperature experiments. Cells were probed using a haptophyte-specific oligonucleotide probe PRYM02 (Simon et al., 2000) modified with an Alexa488 fluorophore (Invitrogen, City, State). Cells were concentrated via centrifugation and preserved in 80% ethanol for three days at -20°C to remove as much autofluorescence as possible. Cells were vortexed in hybridization buffer (18µl 5M NaCl, 2 µl 1M Tris-HCL pH 7.4, 1 µl 1% SDS, 20µl 100% formamide, 1 µl Probe (0.2 nmol/µl), 58 µl distilled H₂O) and incubated at 46°C for 2-hours. Cells were centrifuged and resuspended in wash buffer (4.3µl 5M NaCl, 2µl 1M Tris, 1µl 1% SDS, 92.7µl distilled H₂O) and incubated at 48°C for 15 min. Cells were centrifuged and resuspended in wash buffer, mounted onto an agar coated slide, and dried at room temperature for 1-hr. Slide was mounted with Citiflor:Vectashield (4:1 v/v) (London) to preserve probe fluorescence. Slides were viewed and photographed on a Zeiss Axioskop 2 MOT.

2.4. Lipid analysis

Freeze-dried water and sediment samples were homogenized and extracted with dichloromethane (DCM):methanol (MeOH) (9:1, v/v) using an Accelerated Solvent Extractor ASE200 (Dionex). Fifty microliters of C₃₆ *n*-alkane standard (72.43 µg ml⁻¹) were added, and the total lipid extracts (TLEs) were run on a GC-FID for detection and quantification of alkenones using the internal standard. An Agilent DB-1 GC column (60 m × 320 µm × 0.10 mm) was used with the following temperature program: an initial temperature of 40°C (hold 1-min), ramp 30°C min⁻¹ to 290°C (hold 1-min), ramp 5°C min⁻¹ to 300°C (hold 0-min) then 2°C min⁻¹ to 325°C, GC temperature program (hold 10-min) (Toney et al., 2010). The same temperature program was used on the GC-MS, and samples were run to confirm the identity of the alkenones using the known ion chromatograms and by comparison of mass spectral data with published data and GC retention times (de Leeuw et al., 1980, Marlowe et al., 1984). The final GC program described here is the result of testing many permutations of GC parameters, including the switch from helium as the carrier gas to hydrogen. As such we are able to resolve LCAs with >4µg per sample (i.e. grams of dry sediment or liter of water). Alkenone standards of known temperature calibration were run on the GC-MS to ensure analytical precision (<0.1°C).

3. RESULTS

3.1. Long-chain alkenone distribution and temperature dependence

3.1.1. Experiment 1: confirming LCA production in enrichments

Of the six enrichments (A, B, C, D, E, and F), none produced LCAs based on organic analysis of the 15-ml subsamples collected on June 24, July 15, August 15, and November 15 of 2009 (Table 1). However, LCAs were present in relatively high concentrations (ranging from 10.4 to 1191 $\mu\text{g L}^{-1}$) in all enrichments, except enrichment F, in subsamples that were collected on April 18, 2010. Notably, the alkenone distributions for enrichments A-D were very similar with $\text{C}_{37:3}$ dominance ($76\pm 2\%$) over other C_{37} LCAs and $\text{C}_{38:3}$ both ethyl and methyl forms dominant over other C_{38} LCAs. Whereas, in enrichment E, $\text{C}_{37:4}$ was dominant (54%) and $\text{C}_{38:3}$, the ethyl homologue, dominated the C_{38} LCAs (Figure 1; Table 1). The temperature was inferred from the LCA unsaturation index (U_{37}^K) calibration using a linear regression model ($T = 48.4982 \times U_{37}^K + 42.1494$) that was specifically developed using an *in situ* relationship derived for the LCAs of Lake George (Toney et al., 2010). Using this relationship, inferred temperatures from the U_{37}^K for enrichments A-D produce temperatures much higher ($38\pm 1.6^\circ\text{C}$) than the diurnal growth temperature range of 18°C to 20°C . Inferred temperature from enrichment E produces a temperature of 18.9°C , which is within the range of the diurnal temperature cycle of the growth chamber. None of the enrichments produced LCAs following the increase in temperature to 25°C .

3.1.2. Experiment 2: testing temperature dependence of LCAs in enrichments

New experiments were designed to determine if production of LCAs could be replicated using the same enrichment techniques as above and to test the temperature dependence of the LCAs. The control treatment at 20°C produced no LCAs throughout the sampling period, however, the controls at 4°C and 10°C both produced LCAs with distributions similar to enrichments A-D on July 13, July 27 and August 10 (Table 3). On August 25, only the control at 4°C contained LCAs. Flasks that were started with unfiltered lake water collected at 5-m depth (herein unfiltered enrichments) also did not produce LCAs at 20°C, while the 4°C unfiltered enrichment produced LCAs in similar distributions as enrichments A-D on July 27, and the 10°C unfiltered enrichment produced similar LCAs on July 27 and August 10 with barely detectable amounts on August 25. The enrichment started from the deep sediment (herein deep enrichments) produced LCAs on all collection days with distributions similar to enrichment E at all temperatures until August 25, when only the 10°C enrichment produced LCAs. On August 25, agitated water/sediment samples were collected from the deep enrichment. Only deep enrichments at 4°C and 10°C contained LCAs. The major anion and cation concentrations of the enrichment water are presented in Table 2.

3.2. 18S rRNA gene analyses

From enrichments A-E, the control, unfiltered, deep-sediment enrichments and isolated subsamples of enrichment C (246, 478, 481, 484), we identified two Operational Taxonomic Units (OTUs). Each enrichment had only one of the two OTUs. The OTU associated with enrichments started from the water column and shallow sediments is referred to as Hap-B, whereas the OTU associated with enrichments started from deep sediments are referred to as Hap-A.

We constructed a phylogenetic tree using a representative sequence from each OTU with previously published haptophyte 18S rRNA genes (Figure 2). The tree topology is similar to that published by Theroux et al., (2010) with the new Lake George, ND OTUs branching with OTU7 and OTU8, which were previously reported from the surface sediments of Lake George and other U.S. and Chinese lakes.

4. DISCUSSION

In this paper we present the results of two separate enrichment experiments. The first experiment consisted of starting enrichments from different parts of the lake system (e.g. water column, sediment) in an attempt to produce LCAs under controlled conditions (Section 4.1.). The second experiment used the information gained from the initial enrichments to test the temperature

dependency of LCAs (Section 4.2.). We combined these techniques with 18S rRNA gene sequencing (Section 4.3.) to confirm the identity of the haptophyte species present in the enrichments. We also used FISH (Section 4.4.) to visualize haptophytes in the enrichments.

4.1. Long-chain alkenone production in enrichments

4.1.1. The role of water chemistry on LCA production in enrichments

Our enrichment data show that water chemistry strongly affects the production of LCAs. LCAs were present in relatively high concentrations in enrichments A-E that were grown in native Lake George water. However, LCAs were not produced in enrichment F, which was initiated by adding 50 mL of Lake George water to f/2 medium prepared with a seawater base that was adjusted to Lake George salinity. The base water is the only variable in the culture medium used to establish enrichments A and F, which suggests the lake water chemistry is important for LCA production. The major ion chemistry is significantly different for seawater and Lake George (Table 2). The cation chemistry is similar for the seawater and Lake George. Both are dominated by Na^+ , but in seawater it is ~28-times more abundant than Mg^{2+} , Ca^{2+} , and K^+ . For Lake George, Na^+ is 8-times more than Mg^{2+} , 12-times more than K^+ , and 260-times more than Ca^{2+} . The anion chemistry is completely different, with the dominant anion in seawater, Cl^- , and the dominant anion in Lake George, SO_4^{2-} . We diluted the seawater so that the salinity was

equivalent (Mg^{2+} and Na^+), thus it is the anion chemistry that may control the presence and production of LCAs, although Ca^{2+} may also be important. The anion chemistry supports previous findings from the Northern Great Plains, where the absolute concentration of major anions did not appear to affect the LCA distribution, but the $\text{C}_{37:4}$ -producing haptophytes were only found in lakes with sulfate-to-carbonate ratios (SCR) greater than one (Toney et al., 2010). Here the diluted seawater has a SCR of 0.14, whereas, the Lake George water SCR is 21 (Table 2).

4.1.2. Light as an important trigger LCA production in Hap-A enrichments

Enrichments did not produce LCAs until nearly a year following their start date with light intensity held constantly at $\sim 100 \mu\text{mol m}^{-2} \text{s}^{-1}$. The field water used to start these enrichments was collected at end of bloom season and suggests that we captured the resting phase of the haptophyte that does not produce LCAs. Although nutrients and trace metals were ample in the enrichments beginning in June of 2009, the haptophytes were not triggered into an active, LCA-producing phase until sometime between November 2009 and April of 2010. The only changes that occurred in this window of time were the passing of time and the intensification of light. It is possible that the resting phase of the haptophytes is time sensitive and that overwintering occurs for a certain length of time each year. However, our enrichment methods did revive haptophytes from the deep sediments, regardless of the

time of year in subsequent experiments (see Section 4.2.), which suggests time is not likely the trigger. Alternatively, the intensification of light from $\sim 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ in February of 2010, may have triggered the haptophytes into an active stage that produces LCAs. This supports the hypothesis by Toney et al., (2010) that increased light penetration following ice-off in the spring causes the haptophytes to rise from the bottom of Lake George and produce LCAs during the haptophyte bloom.

4.1.3. Enrichment starting materials on LCA distributions

Based on lipid signatures and 18S rRNA gene sequencing, we enriched for what appear to be two distinct phylotypes of haptophyte algae, Hap-A and Hap-B. Among the five, LCA-producing enrichments, enrichments A through D (Hap-B) produce LCAs with the same distribution, while enrichment E (Hap-A) produces LCAs with a different distribution (Figure 1). 18S rRNA gene analysis confirms that the haptophyte present in enrichment E (Hap-A) is genetically distinct from the haptophyte present in enrichments A-D (Hap-B) (See Section 4.3. and Figure 2). The DNA sequencing results from each enrichment yielded only one haptophyte phylotype each, and suggest that Hap-A and Hap-B do not coexist in the enrichments. The distribution of LCAs in enrichment E is most similar to the sedimentary alkenone profile (Figure 1) and the water column LCAs during *in situ* sampling of the 2008 and 2009 spring blooms (Toney et al., 2010). The concentration of LCAs produced by

haptophytes in enrichments A through D ranged from ~ 10 to $150 \mu\text{g L}^{-1}$, while the concentration of LCAs was an order of magnitude higher in enrichment E ($\sim 1200 \mu\text{g L}^{-1}$) (Table 1). The similar profiles of LCA distributions suggests that the LCAs produced by Hap-A are the dominant contributor to the sedimentary LCAs, which may be due primarily to the vast production rates or competitive exclusion of Hap-B. However, it is also possible that predation may also be important in delivering alkenones to the sediment via fecal pellets. Not enough is known about the community ecology during the bloom or the form of delivery to sediments to speculate further at this time.

The organism producing the Hap-A phylotype and the predominant $\text{C}_{37:4}$ LCA distribution, appears to have different initial enrichment condition requirements than Hap-B. These differences suggest a possible different ecological strategy for the two haptophytes within the lake. The Hap-A phylotype is found in the deep surface sediments (~ 47 m), while Hap-B appears to be enriched from various parts of the lake water column and shallow sediments. It is possible that Hap-A needs a substrate in its resting phase, but if this were the only requirement, then we should also find Hap-A in the enrichments started from the shallow sediments. Instead, the deep sediments may provide refuge from predation over the winter for Hap-A.

These enrichment data provide new insights into the previous *in situ* sampling and temperature calibration from Lake George. Two statistical outliers occur in the Toney et al., (2010) calibration. Upon reanalysis, these outliers' chromatograms resemble the distributions from Hap-B with the presence of the C_{38:3} methyl ketone. These data points were collected in the water column at the surface and at 5 m, when we hypothesize the bloom of Hap-A had already moved down to 10-m depth. This suggests that Hap-B is adapted to thrive following the Hap-A bloom when lower nutrient conditions prevail. These Hap-B characteristic LCAs were present only in very low concentrations (< 27 µg L⁻¹) and resulted in inferred-temperatures that were too high for the Hap-A-based calibration. As a result, this led us to believe that lacustrine environments are subject to similar issues as marine environments, where low concentrations of alkenones produce high inferred-temperature, which is often attributed to instrumental bias when concentrations of LCAs are at or below the detection limit (see Rosell-Melé et al., 1995 and Rosell-Melé et al., 2001). At Lake George, however, low concentrations and high-inferred temperatures are the result of a different LCA-producing haptophyte species.

The requirement of different starting materials and enrichment conditions suggest that the sedimentary resting phase, potential cyst production, is important for phylotype Hap-A's adaptive strategy. Although little is known

about the benthic stages of haptophytes or the factors that affect the transition between different stages (Boalch 1987; Rousseau et al., 1994), our enrichment results suggest that Hap-A and Hap-B have two very different survival strategies. Based on the information that we have at present, Hap-A rests at the bottom of the lake until conditions (e.g., light, nutrients, etc.) are favorable, blooms, and then settles back to the resting phase when nutrients become scarce. Hap-B, alternatively, is adapted to survive under lower nutrient conditions after the onset of thermal stratification and increased competition with diatoms, green algae, and cyanobacteria following Hap-A's bloom. Previous studies have shown that Lake George is N-limited at this time in the seasonal lake cycle (Salm et al., 2010) and suggests that under N-limiting conditions, Hap-B would be more abundant than Hap-A.

4.1.4. Relationship of LCA distribution and indices to enrichment conditions

Two distinct groups of haptophytes have previously been identified based on their LCA distribution in the Northern Great Plains lakes (Toney et al., 2010) and their DNA sequences from surface sediments (Theroux et al., 2010).

Carbonate-dominated lakes in Nebraska produced LCA distributions that lacked C_{37:4}, while northern, sulfate-dominated lakes produced LCAs dominated by C_{37:4}. It is unclear how similar the C_{37:3}-producing Hap-B phylotype is to the haptophyte(s) in the Nebraska sites that appear to lack C_{37:4} because attempts to amplify DNA from the sediments of Nebraska lake

sites during analyses by Theroux et al., (2010) were unsuccessful. The Nebraska lake LCA profiles are likely produced by a different haptophyte and suggests that there maybe at least three different haptophyte species in the lakes of the Great Plains. Theroux et al., (2010) did find that two different haptophyte phylotypes amplified from Lake George surface sediments. These data are compared with the enrichment DNA sequences detailed below in Section 4.3. Despite the existence of two haptophyte species in Lake George, it appears that the sedimentary profile is dominated by the Hap-A profile in the modern environment (Figure 1). It is possible, however, that at times in the past lake environmental conditions favored Hap-B and it is important to know how their presence may affect LCAs as a paleotemperature proxy.

The ratio of $C_{37}:C_{38}$ LCAs has been used to distinguish different haptophyte species in other studies (e.g., Pearson et al., 2009). There is a large difference between the $C_{37}:C_{38}$ ratio of LCAs from the Nebraska (2.4) versus Northern Great Plains (4.6) lake sites (Toney et al., 2010), however, there is only a subtle difference between the $C_{37}:C_{38}$ ratio between Hap-B (1.3 ± 0.1) and Hap-A (1.8). The overall ratios from the enrichments are lower than the samples collected in the field, however, which suggests that there are secondary controls on this ratio (i.e. nutrients, dissolved oxygen, salinity). U_{37}^K , $U_{37}^{K'}$ and U_{38}^K indices describe the degree of unsaturation in the LCA distribution. These indices have been related to growth temperature in previous studies,

although only U_{37}^K was temperature sensitive in the *in situ* work from Lake George (Toney et al., 2010). These indices were calculated for LCAs in all the enrichments (Table 1). The U_{37}^K and U_{38}^K indices for Hap-B are not significantly different from Hap-A, however, the U_{37}^K index is significantly different (-0.08 ± 0.03 versus -0.48). Using the linear regression model ($T = 48.4982 \times U_{37}^K + 42.1494$) specifically developed for the LCAs of Lake George on the unsaturation indices calculated from Hap-B produces temperatures much higher ($38 \pm 1.6^\circ\text{C}$) than the diurnal growth temperature range of 18°C to 20°C . Inferred temperature from Hap-A produces a temperature of 18.9°C , which is within the range of the diurnal temperature cycle of the growth chamber. This lends support to the observation that the Hap-A phylotype is the major contributor to the sedimentary LCAs, and is also the haptophyte responsible for LCA production during the spring bloom from which the calibration was derived.

4.2. Experiment 2: Replicating enrichment methods and LCA temperature dependence

New experiments were designed to show that production of LCAs could be replicated using similar culturing techniques as above and to test the temperature dependence of the enrichment-produced LCAs. A control that contained only Lake George $0.2\ \mu\text{m}$ -filtered water was not expected to contain LCAs throughout the experiment, however, at 4°C and 10°C the controls

produced LCAs with distributions similar to Hap-B enrichment (Table 3).

One possibility is that because LCAs are 62Å in size, they could have passed through in the filtering process, however, none were detected at 20°C and the concentration of LCAs in the 4°C and 10°C controls increased with time.

These results suggest that active production of LCAs occurred in the controls. Similarly, the enrichments started from unfiltered lake water produced Hap-B-type distributions at 4°C and 10°C.

Based on our E enrichment, we hypothesize that Hap-A and Hap-B phylotypes compete with each other during temperature experiments. The enrichments started from the deep sediment produced Hap-A LCAs at all temperatures until week 8, when only the 10°C enrichment produced a trace amount (34µg L⁻¹) of LCAs that resembled the Hap-B LCA profile. The agitated water/sediment samples were collected from the deep enrichment in week 8 when relatively high concentrations (>600µg L⁻¹) of LCAs were found in the 4°C and 10°C enrichments. These distributions were dominated by C_{37:4} at 4°C and by C_{37:3} at 10°C. The 4°C enrichment produced an inferred temperature of 4.9°C using the *in situ* water column calibration, while the 10°C enrichment produce a temperature of 25°C with the *in situ* calibration and 11.9°C with the known *C. lamellosa* calibration (Sun et al., 2007). Notably, the 10°C deep enrichment's agitated, lipid profile showed clear methyl- and ethyl-C_{38:3} peaks and indicates that under the enrichment

conditions, the Hap-B phylotype out-competed the Hap-A phylotype at 10°C. The enrichments were not set up under chemostat conditions, meaning that the nutrients were allowed to decline throughout the experiment. This is likely why Hap-B was able to out-compete Hap-A as time progressed. As the bloom period progressed in all sediment enrichments, the percentage of C_{37:4} decreased (Figure 3), suggesting that the Hap-B phylotype became active once the Hap-A phylotype began to decline. This suggests that Hap-B is adapted to conditions with decreased nutrients and potentially warmer temperatures that prevail toward the end of the bloom as observed in the environmental, *in situ* calibration (see Section 4.1.3.).

Only the Hap-A enrichment U_{37}^K and U_{38}^K indices were temperature dependent during the experiments (Figure 3). The unfiltered and control enrichments did not produce LCAs at 20°C, so a calibration is not possible and there are no significant differences in U_{37}^K , $U_{37}^{K'}$ or U_{38}^K at different temperatures. For the Hap-A enrichment, $U_{37}^{K'}$ shows no temperature dependency, but U_{37}^K and U_{38}^K indices show a linear relationship with temperature in week 6 and week 2 respectively ($r^2 = 0.998$ and 0.950)(Figure 3). These two indices have nearly the same relationship to temperature, but are offset from the *in situ* calibration. The relationship is offset to higher unsaturation values and may indicate different relationships between LCAs and temperature at different growth stages. Alternatively, the offset could be due to a larger contribution

from the Hap-B phylotype that produces less $C_{37:4}$ relative to $C_{37:3}$ and is favored under these enrichment conditions, but not in the environment during *in situ* calibration sampling. The presence of Hap-B phylotype is evident in the LCA profile that shows a decreasing percentage of $C_{37:4}$ and an increased presence of $C_{38:3me}$. The similarity in slopes, despite the different phylotype producer has been found in other studies (D'Andrea and Bradley, 2010) and suggests that while a universal, absolute temperature calibration is not possible for lakes, the slope ($dU_{37}^K/d \text{ temperature}$) may be used to derive the change in temperature through time. From the enrichments and Lake George *in situ* samples a 0.013 change in U_{37}^K occurs per 1°C, although the range in U_{37}^K is relatively high, 0.017 for *in situ* samples and 0.011 for enrichments. At Lake George, careful inspection of the $C_{38:3}$ LCAs for the presence of methyl homologues is the most appropriate way to determine if the calibration can be applied to a particular sample. The presence of methyl homologues indicates that temperatures may be influenced by Hap-B LCAs and appear artificially high.

4.3. Phylogenetic relationships with previously reported haptophytes

Our phylogenetic analyses indicated that distinctions observed between the two different lipid profiles are attributable to two different haptophyte phylotypes (Figure 2). Hap-B from the shallow lake environment branches

with two known haptophyte taxa (*Chrysotila lamellosa*, *Pseudoisochrysis paradoxa*) and one unidentified sequence from Greenland's Lake HundeSø (Theroux et al., 2010). In particular, the Hap-B sequence is identical in the region sequenced for the OTU7 representative sequence that was previously reported from Lake George, Medicine Lake, Skoal Lake, Great Salt Lake, Pyramid Lake, Keluke Hu, and Tso Ur². The lipid profiles for *C. lamellosa* and for surface sediments from the Great Salt Lake, Pyramid Lake, and Tso Ur are similar to those from the enrichments from which the Hap-B phylotype DNA sequences were amplified and have C_{37:4} present, but not dominant. The Hap-B lipid profile differs from *C. lamellosa*, however, because it produces both C_{38:3et} and C_{38:3me} homologues, whereas *C. lamellosa* only produces C_{38:3et} (Rontani et al., 2004). The lipid distribution for surface sediments from Lake HundeSø, Lake George, Medicine Lake, and Skoal Lake are all dominated by C_{37:4}, which suggests that although OTU7 is present, LCA production in these lakes is dominated by a different haptophyte species. The masking of the OTU7-type lipid profile in the sedimentary record suggests that, like Lake George, this producer does not produce high concentrations of alkenones.

Based on the placement of the Hap-A phylotype on the phylogenetic tree and comparison of sequences, this species is likely closely related to the OTU8

² Note: Tso Ur is located in the central Tibetan Plateau (31.48, 91.52) and has multiple names including: Cuo E, Tibetan Plateau Central Lake B, and Co Ngoin.

representative sequence that has been reported from Lake George, Medicine Lake, Skoal Lake, Pyramid Lake, Keluke Hu, Tso Ur and Clear Lake. The surface sediments from Lake George, Medicine Lake, and Skoal Lake have lipid profiles similar to Hap-A enrichments, whereas Pyramid and Tso Ur have lipid profiles similar to Hap-B. These differences in lipid profiles suggest that in the first set of lakes (LG, ML, SL), the lipid profile associated with OTU8 phylotype dominates; whereas in the latter set of lakes (PY, TU) the OTU7 phylotype dominates the lipid production. Despite extensive field sampling, we have not found that the OTU7-phylotype lipid profile dominates in any part of the Lake George natural system spatially or temporally. Therefore, it is worth examining the differences between these two sets of lakes to determine if there are conditions that favor one species over the other, aside from the short-term, low nutrient advantage for the Hap-B phylotype as discussed above in Section 4.1.3.

Lake water chemistry plays a major role in determining whether Hap-A or Hap-B phylotype is the dominant producer in lakes where both are present. Physical parameters, geochemistry, and salinity of lakes with OTU8 (LG, ML, SL) have previously been reported (Toney et al., 2010). Similar parameters have also been reported for Pyramid Lake (Galat et al., 1983) and Tso Ur (Wu et al., 2009), where the OTU7 phylotype dominates the lipid production. The physical lake parameters (depth, volume, etc.) vary among

all the lakes, and salinity ranges from 5 to 12 g L⁻¹ with no significant differences between the two sets of lakes (Table 4). The major cation in all lakes is Na⁺. The main difference between the two sets of lakes is the major anion chemistry. Lakes where the OTU8 phylotype dominates the lipid production are dominated by SO₄⁻², whereas, HCO₃⁻ and CO₃⁻² dominates in Pyramid Lake and Tso Ur (Wu et al., 2009). The sulfate to carbonate ratio (SCR) for the first set of lakes is much greater than one (between 32 and 117), while the SCR for Pyramid Lake and Tso Ur is less than one (0.24 and 0.40, respectively). This supports the importance of SO₄⁻² for the production of LCAs dominated by C_{37:4}. Based on previous studies, the lowest SCR value for LCAs dominated by C_{37:4} is 8.2; whereas, the highest value for Hap-B distributions is 2.6 (Toney et al., 2010). This suggests that the *in situ* temperature calibration derived for Lake George should work in mid-latitude, evaporative lakes with OTU8, where SCR is > 8.2. Below SCR of 1, we would expect that the OTU8 phylotype to be outcompeted by the OTU7 phylotype, if present. The exact threshold for the dominance of OTU8 (Hap-A) versus OTU7 (Hap-B) is not determined here, and would require further investigations and manipulation of Hap-A and Hap-B enrichments. We have not yet developed a U_{37}^K calibration for the Hap-B phylotype, but further investigations in lakes where the OTU7/Hap-B phylotype dominates the lipid production would reveal more information about this species, which does not appear to compete as well in our high sulfate lakes. The conclusion from

these two sets of lakes suggests that our outlying, down-core samples from Lake George reflect brief transitions to a lake system dominated by HCO_3^- and CO_3^{2-} anions or are severely nutrient depleted.

4.4. Size differences between Hap-A and Hap-B from FISH analyses

From the FISH analyses, we were able to visualize two different algae that fluoresced with the PRYM02 haptophyte primer. The first was a small ($<10\ \mu\text{m}$) cell (Figure 4) from enrichments A-D, control and unfiltered enrichments from the temperature experiment, and likely represents Hap-B phylotype cells. The second, from the sediment derived enrichments was $\sim 20\ \mu\text{m}$ (Figure 4), and likely represents Hap-A phylotype cells.

5. CONCLUSION

The laboratory enrichment of two distinct haptophyte phylotypes from Lake George, ND provides new insights into conditions necessary to grow the predominant- $\text{C}_{37:4}$ producing lacustrine haptophyte. The enrichments also provide suggestions about the competing environmental controls on the two haptophytes found and their biosynthesis of LCAs. Specifically, we showed that: (1) Lake George haptophytes can survive a wide range of geochemical conditions, but produced LCAs in enrichments only when sulfate is dominant over carbonate; (2) haptophytes began to produce LCAs under enrichment

conditions after being triggered by increased light intensity; (3) the haptophyte responsible for the production of the lipid profile dominated by $C_{37:4}$ (Hap-A) was only found by ‘reviving’ the algae from its resting phase in the deep sediments rather than water column samples; whereas, the haptophyte that produces the *C. lamellosa*-like lipid profile (Hap-B) was only found in enrichments from the water column and shallow sediments; (4) the Hap-A phylotype produces LCAs in concentrations an order of magnitude higher than the Hap-B phylotype; (5) the Hap-A phylotype dominates the sedimentary signature and is the species from which the *in situ* calibration was developed; (6) the Hap-B phylotype is distinguishable by the presence of $C_{38:3me}$; (7) Hap-B may have an adaptive advantage under low nutrient conditions; (8) U_{37}^K and U_{38}^K indices show a linear relationship with temperature, but the relationship appears to be dependent on the growth stage or offset due to interference by Hap-B; (9) the main difference leading to the dominance of the Hap-A signature over the Hap-B signature appears to be the anion geochemistry, mainly Hap-A prefers sulfate.

Despite the enrichment-based findings and evidence for competition between Hap-A and Hap-B phylotypes, the core-top, sedimentary LCAs record the temperature of the surface water bloom for Hap-A. This suggests that as a community, Hap-A LCA production occurs in the surface waters before migrating back down to the sediments for overwintering. Also, the

sedimentary LCAs can be used to infer temperature if caution is used to detect and discard samples with C_{38:3me} LCAs, indicative of Hap-B phylotype alkenone production. Future analyses of Lake George haptophyte enrichments may reveal additional environmental and physiological intricacies of alkenone production by these novel taxa.

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Table 1. Organic geochemical and culture data and observations for
Experiment 1: *producing LCAs in enrichment cultures.*

Culture	Start date	Culture origin	GPS Collection Location	Culture medium	Growth Chamber Conditions (07/12/09 to 02/15/10)	Observations (April 2010)	Alkenone Presence April 2010	C37:4	C37:3	C37:2	[37] (ug/L)	37:38	UK37	UK37	UK38	UK37 Inferred T (°C)
A	6/9/09	10-m depth water column	N46°44.375, W099°29.622	filtered lake water with 1/2 nutrient medium	20°C light, 18°C dark, 68% humidity, 12hr light: 12hr dark	distinct dark green, globular colonies floating, brownish coccooid cells that might be haptophytes; a Nannochloropsis-like greenish coccooid; a flagellate that looks like Ochromonas trianguata	Y	2.103	7.922	0.832	10.42	1.18	-0.12	0.10	0.32	36.47
B	6/9/09	5-m depth water column	N46°45.014, W099°29.468	filtered lake water with 1/2 nutrient medium	20°C light, 18°C dark, 68% humidity, 12hr light: 12hr dark	red cyanobacterial mat on bottom of culture flask, lots of brownish coccooid cells	Y	22.375	132.460	14.656	153.27	1.14	-0.05	0.10	0.13	39.94
C	6/9/09	near shore sediment (4.5-m depth)	N46°45.045, W099°29.442	filtered lake water with 1/2 nutrient medium	20°C light, 18°C dark, 68% humidity, 12hr light: 12hr dark	brown bubbly foam at surface, greenish/gray fatty material below, Chromulina-like cells; Oedogonium filaments, Hateria (a ciliate); Chroococcus and other Cyanobacteria; Arcellus; a lenticle-bearing pedinellid; a very few coccooid brownish cells	Y	26.537	132.688	16.511	140.65	1.40	-0.06	0.11	0.19	39.38
D	6/9/09	6-m depth water column	N46°44.375, W099°29.622	filtered lake water with 1/2 nutrient medium	20°C light, 18°C dark, 68% humidity, 12hr light: 12hr dark	red bacterial mat on bottom of culture flask, Chromulina-like cells; Pavlova-like cells; Brownish cell in gel that looks like it may be a Haptophyte; a flagellate; and the brown mat is a cyanobacterial mat	Y	16.787	83.048	7.067	122.02	1.26	-0.09	0.08	0.16	37.74
E	6/9/09	deep sediment (44-m depth)	N46°44.342, W099°29.376	filtered lake water with 1/2 nutrient medium	20°C light, 18°C dark, 68% humidity, 12hr light: 12hr dark	similar to B, but brown/green mat of bubbles suspended mid-flask; Lots of cyanobacterial filaments; lots of brown coccooid cells that might be haptophytes; some cells that look like dominant green algae; a loricate cell of Paulinella	Y	892.656	674.458	96.063	1191.59	1.80	-0.48	0.12	0.20	18.92
F	6/9/09	10-m depth water column	N46°44.375, W099°29.622	sea water and distilled water with 1/2 nutrient medium	20°C light, 18°C dark, 68% humidity, 12hr light: 12hr dark	thin orange mats; a couple of dead diatoms and a living filament of a cyanobacterium	N									

Table 2. Comparison of geochemical data for Lake George water and seawater.

Ion	Seawater ^a	Lake George ^a	SW _{NORM}	LG _{NORM}	LG relative to SW
Mg ²⁺	1300	400	0.06	0.05	0.31
Na ⁺	11000	3056	0.58	0.41	0.28
Ca ²⁺	400	11.8	0.01	0.00	0.03
K ⁺	380	250	0.01	0.03	0.66
Cl ⁻	19000	724	1.00	0.10	0.04
SO ₄ ²⁻	2600	7370	0.13	1.00	2.83
CO ₃ ²⁻ /HCO ₃ ⁻	142	346	0.00	0.05	2.44

^aUnits are in mg L⁻¹

Table 3. Organic geochemical data from Experiment 2: *testing temperature dependence of LCAs in enrichment cultures*

Collection Date (m/dd)	Culture ID	Growth Chamber Temp (°C)	LCA Presence	C37:4	C37:3	C37:2	Sum 37	C38:4	C38:3	C38:2	sum 38	[C37] (ug L ⁻¹ water)	UK37	UK37	UK38	UK38*	UK3738	C37/C38	%37:4	Inferred Temp. (°C)
7/13 CONTROL	4	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
7/13 CONTROL	10	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
7/13 CONTROL	20	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
7/27 CONTROL	4	Y	4.13	9.83	0.72	14.68	3.64	13.86	2.51	20.01	18.92	0.068	-0.23	-0.06	0.15	0.12	0.73	28	31	
7/27 CONTROL	10	Y	50.44	68.63	13.32	132.38	33.07	35.77	3.92	72.77	164.44	0.162	-0.28	-0.40	0.10	0.14	1.82	38	29	
7/27 CONTROL	20	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
8/10 CONTROL	4	Y	36.36	134.13	1.12	171.61	145.13	643.16	156.24	944.53	179.29	0.008	-0.21	0.01	0.20	0.17	0.18	21	32	
8/10 CONTROL	10	Y	43.25	99.95	4.83	148.04	107.11	274.49	44.79	426.39	122.19	0.046	-0.26	-0.15	0.14	0.12	0.35	29	30	
8/10 CONTROL	20	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
8/25 CONTROL	4	Y	1.41	5.34	0.001	6.75	18.24	22.67	5.96	46.86	49.17	0.00019	-0.21	-0.26	0.21	0.18	0.14	21	32	
8/25 CONTROL	10	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
8/25 CONTROL	20	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
7/13 DEEP	4	Y	48.85	15.98	1.74	66.57	9.56	7.11	1.66	18.33	49.66	0.088	-0.71	-0.43	0.19	0.13	3.63	73	8	
7/13 DEEP	10	Y	19.48	4.97	0.44	24.89	4.36	3.83	1.25	9.44	20.34	0.080	-0.77	-0.33	0.25	0.16	2.64	78	5	
7/13 DEEP	20	Y	16.23	3.91	1.06	21.20	2.90	3.19	1.08	7.17	18.42	0.213	-0.72	-0.25	0.25	0.23	2.96	77	7	
7/27 DEEP	4	Y	48.42	34.66	0.01	83.09	45.79	164.31	45.47	255.57	55.77	0.0003	-0.58	0.00	0.22	0.19	0.33	58	14	
7/27 DEEP	10	Y	6.41	9.25	0.11	15.77	8.57	21.58	3.04	33.19	20.37	0.012	-0.40	-0.17	0.12	0.09	0.47	41	23	
7/27 DEEP	20	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
8/10 DEEP	4	Y	68.79	72.99	5.10	146.88	73.14	250.70	69.78	393.62	204.61	0.065	-0.43	-0.01	0.22	0.19	0.37	47	21	
8/10 DEEP	10	Y	322.80	510.68	7.52	841.00	825.67	2347.00	515.53	3688.20	418.40	0.015	-0.37	-0.08	0.18	0.15	0.23	38	24	
8/10 DEEP	20	Y	43.25	99.95	4.83	148.04	107.11	811.40	2384.76	529.09	122.19	0.046	-0.26	0.69	0.74	0.72	0.28	29	30	
8/25 DEEP	4	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
8/25 DEEP	10	Y	0.36	1.06	0.001	1.42	1.71	4.47	1.44	7.63	34.59	0.745	-0.25	-0.04	0.24	0.34	0.19	25	30	
8/25 DEEP	20	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
8/25 DEEP TURB	4	Y	103.78	253.17	2.74	131.70	18.24	22.67	5.96	46.86	601.06	0.098	-0.28	0.02	0.21	0.15	2.81	79	5	
8/25 DEEP TURB	10	Y	159.90	231.27	16.38	407.55	349.86	1066.00	265.70	1681.56	697.35	0.066	-0.35	-0.05	0.20	0.18	0.24	39	25	
8/25 DEEP TURB	20	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
7/13 UNFILT	4.0	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
7/13 UNFILT	10	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
7/13 UNFILT	20	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
7/27 UNFILT	4	Y	8.89	26.79	0.01	35.69	23.66	102.42	11.75	137.83	24.85	0.00037	-0.25	-0.09	0.10	0.08	0.26	25	30	
7/27 UNFILT	10	Y	49.83	68.75	10.64	129.21	29.06	20.04	3.93	53.03	117.81	0.134	-0.30	-0.47	0.16	0.14	2.44	39	27	
7/27 UNFILT	20	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
8/10 UNFILT	4	Y	19.58	52.49	0.82	72.89	69.47	234.37	48.16	351.99	76.82	0.015	-0.26	-0.06	0.17	0.15	x	x	x	
8/10 UNFILT	10	Y	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
8/10 UNFILT	20	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
8/25 UNFILT	4	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
8/25 UNFILT	10	Y	0.72	1.90	0.001	2.62	1.97	5.64	1.47	9.08	19.21	0.001	-0.274	-0.054	0.207	0.164	0.289	27	29	
8/25 UNFILT	20	N	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	

Table 4. Comparison of lake water chemistry for lakes containing both OTU7 and OTU8.

Lake	Location	Latitude	Longitude	Depth (m)	pH	Salinity (g L ⁻¹)	Na	K	Mg	Ca	CO ₃	HCO ₃	Cl	SO ₄	Source
Lake George	North Dakota, USA	46.74	-99.49	60	9	9.7	3056	250	400	11.83	346.4	nd	1292	12210	Toney et al. 2010
Medicine Lake	South Dakota, USA	44.82	-97.37	8.3	8.4	9	nd	nd	nd	nd	245	nd	605	28680	Toney et al. 2010
Skoal Lake	North Dakota, USA	47.92	-101.47	1.8	8.9	8	nd	nd	nd	nd	276.9	nd	155.2	8955	Toney et al. 2010
Pyramid Lake	Nevada, USA	40	-119.5	59	9.3	5	1720	118	114	9.3	300	860	2080	280	Galat et al. 1981
Tso Ur	Tibetan Plateau, China	31.48	91.52	8.4	9.8	12	3467.9	249.7	110.7	7.9	638	4240	1234.1	1967.8	Wu et al. 2009

nd = not determined

Anion and cation units are mg L⁻¹

Figure 1. GC-FID traces for alkenone-containing enrichments A-E (lower) and surface sediment (upper).

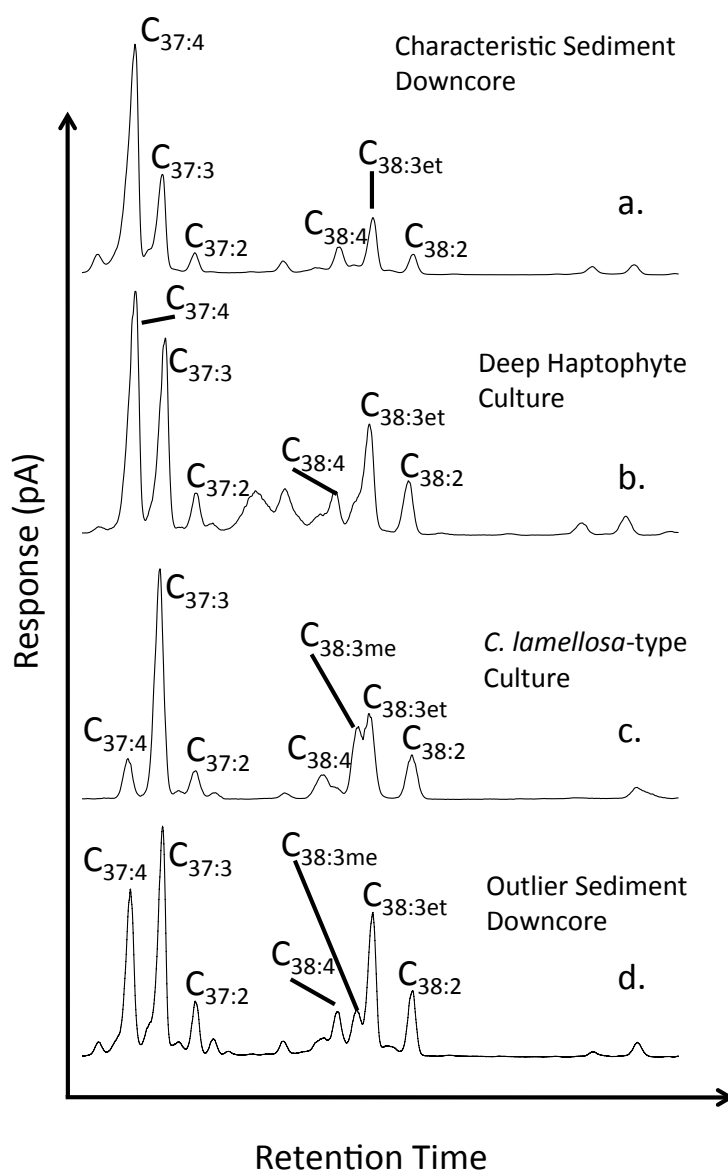


Figure 2. A consensus Bayesian phylogenetic tree showing the 18S rRNA gene-inferred relationships among haptophyte algae in the GenBank database. Sequences reported in this study, Hap-A and Hap-B, are in bold. Details on numbered OTUs and Greenland group are reported by Theroux et al., (2010). Numbers above branch points represent posterior probability values.

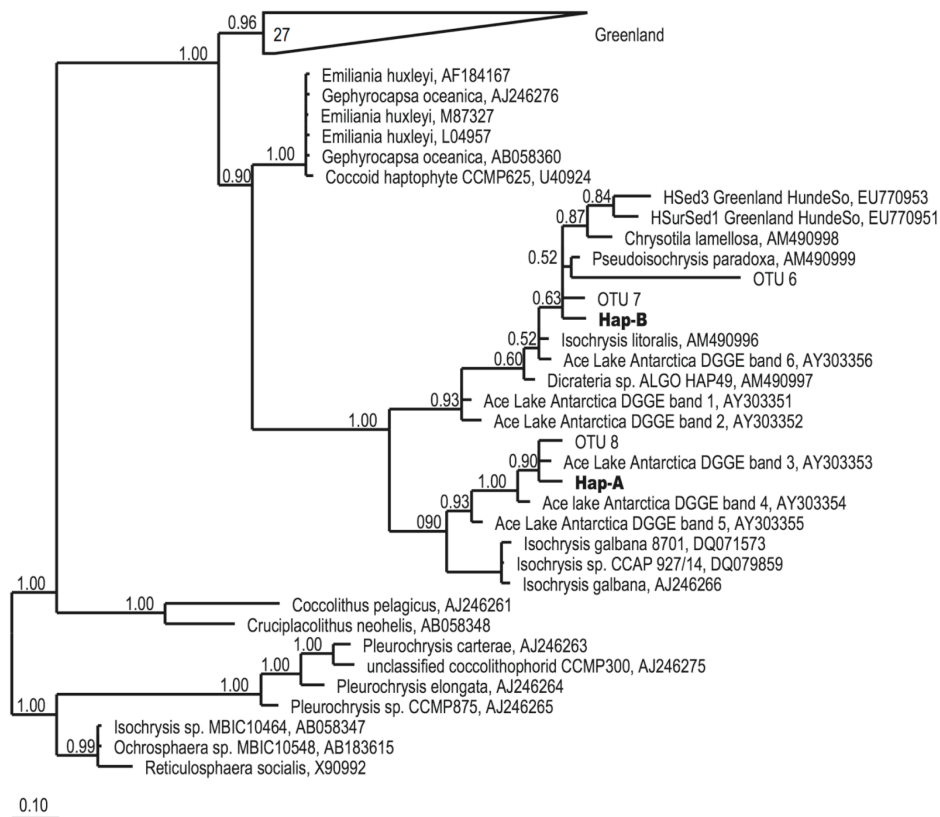


Figure 3. (top panel) Shows the temperature dependence of the U_{37}^K and U_{38}^K indices in week 6 and week 2, respectively for Hap-A enrichments. This is offset from the *in situ* calibration from Lake George (circles) reported by Toney et al., (2010). (bottom panel) Shows the reduction in percent $C_{37:4}$ at all temperatures for Hap-A enrichments.

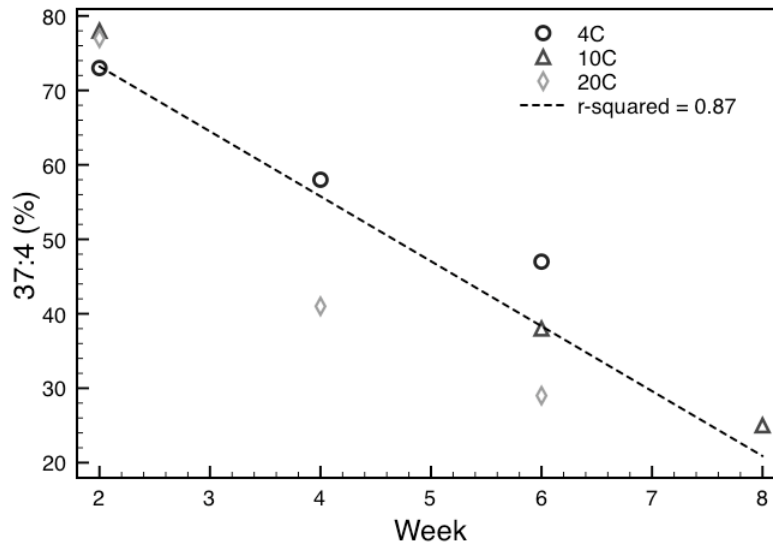
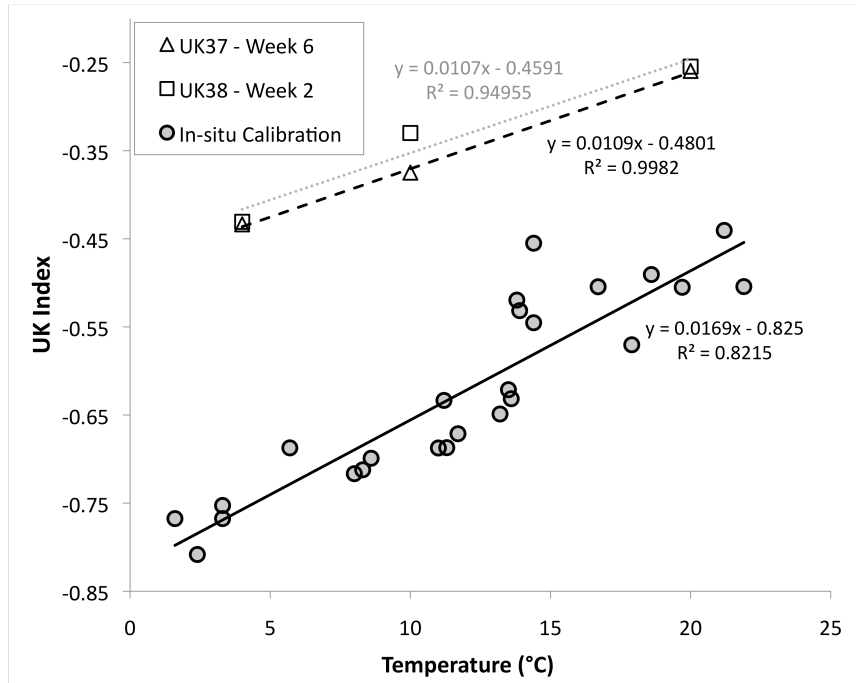
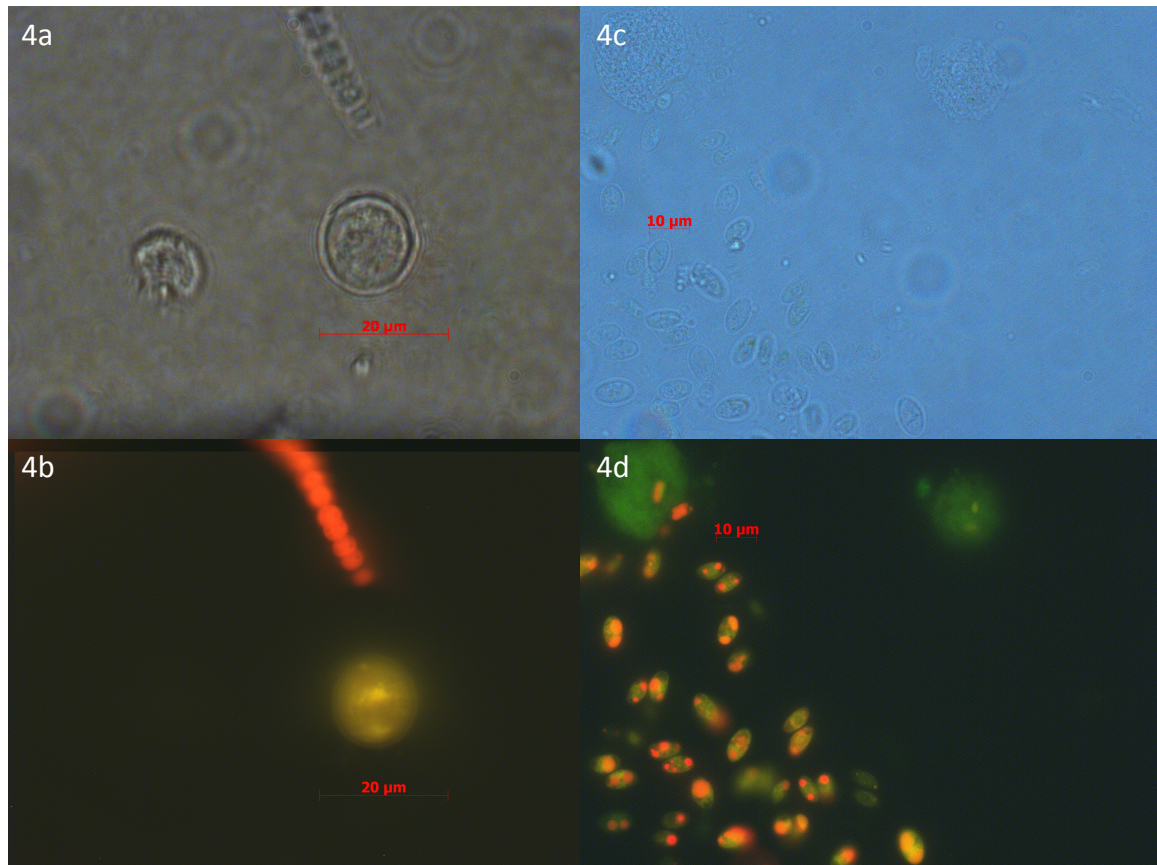


Figure 4. FISH using haptophyte-specific probes. (a) Light microscope image of Hap-A, and (b) the same cell showing probe fluorescence. (c) Hap-B under phase microscopy and (d) green fluorescence from haptophyte probe with red chloroplasts due to natural autofluorescence.



CHAPTER 5

DIFFERENT ALKENONE SIGNATURES ARE PRODUCED IN DIFFERENT LIFE STAGES OF A SINGLE LACUSTRINE HAPTOPHYTE: IMPLICATIONS FOR PALEOTHERMOMETRY

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ABSTRACT

Although long-chain alkenones (LCAs) were first discovered in lakes more than two decades ago, very few paleotemperature reconstructions using lacustrine alkenones are still in its infancy. A major obstacle is the uncertainties associated with the potentially large diversity of haptophyte phylotypes that exist in lakes and the complications of temperature calibrations. Here we report the isolation and culturing of a haptophyte species from Lake George, ND. We found through manipulation of unialgal cultures established from single cell isolations five different life stages. Analysis of the long-chain alkenones in the cryogenically preserved life stages shows that this single haptophyte produces different alkenone profiles: one with the predominance of the C_{37:4}- alkenone commonly observed in lake sediments around the world, and the other dominated by the C_{37:3} alkenone similar to that of ocean distributions. and previous enrichment cultures from Lake George. We propose an ecological succession to depict seasonal haptophyte cycle at Lake George.

1. INTRODUCTION

The long-chain alkenone (LCA) unsaturation index is an established proxy for reconstructing past sea surface temperatures (Brassell et al., 1986; Prah and Wakeham, 1987). Although LCAs were first discovered in lakes more than two decades ago (Cranwell, 1985), using lacustrine alkenones have just begun recently (Wang and Zheng, 1997; Zink et al, 2001; Toney et al., Chapter 7). The sluggish pace of development for this proxy in lakes is due to the uncertainties associated with the unknown diversity of haptophyte algae that exist in lakes and the potential need for separate calibrations for each species. Extensive sampling of the water column at different depths when LCA production is active offers one viable solution to the calibration problem (Toney et al., 2010); however, more information about lacustrine haptophyte algae is needed in order to determine the environmental parameters that control the timing of the haptophyte bloom, production of LCAs, LCA export to the sediments, and possible competitive effects of different haptophytes in the a single lake system.

One aspect of sedimentary LCA distributions in lakes that remains a puzzle is the exceptionally high abundance of tetra-unsaturated C₃₇ LCA (C_{37:4}) (Cranwell, 1985; Volkman et al, 1988; Thiel et al., 1997; Wang and Zheng, 1998; Zink et al., 2001; Sun et al., 2007; Chu et al., 2005; D'Andrea and Huang, 2005; Toney et al., 2010), while signature LCA profiles from cultures

of lacustrine haptophytes have only been reported from *Chrysotila lamellosa* (e.g, Sun et al., 2007), which has not been shown to produce predominantly C_{37:4} LCAs (Sun et al., 2007, Rontani et al., 2004). A breakthrough has recently been reported (Toney et al., Chapter 4) that addresses this problem by stimulating the production of C_{37:4} in enrichment cultures from Lake George, ND.

The study of enrichment cultures shows that the haptophyte SSU rDNA sequence, or phylotype, associated with the dominant C_{37:4} profile is one that has previously been reported from a number of lakes worldwide and named operational taxonomic unit 8 (OTU8)(Theroux et al. 2010). All sites that OTU8 has been reported from also contain another phylotype, that is genetically distinct, OTU7. In Northern Great Plains lakes, where OTU7 and OTU8 coexist, the sedimentary profile is dominated by C_{37:4}; whereas, in Pyramid Lake, NV and Tso Ur, China, where OTU7 and OTU8 coexist, the sedimentary profile is dominated by C_{37:3} with C_{37:4} as a minor component (Toney et al., Chapter 4). Toney et al. (Chapter 4) suggest that the different LCA profiles despite the same phylotypes are due to differences in the sulfate to carbonate ratio (SCR) that allow one phylotype to out-compete the other, namely the OTU8 haptophyte is associated with high SCRs and OTU7 with low SCRs. The controls on these two haptophytes are important, because

shifts in LCA profiles in down core sediments could signal that important environmental changes have occurred.

In this study we were able to isolate individual cells of a haptophyte alga from the enrichment cultures previously reported to have OTU7 (Toney et al., Chapter 4). We analyze the lipid profiles from the single-cell isolate cultures, which produce multiple life stages. We discuss the implications of the new findings in light of previous enrichment culture experiments, haptophytes in the environment, and the implications for LCAs as a paleotemperature proxy.

2. METHODS

2.1. Field and sampling methods

Water and sediment samples were collected from Lake George, North Dakota (46.74°N, 99.49°W) in June 2009 with a Van Dorn water sampler and an Ekman grab sampler. Water samples were collected at multiple depths, while sediment samples were collected from the near-shore oxic environment and from the deep basin of the lake at 44-m depth. One 1-L water sample from each depth was filtered using a vacuum filtration unit with combusted (550°C) GF/F 0.7µm, 47 mm glass filters. Additional water was collected in June of 2010 for ongoing culture experiments (see Toney et al., Chapter 4). Additional deep sediment was collected in November 2010.

2.2. Enrichment Culture Methods

Details for starting enrichment cultures are reported in Toney et al. (Chapter 4). For this study, 4-ml sub-samples of enrichment cultures started from the water column, shallow sediment, and deep sediment were collected in April, 2010 and shipped to one of us (RAA) for establishing unialgal cultures.

2.3 Algal communities in enrichment and isolate cultures

Culture medium was prepared in several ways. In early isolation work, the culture medium was prepared by mixing 1:1 Boothbay Harbor filtered seawater (obtained from CCMP, Bigelow Laboratory, West Boothbay Harbor, ME) with distilled or de-ionized water, and this half-salinity water was nutrified using DY-V medium nutrients except for the MES buffer and the silica stock solution. The pH was adjusted to 9.05 using NaOH and then it was autoclaved. In later work, water collected from Lake George, North Dakota, on October 4, 2010, was used in place of the 1:1 mixture. Nutrification was as described before, however, the pH was not adjusted at all prior to autoclaving. In addition to liquid culture medium, solid medium was also prepared. A 1% (wt:vol) agar solution was prepared by dissolving Bacto-Agar (VWR Scientific) in the liquid medium. The agar nutrient solution was the autoclaved and upon cooling, it was aseptically poured into Petri plates to solidify.

Using the enrichment cultures, biomass was streaked on agar plates. Plates were incubated at temperatures between 10° and 25° C and they were illuminated with approximately 25 - 50 mmol photons · m⁻² · s⁻¹ from fluorescent lights set on a 12:12 or a 13:11 hr light:dark regime. In some cases, cultures were also maintained using natural light from a north-facing window. Growth was examined by light microscopy, and material was re-streaked again until individual uni-algal colonies arising from putatively single cells were obtained. The single-cell colony material was transferred to liquid medium and maintained in liquid culture. In addition, cultures were established by isolation using a micropipette. Using a Zeiss dissecting scope equipped with darkfield illumination, individual cells were isolated, washed with sterile medium and transferred to liquid culture medium in screw-top test tubes. Similarly, sediment collected from 45-m on November 8, 2010, was streaked on agar and enriched in liquid medium. Unialgal and putatively single-cell isolates were established using both the agar streak method and the micropipette method.

Sub-cultures were established with the intent of observing algae via light microscopy and isolating individual algae into pure cultures. Sub-samples (4-ml) of enrichment cultures and from surface sediment

collected at 45-m depth in November 2010. Continued observations and sub-cultures were made since the initial sub-cultures in April 2010 and resulted in the first, pure culture of *C. lamellosa*-type haptophyte in December of 2010. Subsequent manipulation of pure cultures was driven by continual observations and speculated environmental controls on LCA production (see discussion below). Isolations from the deep sediments, which are expected to produce a second haptophyte, namely phylotype OTU8, are ongoing and have not yet resulted in unialgal cultures of a haptophyte.

2.4 Organic geochemical methods

Freeze-dried culture water (mainly salts) were homogenized and extracted with dichloromethane (DCM):methanol (MeOH) (9:1, v/v) using an Accelerated Solvent Extractor ASE200 (Dionex). Fifty- μ l of C₃₆ *n*-alkane standard (72.43 μ g ml⁻¹) was added, and the total lipid extracts (TLEs) were run on a GC-FID for detection and quantification of LCAs using the internal standard. An Agilent DB-1 GC column (60m $\times$ 320 μ m $\times$ 0.10 mm) was used with the following temperature program: an initial temperature of 40°C (hold 1-min), ramp 30°C min⁻¹ to 290°C (hold 1-min), ramp 5°C min⁻¹ to 300°C (hold 0-min) then 2°C min⁻¹ to 325°C, GC temperature program (hold 10-min)(Toney et al., 2010). The same temperature program was used on the GC-MS, and samples were run to confirm the identity of the LCAs using the

known ion chromatograms and by comparison of mass spectral data with published data and GC retention times (de Leeuw et al., 1980, Marlowe et al., 1984). The final GC program described here is the result of many permutations of GC parameters, including the switch from helium as the carrier gas to hydrogen. As such we are able to resolve LCAs with >4µg per sample (i.e. grams of dry sediment or liter of water). LCA standards of known temperature calibration are run on the GC-MS to ensure analytical precision (<0.1°C).

3. RESULTS

3.1 Observations of haptophyte algae in pure algal cultures

The palmelloid (= capsoid) stage had thousands of cells united with a common gel that produced an irregularly shaped colony. Each cell was < 5µm in size and contained a single yellowish green chloroplast that did not contain a pyrenoid. Cells were naked, i.e., they lacked a cell wall. The sarcinoid stage (= *Ruttnera* stage) consisted of regularly shaped cell packets, typically four cells wide, four cells long and two cells deep. The sarcinoid arrangement of cells was maintained by a colonial gel. Cells were surrounded by a distinct cell wall. The flagellate stage consisted of naked swimming cells bearing two approximately equal flagella. Each cell had a single yellowish green chloroplast but no eyespot or pyrenoid was present. The lamellate stage

consisted of cells that were surrounded by a thick gelatinous material with distinct rings or lamella. Vegetative cell division occurred and daughter cells were gradually separated by the production of new gelatinous material. A cyst-like stage consisted of single cells surrounded by a thick cell wall.

While all stages were observed in a single culture tube after manipulation of its growth conditions, the morphogenesis from stage to stage was only documented for one stage change. The palmelloid stage cells converted to flagellate cells by dissolution of the gel and release of cells. The cells first produced two flagella and a haptonema that were inserted subapically into the cell body. With time, the flagella migrated to the cell apex and the haptonema was withdrawn.

Through repeated attempts at isolation of various organisms from the subcultures we have successfully isolated a single haptophyte into pure culture that can be identified via light microscopy. The haptophyte resembles *Chrysotila lamellosa*, after Anand (1937). The cells are about 6-7µm in diameter, have two plastids, and are surrounded by a gelatinous sheath (Figure 1a). This species is present in subsamples of enrichment cultures started from the water column and shallow sediment from Lake George. From additional isolate cultures, we were able to capture four morphologically different life stages: (1) *Chrysotila lamellosa* stage –

lamellate gel layers (Anand 1937) (Fig 1b), (2) *Ruttnera spectabilis* stage – sarcinoid packets (Geitler 1942)(Fig. 1c,d), (3) the flagellate stage (Fig. 1f), and (4) the palmelloid (capsoid) stage (Fig. 1g). A fifth life stage, a cyst phase (Fig. 1e) was later found in a culture started from a pure palmelloid culture.

3.2 Transitions between life stages

Although additional work is necessary and underway for full elucidation of the haptophyte's life cycle (i.e., meiosis and syngamy are not observed), comparisons with similar taxa from culture collections, and determination of the environmental controls that trigger different life stages, in isolate cultures, we have observed that the *Ruttnera*-stage is triggered when palmelloid stage is cooled. We have also observed that the capsoid stage can convert to the flagellate stage and the cyst stage.

3.3 LCA lipid profiles associated with life stages

LCAs analyzed from cryogenically preserved life stages of isolate culture samples including: the capsoid stage, the *C. lamellosa*-type lamellate stage, the *Ruttnera*/sarcinoid stage, and the flagellate stage show that LCAs were not detected in the capsoid and lamellate stages. The *Ruttnera* stage produces LCAs with the dominant C_{37:4} signature (Figure 2a) and C_{38:4}, C_{38:3et}, C_{38:2}, and C_{39:3} also present. The flagellate stage produces LCAs with

the dominant C_{37:3} signature (Figure 2c) and with C_{38:4}, C_{38:3et}, C_{38:2} and C_{39:3} also present.

3.4 Preliminary rDNA amplification

Flagellate and *Ruttnera* stage algal culture rDNA was extracted and amplified by Susanna Theroux (See methods in Theroux et al. 2010). LCAs were detected from both flagellate and *Ruttnera* stage cultures, however, with a first round of amplifications, only the flagellate cultures (n=3) amplified. *Ruttnera* stage cultures did not (n=4).

4. DISCUSSION

4.1 Comparing and contrasting life stage lipid results with previous enrichment culture results

The life stage lipids offer new and surprising results that raise important new questions in the context of the previous enrichment culture results. Here we will summarize the results from Toney et al. (Chapter 4) with the intent to reconcile the new findings with the previous findings. Based on the SSU rRNA and lipid profiles, two haptophyte phylotypes occur in Lake George OTU7 and OTU8 (Theroux et al. 2010). During times of active LCA production in the enrichment cultures, only OTU8 (also referred to as Hap-A)

was amplified during the production of LCAs dominated by C_{37:4} (Figure 2b), while only OTU7 (or Hap-B) was amplified during the production of LCAs dominated by C_{37:3} (Figure 2d). The OTU8 phylotype was exclusive to the enrichment cultures started from the deep sediments, whereas, OTU7 was detected only in samples from the water column and shallow sediments. OTU8 and OTU7 were never found to coexist in enrichment cultures, however, the lipid profile associated OTU7 was present after maximum production of C_{37:4}-dominated LCAs ceased. The OTU7 lipid profile was also observed in the field directly after the C_{37:4}-dominated bloom began vertical descent in the water column. Only one rDNA sample was collected and sequenced during the enrichment culture experiments, and it is possible that OTU7 was present, but not detected if the phylotype was only present in low cell concentrations, or if the life stage at this time required extraordinarily aggressive DNA extraction methods (see below).

What is now apparent from the lipids that were analyzed from cryogenically preserved life stages of a single haptophyte alga isolated from the OTU7 enrichment cultures, is that this haptophyte can produce both the C_{37:4}-dominated profile and the C_{37:3}-dominated profile in different life stages. Also, there are at least two stages, capsoid and lamellate stages, in which LCAs are not present. Additional work is underway to sequence the DNA from each life stage and verify that this is the haptophyte associated with the

OTU7 phylotype. While we never detected OTU8 in the enrichment culture that this haptophyte was isolated from, additional sequencing directly from the life stages will provide absolute certainty as to which phylotype has these life stages. It appears that the haptophyte producing the different stages is capable of accounting for all the environmental and culture lipid profiles observed thus far, just from different life stages. Thus it remains unclear if the second phylotype produces alkenones. We have begun isolation of a second alga that resembles a haptophyte, however, the isolation process likely will not yield results for many months (i.e., the isolation reported here took greater than 10 months of continuous monitoring and careful isolation before pure, unialgal cultures were ready for observation, manipulations, and lipid analysis). Herein we will refer only to the isolated haptophyte and refrain from associating it with OTU7 or OTU8 until future analyses (to be undertaken by S. Theroux) have worked out this problem.

4.2 Isolate observations and life stages dynamics

4.2.1 Ruttnera stage produces C_{37:4} dominant LCAs

With respect to using LCAs as a paleotemperature proxy at Lake George, we are most interested in the life stage that produces the C_{37:4}-dominated profiles, because this is the profile that dominates the sedimentary signal and has been shown to record surface water temperature (Toney et al., 2010). The C_{37:4}-producing stage is the *Ruttnera spectabilis* stage, which is a colonial

stage surrounded by a gelatinous sheath (Figure 1 c,d). Prior to isolating the *Ruttnera spectabilis* stage into a unialgal culture, the colonial stage appeared to only occur in the presence of amoeba and suggested that this colonial stage acts as a defense mechanism against predation, namely the cell clusters are too large for the predator to consume. However, after isolation, in the absence of predators, the sarcinoid shape was maintained and it was found that submitting other life stages to cold conditions also triggered the conversion to the sarcinoid stage. In subsequent observations from unialgal isolations, the lamellate stage converted to the colonial phase upon being transferred to new medium. This suggests that the colonial stage can also be triggered by nutrient or geochemical differences in the water medium, as well.

4.2.2 Triggering the *Ruttnera* stage

Previous haptophyte studies from marine settings have shown that various environmental parameters can trigger the colonial haptophyte stage (e.g., nutrients, predation, viral protection). Some studies show that the colonial phase can be triggered by low phosphate conditions, which does not appear to be a factor in our cultures, because the transfer that triggered the conversion took the haptophyte from a nutrient-depleted medium into a fresh, nutrient-replete medium. This may still be a factor in the environment, however, because primary productivity in Lake George is primarily nitrogen-limited and secondarily phosphorus-limited (Salm et al., 2009). In particular, results

from previous studies of *Phaeocystis globosa* suggest that the haptophyte colonial stage is highly competitive. The colonial stage can out-compete free-living forms under low phosphate and iron conditions due to the higher ability to use organically bound phosphate and iron (Veldhuis and Admiraal, 1987; Veldhuis et al., 1991; Schoemann et al., 2001). In general, the gelatinous sheaths surrounding the colonies have also been reported as advantageous for protecting against grazing and viral infection (Lancelot et al. 2001). While colony size alone may be a deterrent for grazers, *Phaeocystis* has also been shown to produce DMSP that releases acrylic acid in the matrix and converts DMSP to DMS, a grazer deterrent (Noordkamp et al. 2000). The haptophytes at Lake George have been speculated to use DMSP as an osmoregulatory compound under low nitrogen conditions due to their spatial distribution only in lakes with high sulfate content (Toney et al., 2010). The association of the C_{37:4}-dominated LCAs with the non-motile stage that rises through the water column suggests that the compounds may be associated with cell buoyancy, but does not exclude the hypothesis that they play a role in metabolic storage (Epstein et al., 2001). Ultimately, additional work is necessary that monitors algal communities, while also measuring important trace elements, vitamins, day length, and predation.

Our results confirm that the colonial stage does outcompete the flagellate stage based on the high concentrations of C_{37:4} LCAs that are associated with

this phase and the lack of the flagellate lipid signal during the C_{37:4}-dominated bloom in the field and in the enrichment cultures. However, more culture manipulation investigations are necessary to support what causes the conversion from the lamellate phase to the colonial phase. Both in the field and in enrichment cultures we observed that directly after the major bloom dominated by the C_{37:4} LCA, the flagellate signature profile appeared. We did witness the conversion from colony to flagellate, and similar phenomena has been observed in *P. globosa*, where the conversion from colonies to flagellates occurs following the colonial bloom due to nutrient stress and light limitation following vertical transport (Peperzak et al. 2000). In the field studies (Toney et al., 2010), we did witness the colonial LCA signature descend through the water column following the surface bloom, which suggests that the latter may be a contributing factor.

4.2.3 Flagellate LCAs and the C_{37:3} signature

The lipid signature suggests that although the majority of the colonial material descends through the water column and ultimately resides in the sediments, some individual flagellates remain at the surface for about two weeks following the colonial bloom. Studies of haptophyte *Phaeocystis* have shown that the flagellate can out-compete the colonial form when ammonium is limiting, although they rarely form blooms, and are heavily grazed (Riegman et al. 1992). Most of the flagellate-derived material is retained in

the surface waters through the microbial food web, which may be why we do not see this signature in the sediments (Toney et al., 2010).

In culture, we observe that the capsoid stage can convert to either the flagellate stage or cyst stage, although it is unclear if the colonial stage goes directly to the flagellate stage or into the capsoid-intermediate stage. The capsoid stage cannot be detected using lipid profiles because no LCAs are associated with the phase. Likely, the conversion to flagellate or cyst is dependent on the environmental conditions, for example, under nutrient stress the capsoid may favor transformation to the cyst, which then gets exported to the sediments. In other haptophytes, mainly *Prymnesium*, it is speculated that cysts provide an overwintering stage that seeds the bloom in the following spring, or when the appropriate conditions appear (Edvardsen, 2001). Based on what we know about the isolated Lake George haptophyte we propose a tentative ecosystem diagram for Lake George dominated by this haptophyte (Figure 3).

4.3 Preliminary rDNA amplification

In order to amplify rDNA from the *Ruttnera* stage aggressive extraction techniques were necessary. This is likely due to the protection provided by the gel sheath and cell wall (e.g., see Snirc et al. 2010). Without such aggressive techniques, environmental and culture extractions may not

amplify all haptophyte DNA present. In particular this new finding suggests that extraction, amplification, and sequencing of enrichment rDNA (Chapter 4) may not have been complete. Further analyses with aggressive extraction techniques from the C_{37:4}-producing haptophytes to confirm that only OTU8 phylotype was present.

5. CONCLUSION

We show that one single haptophyte species isolated and cultured from Lake George is capable of making two contrasting alkenone profiles: one that has been observed in numerous lakes around the world and the other more similar to ocean profiles. Therefore, conditions favoring one life stage over the other in natural settings would determine what alkenone signatures will be observed in sediments if this alga is the sole alkenone producer. Our results offer new insight into the production of the unusual alkenone lipids in haptophytes.

While elucidation of the full life cycle of this Lake George haptophyte requires further investigation, what we have determined, thus far has important implications for the interpretation of LCAs as a paleotemperature proxy. For instance, we know that the sedimentary signal is produced by the colonial stage of the haptophyte algae, and while we previously thought that the end of the bloom was related to surface water temperature imposed by

stratification, comparison with similar haptophyte colonial stages suggests that nutrient limitation may also play a key role. We know that the second lipid profile, C_{37:3}-dominated, does not necessarily indicate the presence of a second haptophyte species or phylotype. When this profile dominates the sedimentary record, which it does in several discrete samples down-core (see Chapter 7), then conditions were not favorable for the colonial stage and flagellate signature was a better competitor and/or had greater export from the surface waters. Based on this study we were able to construct a preliminary diagram representing the structure of Lake George haptophyte ecosystem to guide the interpretations of downcore lipid analyses and future field research at the site.

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Figure 1. Haptophyte from Lake George cultures. (a,b) Lamellate stage, (c) colonial stage – unialgal, (d) colonial stage – with amoeba and diatoms, (e) cyst stage, (f) capsoid converting to flagellate, (g) capsoid stage.

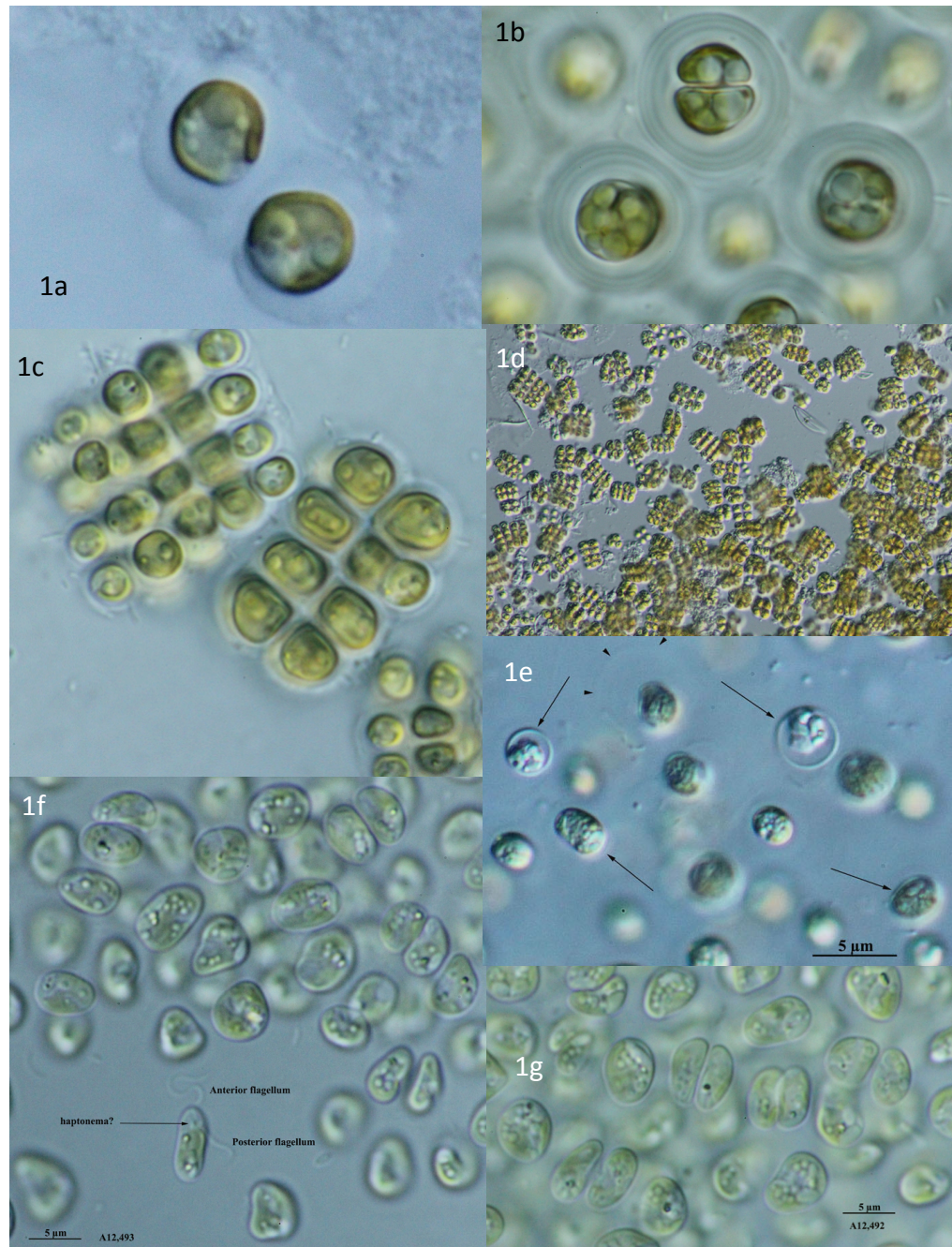


Figure 2. Long-chain alkenone profiles associated with each life stage and comparison with previously reported phylotype lipid profiles from enrichment cultures from Lake George (Toney et al. Chapter 4).

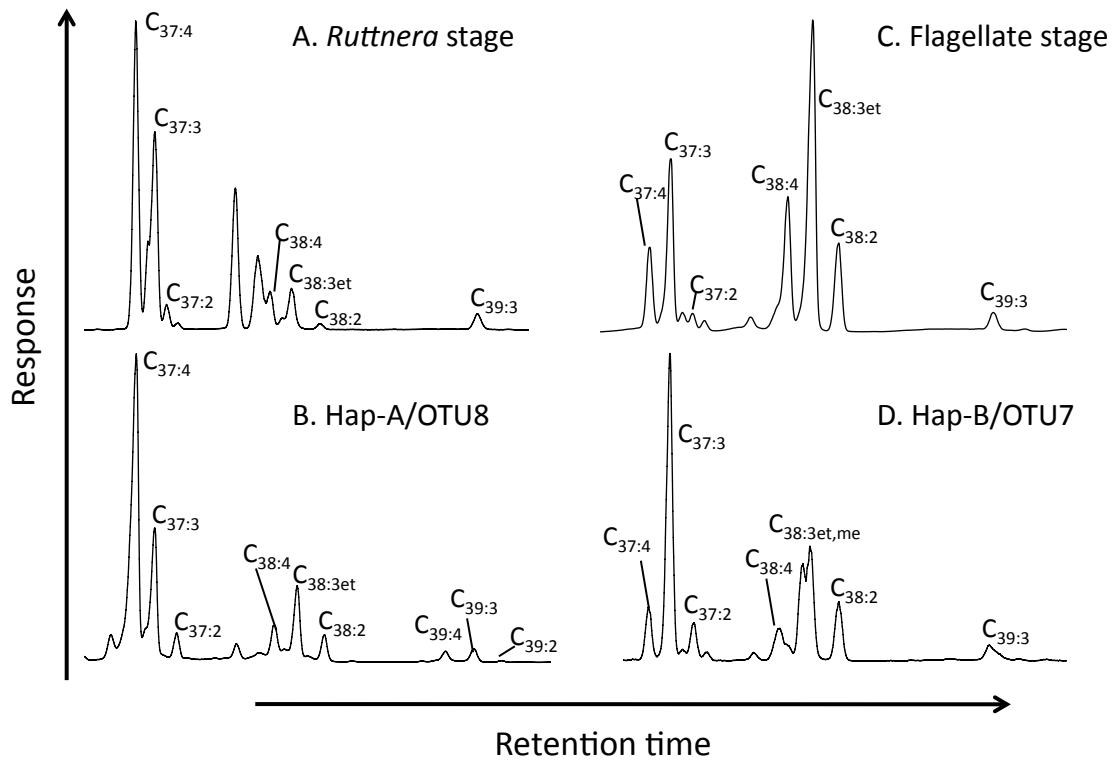
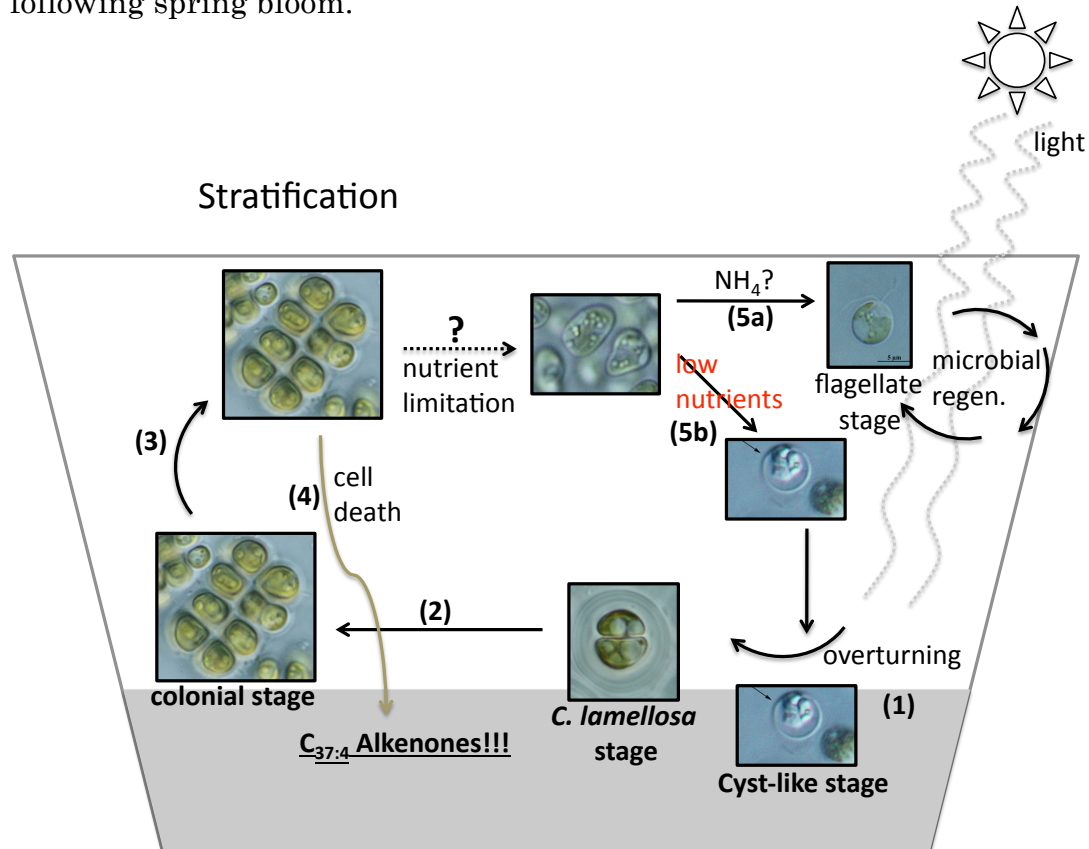


Figure 3. Proposed diagram of haptophyte life cycle in Lake George, ND. (1) Ice-out allows increased light penetration combined with overturning triggers the cyst to convert to the lamellate stage. (2) Lamellate stage continues to aggregate form colonial stage. (3) Colony rises to the surface over 2-weeks, LCAs equilibrate with temperature throughout ascent. (4) Stratification causes unfavorable conditions, colony cells die and begin descent, several individuals break free (possibly as capsoid or flagellates). Majority of colony material exported to sediment. (5a) Under low nutrients (especially low NH_4) capsoid converts to flagellate and remains part of the microbial regeneration cycle with little to no material exported to sediments. (5b) Under complete nutrient depletion, capsoid converts to cyst for overwintering and seeding of following spring bloom.



CHAPTER 6

HAPTOPHYTE *PSEUDOISOCRYSIS PARADOXA* PRODUCES LONG-CHAIN ALKENONES

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ABSTRACT

Although, little is known about the potential diversity of lacustrine haptophyte taxa, speculation regarding their complexity has hindered the progress of using alkenones in lake environments. Here we report that *Pseudoisochrysis paradoxa*, a close relative of lacustrine species *Chrysotila lamellosa* and recently reported lacustrine phylotype OTU7, produces alkenones with similar C_{37:3}-dominated profiles to its relatives, although the C₃₈ homologues vary as a result of culture conditions and likely growth stage. This study adds to the growing number of haptophytes known to produce alkenones, including: *Chrysotila lamellosa*, *Emiliana huxleyi*, *Isochrysis galbana*, and *Gephyrocapsa oceanica*.

1. INTRODUCTION

Long-chain alkenones (LCAs) are important organic compounds, because they are produced by haptophyte algae that modify the level of LCA unsaturation based on growth temperature (Brassell et al. 1986). LCAs are preserved in marine and lacustrine environments as molecular fossils and have been used extensively to reconstruct sea surface temperature (Müller et al. 1998; Lawrence et al. 2005), and more recently lake surface water temperature (Zink et al. 2001; Toney et al., Chapter 7). LCAs are biosynthesized only by algal species belonging to the monophyletic group, Haptophyta, in the order Isochrysidales (Müller et al. 1998; Medlin et al. 2008). Although LCA production is conserved within the haptophyte group, not all members of the group produce LCAs. For example, known marine haptophytes, *Chrysotila lamellosa* (Marlowe et al. 1984), *Emiliana huxleyi* (Volkman et al. 1980, Marlowe et al. 1984), *Isochrysis galbana* (Marlowe et al. 1984), and *Gephyrocapsa oceanica* (Conte et al. 1994) produce LCAs, while *C. stipitata*, *Dicrateria inornata*, *Hymenomonas carterae*, *Imantonia rotunda*, *Ochrospira neapolitana*, and *O. verrucosa* do not (Marlowe et al. 1984a, 1984b), at least in the stage from which they were analyzed.

Recently, with the common use of ribosomal DNA to detect and identify haptophytes in environmental samples, unique haptophyte phylotypes are continually added to the GenBank database (e.g., Coolen et al. 2004; Theroux

et al. 2010). One pseudo-cryptic haptophyte, *Pseudoisochrysis paradoxa* nomen nudum strain CCMP715 and PLY507 (= *Isochrysis* sp. Marlowe et al. 1984a; not *Pyramimonas virginica* Pennik, 1977), shares a phylogenetic branch with known LCA-producers (GenBank sequence AM490999, Sáez et al. 2004), *I. galbana*, *C. lamellosa* and a new phylotype (OTU7) from a number of lakes worldwide (Theroux et al. 2010). *P. paradoxa* is thought to have evolved ca. 50 million years ago (Sáez et al. 2004, Medlin et al. 2008), and was originally isolated and classified as *Pseudoisochrysis paradoxa* (Franklyn Ott) (see Jordan et al., 2004). Subsequent cultures of *P. paradoxa* were reported to produce LCAs, although this finding has never been formally published (Volkman, personal communication; Farmer, 1993).

Here we formally report new analyses of the LCAs produced by *P. paradoxa* from a culture collection, CCMP715, and compare and contrast the LCA lipid profile with the previously unpublished LCA lipid profile of the CCMP715 strain.

2. METHODS

2.1 Lipid analysis for *Pseudoisochrysis paradoxa* – CCMP715

Pseudoisochrysis paradoxa strain CCMP715 was acquired from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton. The culture was maintained for one month in the original L1 medium at 10-15°C. A

subsample was collected, cells were centrifuged and the cell pellet cryogenically preserved (cells were frozen $< -151^{\circ}\text{C}$) for lipid analysis. The culture was freeze-dried under vacuum at <100 millitorrs for 48 hrs. The residual salts and organic matter were homogenized and extracted with dichloromethane (DCM):methanol (MeOH) (9:1, v/v) using a Dionex Accelerated Solvent Extractor ASE200. Fifty- μl of C_{36} *n*-alkane standard ($72.43\ \mu\text{g ml}^{-1}$) was added, and the total lipid extracts (TLEs) were run on a GC-FID for detection and quantification of alkenones using the internal standard. An Agilent DB-1 GC column ($60\text{m} \times 320\mu\text{m} \times 0.10\text{ mm}$) was used with the following temperature program: an initial temperature of 40°C (hold 1-min), ramp $30^{\circ}\text{C min}^{-1}$ to 290°C (hold 1-min), ramp $2^{\circ}\text{C min}^{-1}$ to 325°C (hold 30-min). The same temperature program was used on the GC-MS, and samples were run to confirm the identity of the alkenones and alkenoates using the known ion chromatograms and by comparison with published mass spectral data and GC retention times (de Leeuw et al., 1980, Marlowe et al., 1984). The final GC program described here is the result of testing many permutations of GC parameters, including the switch from helium as the carrier gas to hydrogen.

2.2 Lipid analysis *Pseudoisochrysis paradoxa* from algae previously reported (Farmer, 1993)

P. paradoxa CCMP715 was obtained from culture collection (Farmer, 1993) and was grown in f/2 medium at 20°C on a 12:12 light cycle. Cells were harvested during log phase (cell density of 1.55×10^6 per ml).

3. RESULTS AND DISCUSSION

3.1 Alkenone distributions in *P. paradoxa*

Pseudoisochrysis paradoxa produces long-chain alkenones (LCAs) from pure cultures obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (Figure 1). *P. paradoxa* (CCMP715) produces relatively small concentrations of LCAs (2.3 μl / ml culture medium, this study) in culture and 0.5 pg/cell out of the total lipid content (2.5 pg/cell) (Farmer, 1993). The LCA profiles are similar in that the dominant C_{37} homologue is the $\text{C}_{37:3}$ (Table 1). Differences also occur among the various LCAs including the presence of abundant ethyl esters ($\text{C}_{37:3}$, $\text{C}_{38:4}$, $\text{C}_{38:3}$, $\text{C}_{38:2}$) in the recent cultures versus ethyl ketones ($\text{C}_{38:3}$, $\text{C}_{38:2}$) (Farmer, 1993). The presence of ethyl esters in the recent culture is likely the result of different culture conditions, namely the temperature. The recent culture was grown at cooler temperatures (10-15°C) than the Farmer culture (20°C). Previous studies of the closely related, *Chrysotila lamellosa*, show that ethyl esters are present in the early stationary phase and are produced at low temperatures

(Rontani et al. 2004). The recent culture had been maintained for a month in the same medium and the cells were in a stationary phase at the time of lipid analysis.

3.2 Alkenone profiles in *P. paradoxa* and closely related species

3.2.1 Isochrysidales Group I

Three main groups of haptophytes occur within the clade Isochrysidales (Theroux et al. 2010). Group I consists only of phylotypes detected in lakes in Greenland, China, U.S., and Canada. No haptophytes have been captured, cultured, or described in association with the phylotypes from Group I.

Alkenone production is inferred from sedimentary lipid profiles for this group and contain a mix of characteristic profiles, some dominated by C_{37:4} (e.g., Pyramid Lake, U.S.) and some dominated by C_{37:3} (e.g., Upper Murray, Canada).

3.2.2 Isochrysidales Group II

Group II contains a variety of phylotypes from lakes in Greenland, U.S., Antarctica, and China (Theroux et al. 2010), from which phylotypes OTU7 and OTU8 have been isolated in enrichment cultures (Toney et al., Chapter 4) and OTU7 has been isolated from a single haptophyte cell into unialgal cultures (Toney et al., Chapter 5). Group II also contains cultured *Chrysotila lamellosa*, *Pseudoisochrysis paradoxa*, *Isochrysis litoralis*, *Dicrateria* sp., and

Isochrysis galbana. Of the previously cultured species, *C. lamellosa*, *P. paradoxa* (reported here), and *I. galbana* are known to produce alkenones. We have not found reports of alkenone production in the literature for *Dicrateria* sp. and *I. litoralis*; however, *I. litoralis* is generally accepted as the flagellate stage of *C. lamellosa* (Green and Pienaar, 1977), so in some life stage it does produce LCAs, if not in the flagellate stage. Interestingly, Patterson and others (1994) report that six strains of *Isochrysis* show qualitative differences in long-chain alkenone profiles; two do not produce LCAs, and two different strains of same species, *I. galbana*, show fundamentally different LCA compositions. While all LCAs produced from unialgal cultures in Group II are dominated by C_{37:3} (aside from new manipulated cultures of OTU7, see Chapter 5), the environmental sedimentary LCA profiles are a mix of characteristic profiles, some dominated by C_{37:4} (e.g., Lake George, U.S.) and some dominated by C_{37:3} (e.g., Tso Ur, China) (Theroux et al. 2010). Toney et al. (Chapter 5) show that different LCA profiles can consistently be associated with a distinct life stage, which begs the question: From which life stage were LCAs analyzed previously? Here, the *P. paradoxa* profiles were collected from flagellate cells (Farmer, 1993) and stationary phase (this study) and both contain LCAs. Additional work is necessary to determine if the lipid profiles previously reported from various members of the Isochrysidales are associated with a

particular life stage and if manipulation of previously reported strains can induce life stage changes and correspondent lipid profiles.

3.2.3 Isochrysidales Group III

Group III contains the well-known marine haptophyte strains *Emiliania huxleyi* and *Gephyrocapsa oceanica*, and another coccoid haptophyte phylotype (CCMP625) not yet described. The coccoid haptophyte is distinct visually from *E. huxleyi*, but has an identical gene sequence and is thus, a new life stage (RAA unpublished data). Lipids have not been analyzed directly from this life stage of *E. huxleyi*, so the LCA lipid profile is not known. *E. Huxleyi* and *G. oceanica* produce C_{37:3}-dominated profiles. It is common for manipulation of cultures (i.e., temperature, salinity, nutrients) to take place in the absence of visualization via microscopy, so little is known about the triggers between haptophyte life stages and with which life stage the reported profiles are associated (i.e. Prahl et al. 2003; Toney et al., Chapter 4). These visualizations are key in future studies for developing a deeper understanding of LCA production and export to the sediments, for example, Toney et al. (Chapter 5) show that for a particular lake, although both the flagellate and colonial life stages exist in the water column throughout parts the year, only the colonial stage lipids are exported to the sediments.

4. CONCLUSION

Here we report that *Pseudoisochrysis paradoxa*, a close relative of lacustrine species *Chrysotila lamellosa* and recently reported lacustrine phylotype OTU7, produces alkenones with similar C_{37:3}-dominated profiles to its relatives, although the C₃₈ homologues vary as a result of culture conditions and likely growth stage. The capability of *P. paradoxa* to produce alkenones suggests that in environmental settings, where it is visually or genomically detected, it may contribute to the sedimentary alkenones. With this in mind, future manipulation of pure cultures and resulting analysis of temperature dependency of alkenone unsaturation would be useful. Furthermore, *P. paradoxa* is the closest relative to Hap-B (OUT7, Theroux et al., 2010), which reportedly produces both C_{37:4} and C_{37:3} dominated profiles (Toney et al., see Chapter 6), and is thus a key species for testing whether different other haptophyte taxa produce different alkenone profiles during different life stages.

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Figure 1. Long-chain alkenone lipid profile from (A.) *Pseudoisochrysis paradoxa* (CCMP715) and (B.) digital trace of *Pseudoisochrysis paradoxa* (unpublished thesis, Farmer 1993). [Methyl ketones = a ($C_{37:4}$), b ($C_{37:3}$), c ($C_{37:2}$), g ($C_{39:3}$); Ethyl ketones = e ($C_{38:3}$), f ($C_{38:2}$); Methyl esters = d ($C_{37:3}$); Ethyl esters = h ($C_{38:4}$), i ($C_{38:3}$), j ($C_{38:2}$); peak labeled “?” appears to be an impure ethyl ketone $C_{38:3}$ (m/z 542, -29) peak].

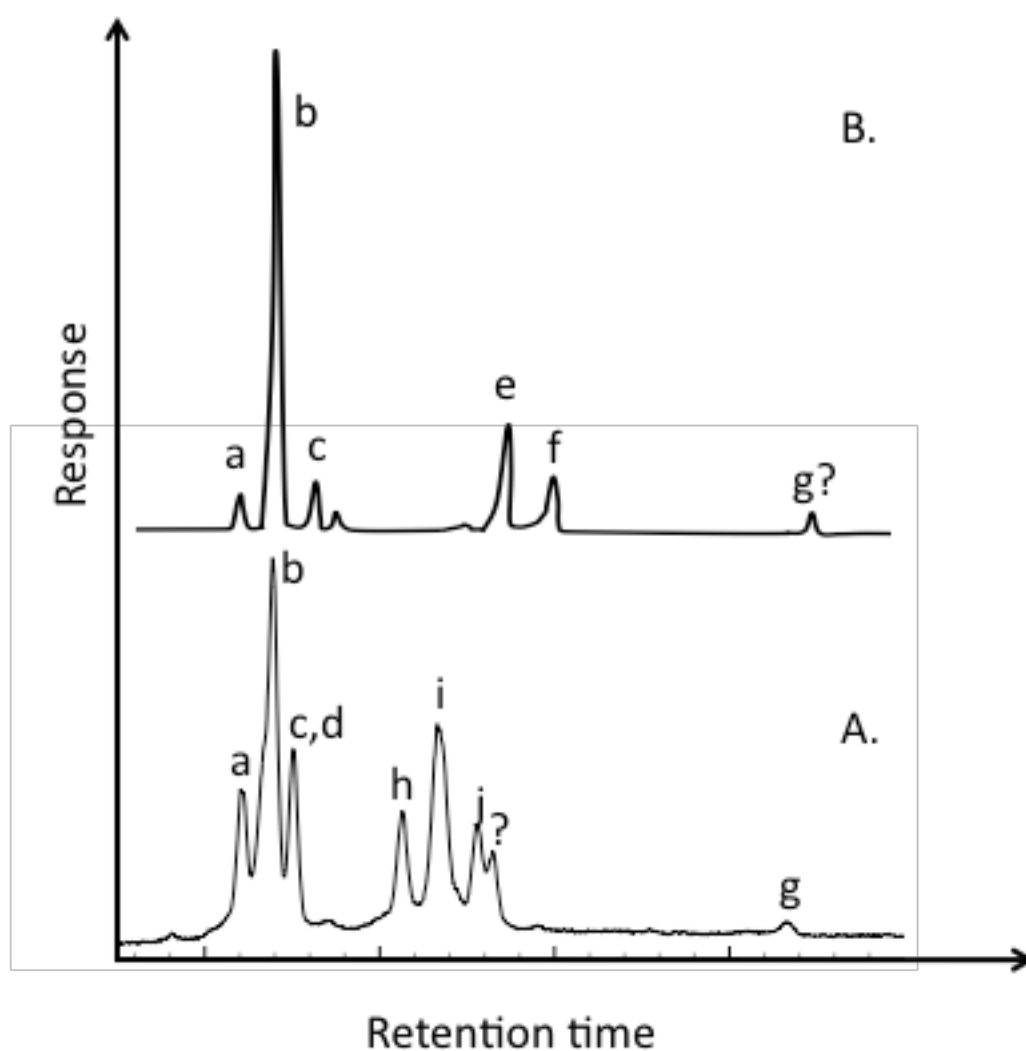


Table 1. A comparison of lipids detected from two different cultures of *P. paradoxa*.

Species strain:		<i>P. paradoxa</i> (CCMP715)	<i>P. paradoxa</i> (Farmer 1993)
a	C37:4 methyl ketone	7.2	x
b	C37:3 methyl ketone	22.8	65.2
c	C37:2 methyl ketone	x	5.6
d	C37:3 methyl ester	x	nd
e	C38:3 ethyl ketone	3.8	11.3
f	C38:2 ethyl ketone	nd	6.7
g	C39:3 ethyl ketone	nd	x
h	C38:4 ethyl ester	7.9	nd
i	C38:3 ethyl ester	16.4	nd
j	C38:2 ethyl ester	5.8	nd

Note: x = not quantified, nd = not detected

Letters (a-j) correspond to peaks in Fig. 1

CHAPTER 7

HOLOCENE SPRING TEMPERATURE OF NORTHERN GREAT PLAINS: INSOLATION AND OCEANIC INFLUENCES

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ABSTRACT

Long-chain alkenone (LCA) unsaturation indices (U_{37}^K) from two lakes in the Northern Great Plains (NGP), U.S., provide a proxy for spring temperature for the past 9.4ka. The records from two sites 300 km apart show consistent trends, indicating that they represent regional temperature. The long-term trend is forced indirectly and directly by precession through changes in seasonality (lengthening of spring). The presence of the Northern Hemisphere ice from 9.4 to 7.0ka suppressed temperatures in the NGP leading to corresponding changes in N. Atlantic Deep Water formation inferred by magnetic minerals off the Icelandic margin. After 5.0ka the millennial-scale spring temperature exhibits similarities to sea surface temperatures in the W. tropical Pacific Ocean, suggesting a major influence of ENSO related processes.

1. INTRODUCTION

Climate model projections of future climate change show unequivocal warming of global temperature (IPCC 2007), yet regional predictions are uncertain for many parts of the earth, including the Northern Great Plains (NGP), U.S., a major agricultural area. New long-term records of continental temperature and precipitation are crucial for developing a mechanistic understanding of likely ranges of future climate response.

Advances in continental paleoclimate are hindered by the lack of high-resolution temperature reconstructions. Extant proxies are influenced by multiple factors (e.g., chironomids respond to salinity, temperature, hydrology, and nutrients (Porinchu and MacDonald, 2003)). In the NGP speleothem $\delta^{18}\text{O}$ and tree-ring records are rare or discontinuous, so lakes provide the best continuous archive of climate change. In-lake proxies are well developed for drought (e.g. diatom- and ostracode-inferred salinity (Xia et al. 1997; Fritz et al. 2010)), but pre-instrumental temperature proxies are scarce. Organic biomarkers, e.g., TEX₈₆ have been used to reconstruct sea-surface temperature (SST) (Schouten et al., 2002; Kim et al., 2008; Rueda et al., 2009), but appear to be only applicable in a limited number of large lakes (Powers et al., 2004; Tierney et al., 2008; Sinninghe Damasté et al., 2009). Other indices (e.g. MBT and CBT ratios) derived from soil bacteria GDGTs (Weijers et al., 2007) are correlated to soil temperature and pH, but their

applicability in lakes is complicated by in-lake production of GDGTs (Tierney and Russell, 2009).

Long-chain alkenones (LCAs) have been successfully used as a SST proxy for decades (Müller et al. 1998; Lawrence et al. 2005) and are found in lakes around the world (Cranwell, 1985; Zink et al. 2001; Sun et al. 2007). LCAs record lake water temperature (e.g., Zink et al. 2001; Sun et al. 2007, Toney et al. 2010) and are produced by haptophyte algae that occur in saline lakes in the NGP (Toney et al. 2010). LCAs are only produced in lakes and do not have the complications of terrigenous versus aquatic input as tetraethers do. Toney et al. (2010) developed a linear calibration between alkenone unsaturation (U_{37}^K index, Brassell et al., 1986) and *in situ* water temperature at Lake George (LG), ND. In this system, alkenone-producers grow following ice-off in early May. Populations rise to the lake surface and decline by late June with the onset of thermal stratification. Therefore, the U_{37}^K index at LG represents spring surface-water temperature between ice-off and thermal stratification. Here we apply the LCA calibration to a Holocene-length record from LG³ to develop the first quantitative spring temperature reconstruction from the NGP. We also assess links to orbital-scale insolation forcing and Atlantic and Pacific climate variability over the past 9.4ka.

³ Supplemental information (Figure DR1)

2. SITE INFORMATION, SAMPLES AND METHODS

Lake George (46.74°N, 99.49°W) is a closed-basin, kettle lake in glacial drift underlain by the Hell Creek, Fox Hills and Pierre formations of the Upper Cretaceous (Whitehead, 1996). The Pierre shale is rich in sulfate from the oxidation of pyrite, and LG has a modern sulfate concentration of 7370 mg/L. LG is surrounded by mixed-grass prairie (Barker and Whitman, 1989) and has a maximum depth of 60-m.

A 13-m core was collected from a broad shelf at 14-m depth in 2009 with a Wright square-rod piston corer (Wright et al. 1984). Nine AMS radiocarbon ages on charcoal provide chronological control (Table 1). The mixed-effect regression method developed by Heegaard et al. (2005) was implemented in R version 2.11.0 ©2010 to create an age model.⁴ Sedimentation rates were $\sim 0.12\text{-cm yr}^{-1}$ prior to 3ka and $\sim 0.23\text{-cm yr}^{-1}$ post-3ka. Samples every 8-cm post-3ka and every 4-cm prior to 3ka yield an average sample spacing of 40-years. Organic analyses were performed using derivitization schemes after Huang et al. (2002).⁵ The derivatized alkenone (DCM) fraction was rerun on the GC-FID.

⁴ Supplemental information (Figure DR2)

⁵ Supplemental information (Methods Text)

Temperature (T) is inferred from the U_{37}^K calibration using a linear regression model ($T = 48.4982 \times U_{37}^K + 42.1494$). This relationship was developed using an *in situ* relationship derived specifically for the LCAs of LG (Toney et al. 2010). In this study we remove two outliers following jackknife analysis using JMP® 8.0.2, which improves the statistical reliability ($r^2 = 0.82$, $P < 0.0001$), with a RMSE of 2.4°C.⁶

3. ALKENONE TEMPERATURE PROXY: VALIDATION AND SIGNIFICANCE

LCAs in the modern surface sediments record an inferred-T of 11.2°C, which corresponds with the surface water temperature (11.8°C) during the time of highest LCA concentrations ($\sim 280 \mu\text{g L}^{-1}$) (May, 28, 2008).⁷ Furthermore, regression of air temperature with weekly surface-water temperature (April to August 2008) shows a linear relationship ($T_{\text{air}} = 1.2944 \times T_{\text{lake}} - 0.5968$; $r^2 = 0.97$)⁸. The *in situ* calibration is applied down-core to infer temperature for the past 9.4ka (Fig. 1). The pattern of variation in LG LCA-inferred temperature (LGT) is similar to the LCA-inferred-T from Brush Lake, MT (300 km NW of LG)¹ (Fig. 1), which confirms that the LGT depicts regional temperature trends. Climate parameters that may influence lake temperature at the timing of maximum LCA production include: 1) changes

⁶ Supplemental information (Figure DR3)

⁷ Supplemental information (Figure DR4)

⁸ Supplemental information (Figure DR5)

in the rate of spring temperature increase due to insolation and radiative forcing; and 2) the length of the window during which LCA production occurs including: blooms later into the summer due to changes in the timing of lake thermal stratification (ex. by insolation or enhanced wind mixing).

Noteworthy centennial-scale deviations in U_{37}^K at LG equate to anomalously high temperatures. Eleven of our 240 data points produce temperatures above 22°C, are outside the calibration range, and are treated as outliers following jackknife analysis (Fig. 2). These changes in U_{37}^K may indicate that (1) the calibration is not linear at high temperatures, (2) late stratification may extend the duration of the bloom later into the summer and record higher temperatures, or (3) another environmental parameter (e.g., salinity, nutrients, $\text{SO}_4:\text{CO}_3$) may affect the U_{37}^K or the species composition of LCA producers in the lake.

4. HOLOCENE TEMPERATURE: INSOLATION AND ICE SHEET FORCING

The LGT record (Fig. 1) indicates that the cool spring temperatures in the early Holocene were followed by gradual spring warming (increased spring duration) starting at 7.8ka. Prolonged spring continued until 4.8ka, followed by a relatively stable long-term mean spring temperature from 4-1ka, with variability around the long-term mean. After 1ka, the rate of warming

increased to 0.2°C per century. The long-term LGT increases over the past 9.4 kyr by ~4°C (Fig. 1) indicating that spring temperatures increased or the haptophyte bloom continued longer into the season. However, spring insolation (47°N) declines over the Holocene and cannot directly explain the trend in LGT (Fig. 2).

We propose that the long-term trend of LGT is controlled by total spring insolation (rather than maximum insolation). In addition, the presence of Northern Hemisphere (NH) ice also suppressed LGT between 9.4 and 7.8ka (Fig. 2). Total insolation received by the lake surface is determined by the number of days between ice-out and thermal stratification, which was shorter in the early Holocene (14d versus 27d). Using a simple conceptual model, we show that the daily insolation totaled over the days that favor the haptophyte bloom increases over the Holocene due to contrasting seasonality. We assume that ice-out and thermal stratification respond over long time-scales to insolation forcing. Modern ice-out occurs when daily insolation values reach 439 W/m² (May 9), and the onset of thermal stratification occurs when daily insolation reaches 477 W/m² (June 5). We use these insolation values as constraints on the number of days favorable for the spring bloom at 1kyr intervals from 10ka to present (e.g., the bloom occurs from May 9th to June 5th, which is 27d, the sum of daily insolation over these 27d equals total insolation during the spring bloom window). The trend in total insolation

during the bloom window increased from ~6000 to 12000 W/m² (Fig. 2). This trend toward prolonged spring can sustain the bloom longer, and it equilibrates to warmer surface water conditions. Before 8.2ka, the anticyclone associated with the NH ice sheet likely contributed to reducing spring temperature by restricting warm air from the Gulf of Mexico from entering the NGP (COHMAP, 1988).

Superimposed on the long-term trend are millennial and centennial changes. Some periods have an 8°C shift over 30-yrs, which is reasonable given the instrumental avg. May temperature commonly deviates up to 6°C from year to year. Cool intervals interrupt the long-term warming from 5.7-5.5ka, 4.7-4.4ka, 3.2-3.0ka, at 2.0ka, and from 0.6-0.45ka, at the onset of Little Ice Age (LIA).

5. COMPARISON WITH REGIONAL CLIMATE RECORDS

LGT shows general agreement with long-term trends in temperature inferred from other regional records (Wright et al. 1992, COHMAP 1988). The full development of the prairie occurred later from 9.4-7.9ka (Wright et al., 2004), and wet conditions at Coldwater Lake and Moon Lake, ND gave way to increased salinity by ~9.0ka (Laird et al. 1996; Xia et al. 1997; Fritz et al. 2000). The trend toward warmer LGT, began at 7.8ka, however, the LG core bottomed in a thick sand layer that indicates a major lake drawdown at

~9.4ka⁹, hence a drought exceeding any since that time. We did not recover this interval, so LGT associated with this event is not known. Recent geochemical and pollen work at Kettle Lake, ND (Grimm et al. accepted) indicates that this was the most severe drought of the Holocene (~9.25ka). LGT records spring temperature, not drought, thus evidence for maximal spring temperatures after 7.8ka is not in conflict with evidence for lowest lake levels and maximal drought ~1.5kyr earlier. Lake levels likely respond to annual or winter precipitation, not spring temperature. A subsequent short excursion in LGT at ~7.4ka is correlated with independent evidence for regional warming from 7.4 to 7.2ka by the presence of *Iva annua* (Marsh elder) pollen at neighboring Moon Lake (Laird et al., 1996).

The long-term trend and variability about the mean are similar to the total carotenoid reconstruction from Elk Lake, MN (Fig. 3)(Sanger, 1988). The carotenoids infer fluctuations in productivity and cyanobacteria community structure, and concentrations are high during episodes of spring warmth (prolonged spring) inferred from LCAs. Subsequent stabilization of LGT after 4.8ka correlates with an abrupt and regionally coherent shift to less arid conditions post-4.5ka (Laird et al. 1996; Xia et al. 1997, Dean et al. 2002). Regional drought records are less coherent after ~3.5ka.

⁹ The error associated with this age at Lake George is ± 19 years; however, it is possible that it is the same event reported by Grimm et al. (submitted) at 9.25ka.

The majority of highly resolved records of NGP drought span the last 2ka, during which a positive association between drought and LGT is evident. The Medieval Climate Anomaly (MCA) occurred from 1.0 to 0.65ka, when Europe, Greenland, and Asia showed evidence of warmer temperatures (NRC, 2006). At LG elevated spring warmth occurs from ~1.0 to 0.2ka with a cold excursion from ~0.6 to 0.45ka, which may be an expression of the LIA. A synthesis of regional drought records (Yu et al. 2002) suggests that the MCA contained two dry events, MCA1 and MCA2, which coincide with high temperature events at 1ka and 0.6ka at LG and suggests that temperature-induced evaporation may have enhanced the severity of these droughts. It is also possible that the changes in wind strength and direction (Sridhar et al. 2006) during the MCA in the NGP could have extended the LCA bloom into the summer season.

6. INFLUENCE OF THE LAURENTIDE ICE SHEET AND THE NORTH ATLANTIC

Common broad-scale patterns are evident in records from the N. Atlantic Ocean for the past 9.4ka and may impact LGT (e.g., Rousse et al. 2006) (Fig. 4a,b). Variability superimposed on the long-term LGT trend matches variability in the anhyseric magnetic resonance (ARM) record from the N. Atlantic and a proxy for N. Atlantic Deep Water Formation, including enhanced variability between ~7.8 and ~4.8ka. After 3.0ka the correlation

becomes less apparent. Both LGT and ARM show stable early Holocene climate conditions with low variability relative to post-6ka. These sites are both sensitive to anticyclonic flow around the NH ice sheet. This atmospheric configuration suppressed LGT, while also affecting sea-ice formation through stronger northwesterly winds in the N. Atlantic (COHMAP 1988). These patterns show strong support for a link between NH ice and suppression of LGT during the early Holocene. The effects of NH ice on atmospheric circulation complement the reduction in the length of the spring, which are tied to precessionally driven orbital changes. Whereas, we posit that spring temperatures in the NGP were influenced by the size of NH ice in the early Holocene, Grimm et al. (accepted) suggest that early Holocene drought was more influenced by the size and proximity of Lake Agassiz (Teller and Leverington, 2004). Thus, during the early Holocene, temperature and precipitation may have been decoupled and responded differently to the ice sheet, proglacial lake, and insolation forcings.

7. COMPARISON WITH PACIFIC PROCESSES

As the N. Atlantic correlation fades in the mid-Holocene, a strong similarity between patterns of multidecadal LGT variability and a SST record from the Indonesian through-flow region (Stott et al., 2007) starting ~7.0ka suggests that the tropical Pacific plays an increasingly important role in the heat distribution to the interior U.S. (Fig. 4c). Reduced SST and salinity

correspond to warmer LGT. This Pacific site cools during El Niño events, when the heat is distributed across the equatorial Pacific, which is consistent with modern observations of increased spring heating of our site at the end of an El Niño year (Green et. al 2007). While the resolution of the records prohibits inter-annual comparisons, the direction of change supports the Stott et al. (2004) hypothesis that reduced SST and salinity alters atmospheric convection and allows for greater heat transport to the mid-latitudes. These patterns suggest that after 7.0ka the NGP became more strongly influenced by Pacific climate drivers, such as ENSO.

8. CONCLUSIONS

Here we generated the first Holocene lacustrine, LCA-inferred temperature record for the interior U.S. The Holocene temperature trend is consistent with earlier ice-out and later onset of thermal stratification in LG and shows the strong influence of seasonality on spring conditions in the NGP. The results provide new support for the importance of the NH ice on NH climate and links between the N. Atlantic climate and NGP prior to 4.8ka. The reconstruction shows a shift in boundary conditions after 5.0ka and suggests that teleconnections with the tropical Pacific became important around the same time that ENSO purportedly intensified (McGlone et al. 1992; Rodbell et al. 1999; Moy et al. 2002).

Spring is a key time for agricultural decision making in the NGP. Here we show that changes in seasonality (the lengthening of the spring season) during the Holocene strongly impact the regional temperature. We show that spring temperature was elevated during the MCA droughts and may have enhanced the drought severity. Because the NGP is sensitive to winter temperature change (e.g., 1.1°C winter warming per decade over the past 30- yrs), the link between decreased seasonality and spring temperature exhibited here holds potential consequences for agricultural production and warrants further investigation.

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Table 1. Radiocarbon data used for age model.

DBLF* (cm)	Date type	Material dated	NOSAMS Accession #	¹⁴ C Age	¹⁴ C Age Error	Age (cal BP) ^b	Cal. Age Error
20 ^a	²¹⁰ Pb	x	x	x	x	18	x
66	AMS ¹⁴ C	Charcoal	OS- 79421	480	30	524	10.5
185	AMS ¹⁴ C	Charcoal	OS- 74739	675	30	664	11
338	AMS ¹⁴ C	Charcoal	OS- 74740	1320	30	1279	18
490	AMS ¹⁴ C	Charcoal	OS- 79422	1890	30	1854	34
678	AMS ¹⁴ C	Charcoal	OS- 74741	2800	55	2911	61
790	AMS ¹⁴ C	Charcoal	OS- 79423	3580	30	3908	74.5
974	AMS ¹⁴ C	Charcoal	OS- 74742	4770	50	5521	45
1165	AMS ¹⁴ C	Charcoal	OS- 74861	6150	40	7059	31
1297	AMS ¹⁴ C	Charcoal	OS- 79424	8450	50	9488	20

*Depth Below Lake Floor (DBLF)

^aThis point is tied to a ²¹⁰Pb dated chronology from the short core analyzed by Dan Engstrom.

^bCalibrated using Calib 5.0.2 (Reimer et al. 2004), ages reported in years before 1955 AD.

Figure 1. LCA-inferred temperature for Lake George, ND (black) plotted with LCA-inferred temperature from Brush Lake, MT (gray) showing similar patterns from 2-9.0ka. Circles indicate outliers that have been removed from the LG dataset.

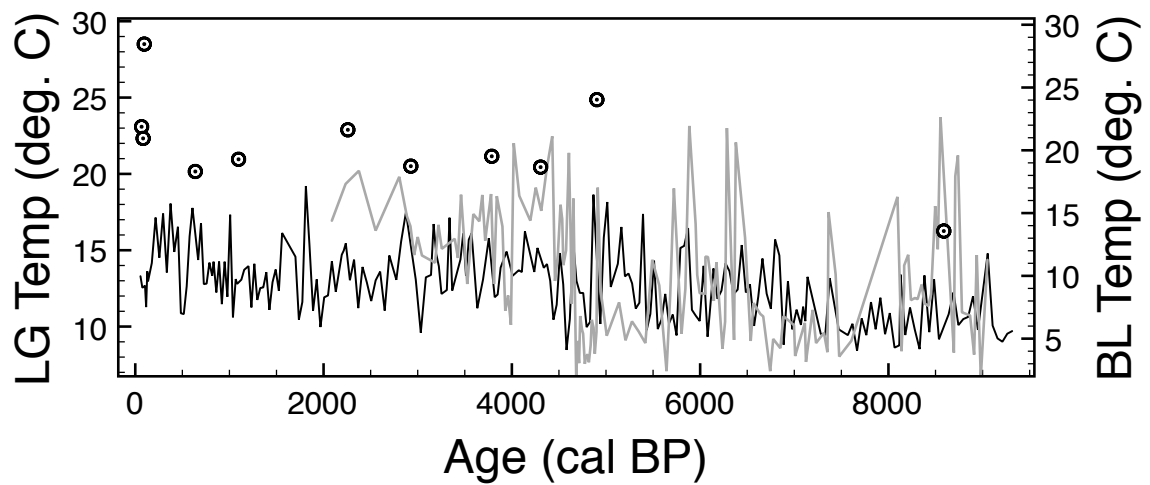


Figure 2: (a.) Total northern hemisphere ice area (Paterson, 1972) versus LGT (black line), (b.) the total insolation (thick black line) during the spring window favorable for the haptophyte bloom based on daily insolation values (439 Wm^{-2} = ice-out, 477 Wm^{-2} = onset of stratification) versus the LGT (thin black line), (c.) May insolation.

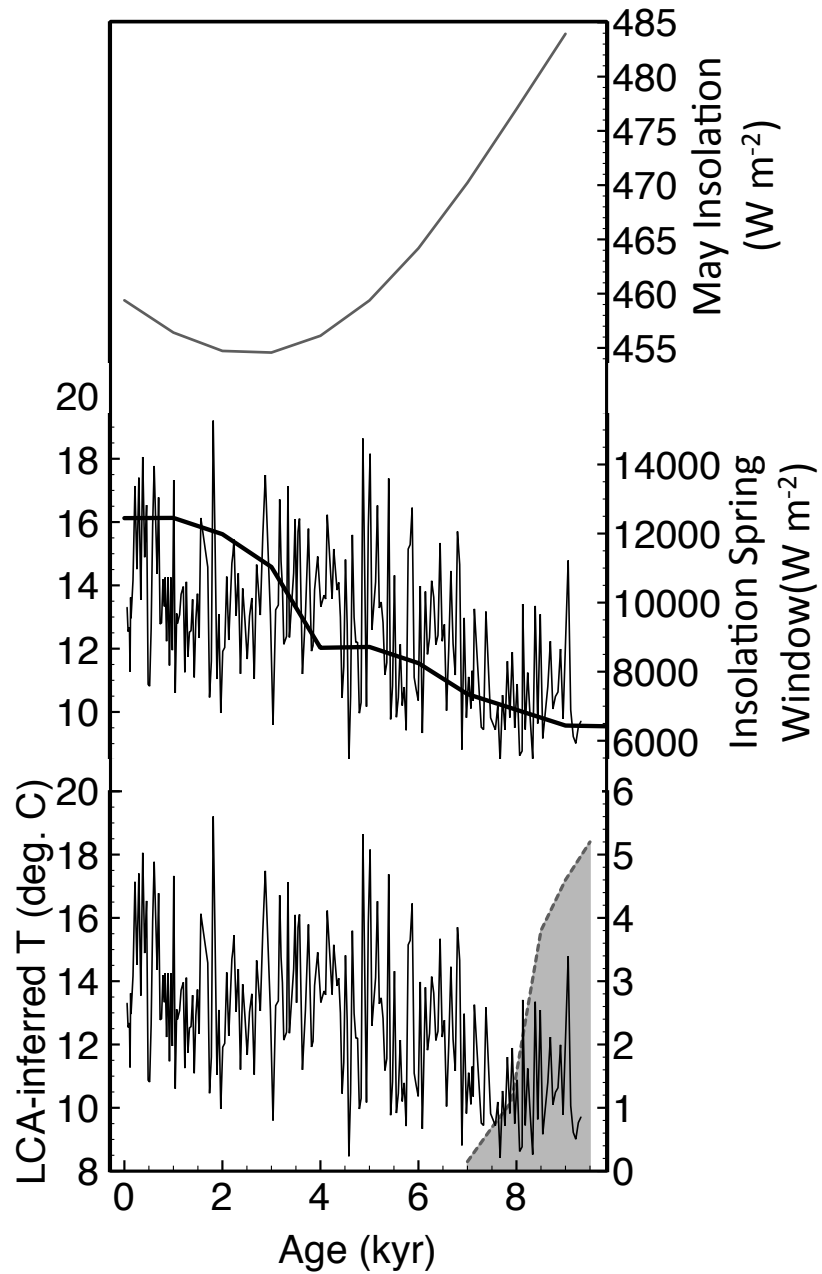


Figure 3. Lake George LCA-inferred temperature (black) plotted with the total carotenoids (gray) from Elk Lake, MN (Sanger et al. 1988). Note: Wright et al. (2004) suggest that there may be errors associated with the chronology for Elk Lake.

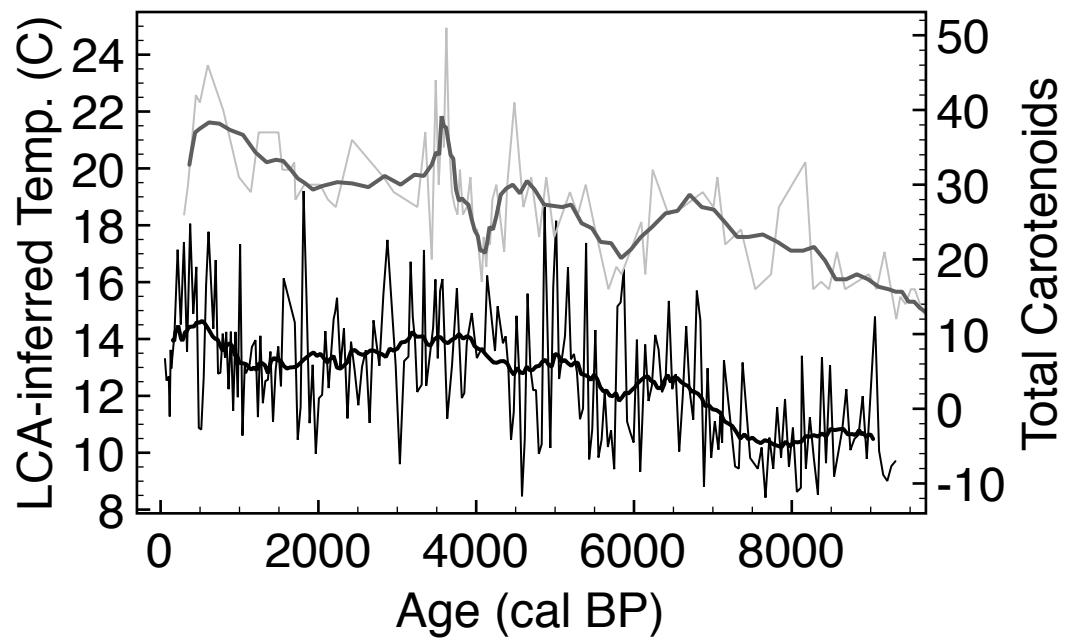
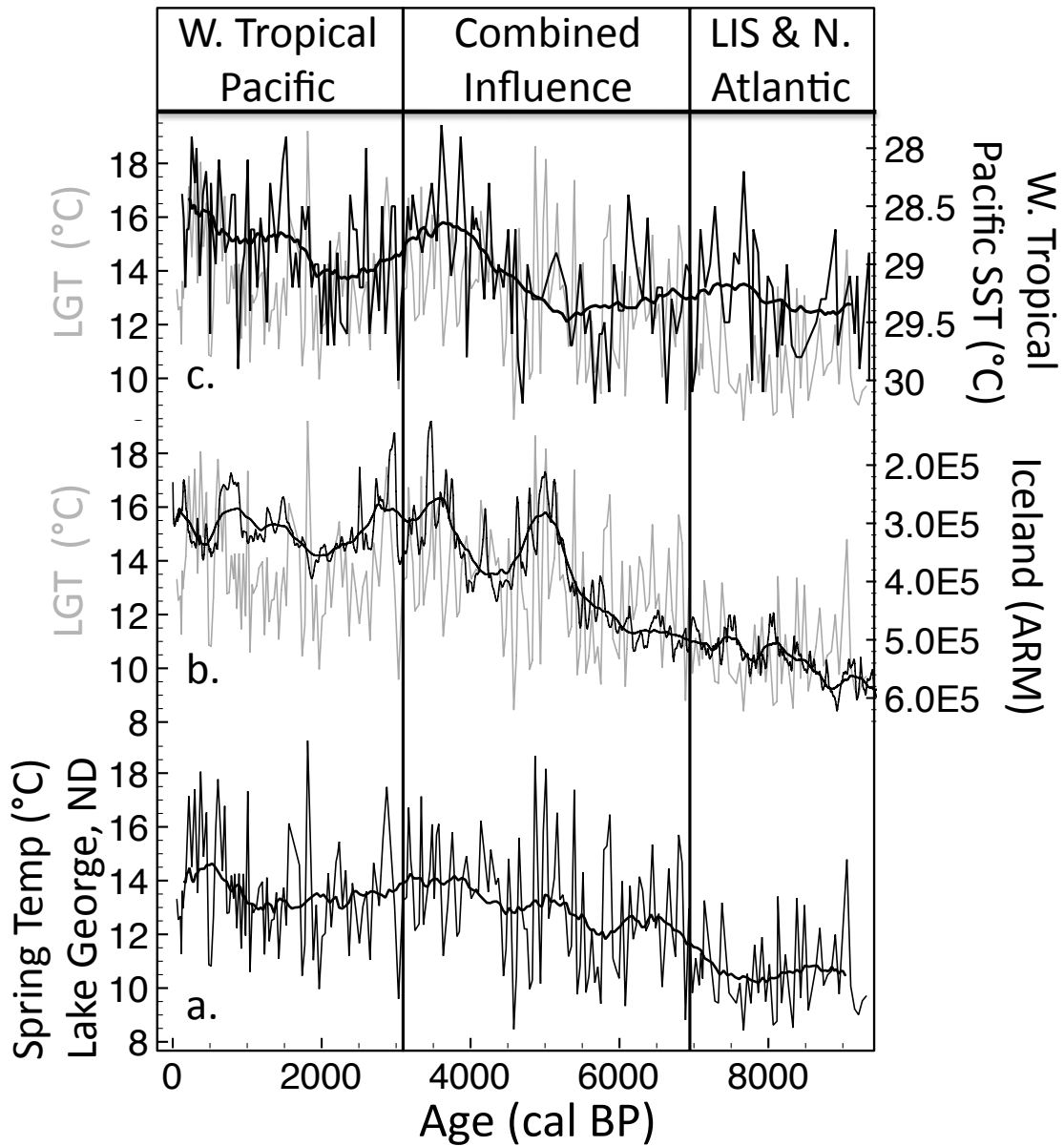


Figure 4. (a.) LCA-inferred temperature from LG, ND with 10-point smoothing, (b.) Anhysteretic magnetic remanence from the Northern Icelandic Margin (Rousse et al. 2006), (c.) SST derived from the Mg/Ca of planktonic foraminifera from the W. Tropical Pacific (Stott et al. 2004). Note: LGT is plotted in gray (b,c) to promote high-resolution comparison.



SUPPLEMENTAL TEXT

Sediment and filter samples (~1 g) were freeze-dried, homogenized, and extracted with dichloromethane (DCM):methanol (MeOH) (9:1, v/v) via an Accelerated Solvent Extractor ASE200 (Dionex). C₃₆ *n*-alkane standard was added, and the total lipid extracts (TLEs) were run on a GC-FID for detection and quantification of alkenones using the internal standard. All samples were further separated into neutral and acid fractions by elution through a LC-NH₂SPE column using DCM:isopropyl alcohol (2:1, v/v), followed by ether with 4% acetic acid (v/v) (Huang et al., 2002). The neutral fraction was separated into the aliphatic (*n*-hexane), alkenone (DCM) and polar (MeOH) fractions using silica gel column chromatography.

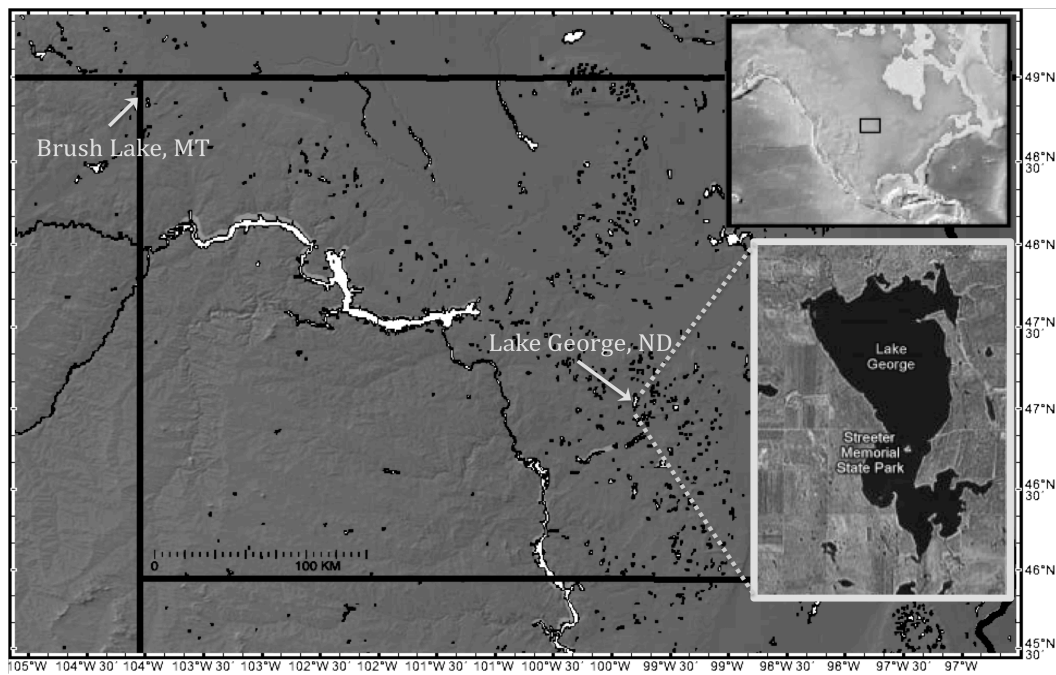
An Agilent DB-1 GC column (60m × 320μm × 0.10 mm) was used with the following temperature program: an initial temperature of 40°C (hold 1 min), ramp 30°C/min to 290°C (hold 1 min), ramp 5°C/min to 300°C (hold 0 min) then 2°C/min to 325°C, GC temperature program (hold 10 min)(Toney et al. 2010). The same temperature program was used on the GC- MS, and samples were run to confirm the identity of the alkenones using the known ion chromatograms and by comparison of mass spectral data with published data and GC retention times (de Leeuw et al.,1980, Marlowe et al.,1984). The final GC program described here is the result of many permutations of GC parameters, including the switch from helium as the carrier gas to hydrogen.

As such we are able to resolve LCAs with $>4\mu\text{g}$ per sample (i.e. grams of dry sediment or liter of water). Alkenone standards of known temperature calibration are run on the GC-MS to ensure analytical precision ($< 0.1^\circ\text{C}$).

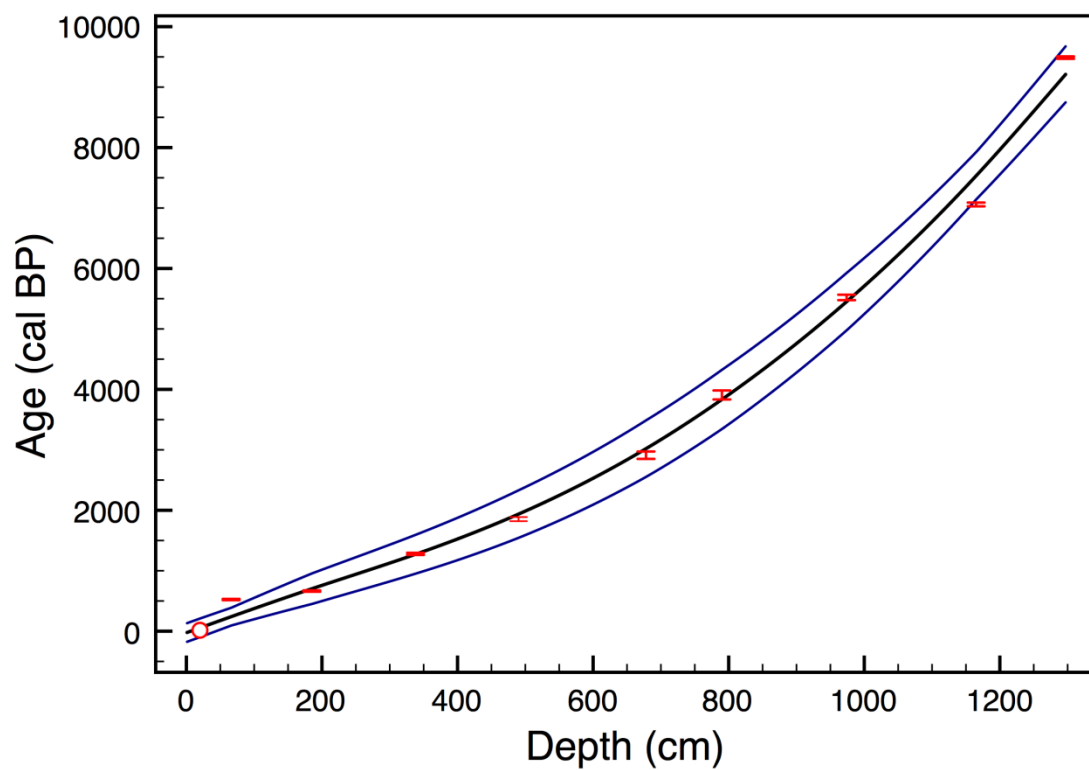
The variance of the dataset is 5.3°C , and the standard deviation is 2.3°C , so variability of the data is within the error of the calibration. Clear patterns exist in the data that are reproduced in a second site, Brush Lake, MT.

Insolation values presented here were calculated using the Matlab routine generated by Huybers et al. (2006) for 47°N latitude

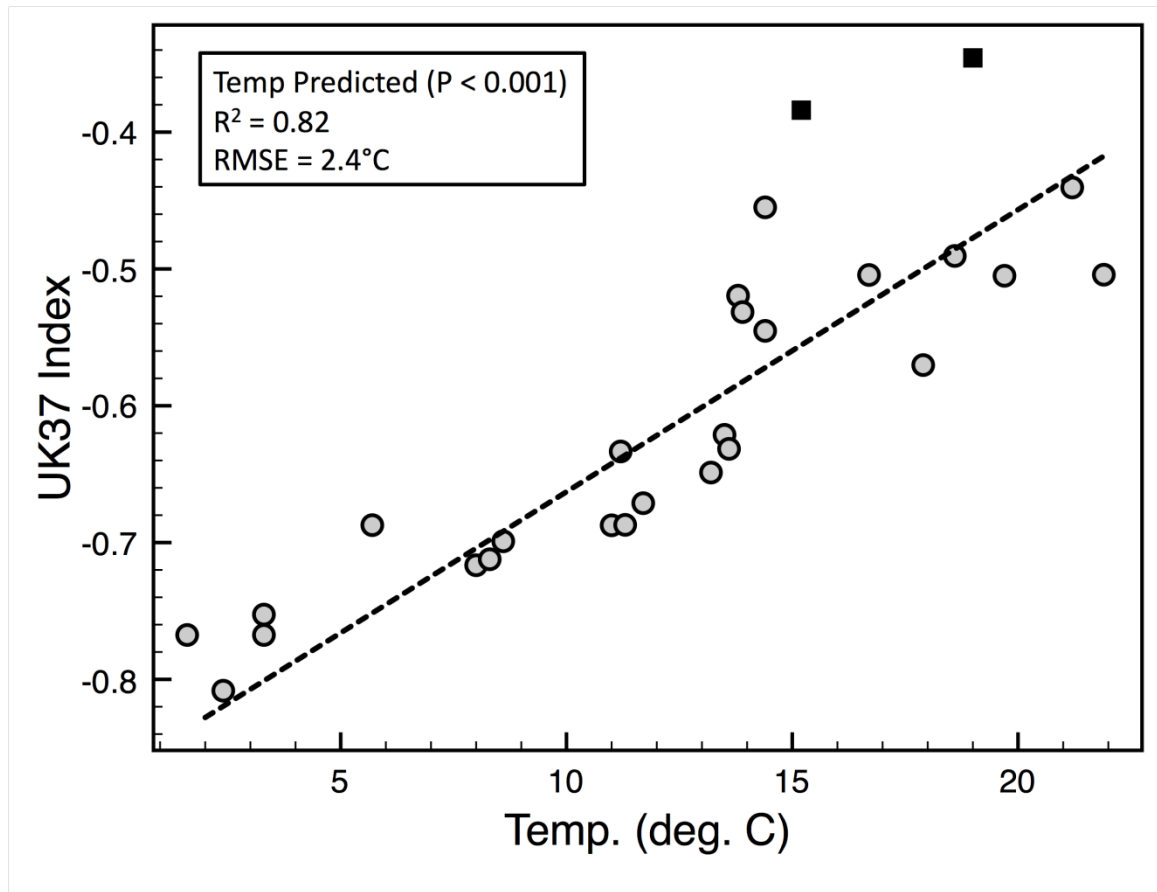
Supplemental - Figure DR1. Map showing the location of Lake George, ND (46°74', 99°49') and Brush Lake, MT (48°60', 104°11'). Lake and rivers are indicated by a black outline and white infill. The map was created using GeoMapApp Software © 2010 and Global Multi-resolution Topography data provided by Ryan et al. (2009).



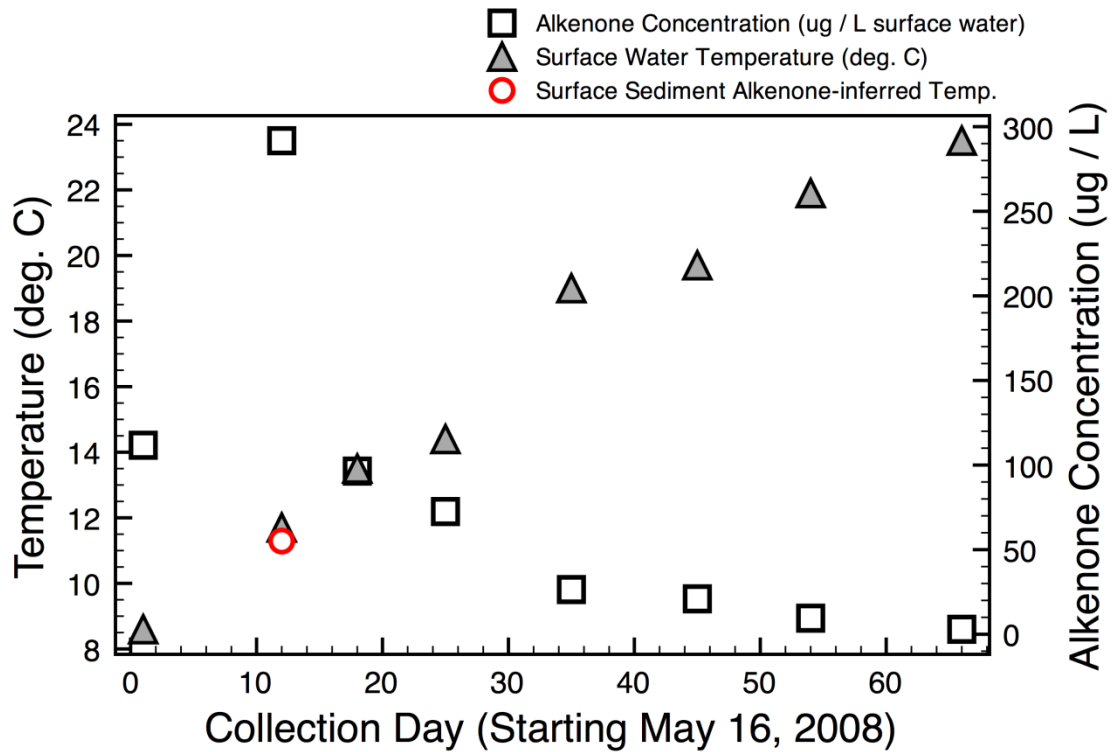
Supplemental – Figure DR2. Age model for Lake George, ND based on the nine AMS ^{14}C dates provided in Table 1. The age model was calculated using the Cagedepth and Cagenew functions developed by Heegaard et al. (2005) with the statistical software R version 2.11.0 ©2010



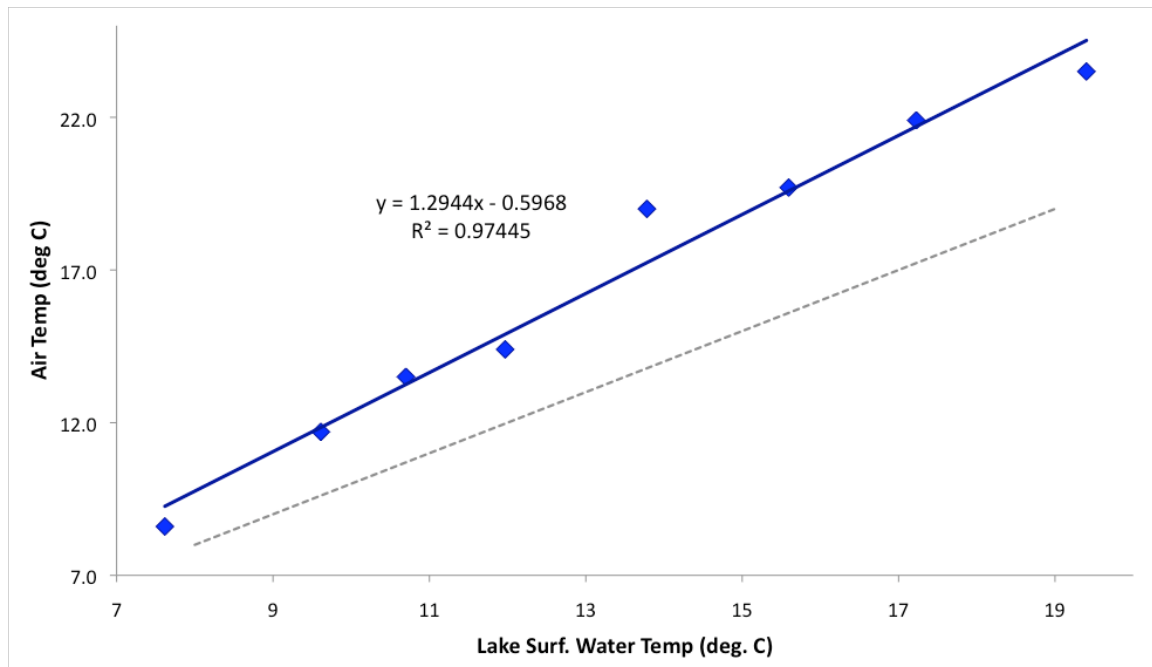
Supplemental - Figure DR3: The gray-filled circles show U_{37}^K index calculated from individual measurements of alkenones from water column filters plotted versus the *in-situ* water temperature (data from Toney et al. 2010). The black squares represent outliers that were removed following jackknife analysis. The regression is significant ($P < 0.001$) with a predictive error of $\pm 2.4^\circ\text{C}$.



Supplemental - Figure DR4: Alkenone concentration (squares) peaks on May 28, 2008 in the surface water of Lake George, ND. The alkenone-inferred temperature from surface sediments collected the previous summer record a temperature of 11.2°C (circle), which is on par with the surface water temperature (shaded triangles) of 11.7°C at the time of the surface bloom.



Supplemental - Figure DR5: Modern relationship between air temperature (data from CGREC) and measured lake surface water temperature for 2008 AD.



CHAPTER 8

INFLUENCE OF TROPICAL PACIFIC CLIMATE ON SUMMER DROUGHT IN THE NORTHERN GREAT PLAINS STRENGTHENS OVER THE HOLOCENE

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ABSTRACT

The severe and sustained droughts of the 1930s devastated the Great Plains, a major agricultural region of North America, leaving a long lingering question of how frequently and why such extreme events occur. Existing proxy records from regional climate archives are ambiguous and present a perplexing picture for formulating long-term management decisions. Here we demonstrate that hydrogen-isotope ratios (δD) from leaf waxes from the sediments of Lake George, North Dakota record growing season droughts, such as those during the Dust Bowl period. Our leaf wax δD record for the Holocene is highly consistent with regional hydrological records from lakes and the Gulf of Mexico. High-resolution (decadal to multidecadal) analyses indicate that growing season droughts with the severity of the 1930s Dust Bowl are relatively uncommon throughout the Holocene. We also show that growing season droughts in the NGP often correspond to periods when moisture is confined to a more southerly position in the southeastern U.S. There are also significant correlations between summer precipitation in the NGP and the intensity of both El Niño and La Niña.

The Northern Great Plains (NGP), U.S. is an important agricultural region accounting for 13% of global wheat production and 60% of U.S. livestock production. NGP agricultural yield is dependent upon spring and summer rainfall, where deficits of 27% can lead to a 75% drop in production. Because the region is situated near the northern limit of summer rainfall sourced in the Gulf of Mexico (GOM), rainfall fluctuates greatly from year-to-year and is highly sensitive to climate perturbations. Understanding drought occurrence is essential, especially for severe droughts like the 1930s Dust Bowl. This infamous drought extended to the whole interior North American continent and was characterized by extreme rainfall deficits (-25.4-cm for ND¹), anomalously hot summers (49°C for Aug.²) and cold winters (min -51°C), which caused major economic losses and the failure of 3 million farms. For the NGP, the impact of the Dust Bowl was especially severe, because the droughts occurred during the growing season. The exceptional severity of the 1930s drought has brought about two scientific questions: 1) how common are such extreme droughts and 2) why isn't the severity represented in extant drought reconstructions?

Despite extensive modeling efforts to improve drought prediction, even the best models cannot agree on the sign of projected precipitation change over the next century for the NGP³. While models have related the 1930s to sea surface temperature patterns in Pacific and Atlantic Oceans^{4,5}, they cannot

reproduce the drought in the NGP⁴. Resolving these problems requires long, high-resolution drought records in the NGP region that can be closely related to global climate processes. Existing NGP drought reconstructions (e.g., diatom-inferred salinity^{6,7} and ostracode Mg/Ca⁸) integrate the effects of annual temperature, evaporation and rainfall, from which limited conclusions can be drawn regarding the relative importance of various forcings. New records need to isolate drought parameters (i.e. temperature, rainfall) or offer sufficient seasonal information to identify processes causing severe growing season droughts.

Here we present the first compound-specific H-isotopic (δD) record of higher plant leaf waxes (δD_{WAX}) from a sediment core with high sedimentation rates from Lake George (LG), ND (Fig. 1). Multiple studies demonstrate that δD_{WAX} track growing season precipitation $\delta D^{9,10,11,12,13,14}$. LG is situated on the Missouri Coteau and surrounded by a mixed-grassland. Pollen suggests that LG was surrounded by grassland and steppe vegetation during the entire Holocene⁶. The stable grassland-dominated ecosystem minimizes the effect of vegetation change on δD_{WAX} ¹⁵. Instead, because grasses grow from April to September, δD_{WAX} primarily record growing season rainfall. In mid-continental regions of North America, precipitation δD respond to both precipitation amount and temperature. The amount effect is relatively strong, because the summer moisture from GOM constitutes ~77% of annual

rainfall. Importantly, higher summer precipitation δD values during dryer conditions are generally reinforced by enhanced δD values as a result of higher temperatures, resulting in greater amplitude of isotopic change and enhanced sensitivity of our leaf wax isotopic proxy.

To validate that our δD_{WAX} proxy records droughts in our study region, we analyzed samples at near-annual resolution from a ^{210}Pb -dated freeze core from LG and compared it with the instrumental record (Supplementary F4). Trends in the freeze core overlap instrumental rainfall and show that δD_{WAX} documents the sustained rainfall deficits during the 1930s. Other observed rainfall deficits (i.e. 1918, 1950s, 1988AD) show peaks in δD_{WAX} , however, the magnitude is less than the observed record. These differences likely reflect the lower sample resolution (~ 3 -yrs per sample) and the effect of temperature on precipitation δD . Correcting δD_{WAX} for MJJ temperature brings the magnitude of δD_{WAX} record closer to the observed rainfall amount (See Supplementary text), confirming the controls on δD_{WAX} for this region. The uncorrected LG δD_{WAX} reconstruction shows regionally coherent changes by strong correlation with an overlapping δD_{WAX} and aragonite-inferred¹⁶ drought record from nearby Kettle Lake (KL) (Fig. 1).

Our long-term isotopic record is consistent with regional climate reconstructions. An exceptionally strong relationship exists between the

δD_{WAX} reconstruction and clay provenance in the Pigmy Basin, GOM¹⁷. Times of high δD_{WAX} (drought) match periods of low smectite input to the Pigmy Basin, a marker for Missouri River valley, or NGP, input (Fig. 2).

Remarkable similarities occur between the NGP growing season drought and increased precipitation to southerly U.S. sites shown by high K intensity in the Pigmy Basin¹⁷ (i.e. 8.2ka, 4.8ka, 2.8ka, 1.0ka, 0.6ka)(Fig. 2). This pattern of wetter southern U.S. and drier interior U.S. also persisted during the 1930s¹, 1988AD^{2,18} and at 8.2ka^{19,20} (See anomaly maps in refs. 1,19). In 1936AD the U.S. interior experienced a 10-20 cm rainfall deficit, while the Atlantic seaboard experienced enhanced precipitation anomalies (>51-cm for NC, SC and FL)¹. The severe NGP 8.2ka drought coincided with a wetter Atlantic seaboard indicated from pollen in Lake Tulane, FL²¹, lake-level¹⁹ and continental moisture balance models¹⁸, a fresher GOM²², and clay minerals in the Pigmy Basin¹⁷ (Fig. 2). These spatial patterns coincide with the southern displacement of the Jet Stream and a northeastward position of the Bermuda high that limits the advection of moisture to the interior U.S.^{17,23,24,25}.

Processes in the Atlantic Ocean affect precipitation in NGP. Complimentary to the atmospheric configurations, NGP drought periods also correspond to isotopic changes in the Florida Current (FC) (Fig. 3)²⁶ from 3.0ka to present that reflect the strength of meridional overturning circulation (MOC).

Droughts correspond with reduced MOC (e.g., ~2.8ka), while major wet periods (e.g., ~2.0ka and 0.3ka) correspond with enhanced MOC²⁷. This supports that the strength of MOC plays an important role in heat distribution from the GOM to the N. Atlantic during the Holocene^{28,29} and further suggests that reduced MOC plays a role in trapping moisture in the GOM and along the Atlantic seaboard leading to mid-continental drought. The reduction of the MOC during growing season droughts at LG is coincident with cold conditions in Greenland (i.e. at 8.2ka, 0.6ka, 1930sAD)³⁰. Fluctuations in sea salt Na and terrestrial dust fluxes to summit Greenland are also coherent with the LG δD_{WAX} record²³ (Fig. 2). Thus, it is clear that the NGP growing season droughts are linked to the Atlantic modes of marine and atmospheric climate change. Changes in the North American Monsoon (NAM) is one mechanism for enhancing southern U.S. precipitation and freshening the GOM³¹, however, our site is not sensitive to the NAM. LG is sensitive to the northward advection of moisture from the GOM, which is linked to changes in the subtropical N. Atlantic. Based on evidence of increased hurricane activity during the 1930s and 1988AD droughts and the strong similarities between the timing of high Accumulated Cyclone Energy (ACE) index³² and high δD_{WAX} from LG (Fig. 4), we hypothesize that Atlantic tropical cyclones are likely a key player in severe NGP growing season droughts.

If this connection is robust, we would expect to see links to ENSO through the modulation of N. Atlantic tropical cyclone formation with El Niño events preventing cyclone formation and La Niña events promoting cyclone formation (promoting NGP summer drought)^{31,33}. During the Holocene our inferred growing season droughts occur during enhanced La Niña conditions (i.e. after 2ka^{34,35,36}), while in the instrumental time period El Niño events increase winter precipitation in the NGP³⁷. A novel ENSO record shows concomitant increases in El Niño and La Niña intensity in the late Holocene (Fig. 3). The increased intensity of both phases of ENSO reconcile the differences between the late Holocene (post-2ka) δD_{WAX} -inferred increase in growing season drought and the inferred P-E indicators that show sustained annual moisture^{6,7,16} post-2ka. Namely, increased winter moisture³⁸ related the intensified El Niño balances the δD_{WAX} -inferred, decrease in growing season moisture related to La Niña. This may also explain why the magnitude of the 8.2ka and 1930s droughts are reduced in the annual P-E record from nearby Moon Lake suggesting that while summers were dry, winter precipitation was likely sufficient to recharge lakes⁷. Our δD_{WAX} 8.2ka drought is consistent with increased eolian activity³⁹, the full development of the prairie period^{36,40,41,42}, and increased varve thickness from Deep Lake, MN (Fig. 2)³⁶. The mid-Holocene overlapping section of δD_{WAX} matches well with aragonite-inferred P-E from nearby Kettle Lake^{16,43} (Fig.1), suggesting that for the these droughts, the intensity of La Niña was greater than El

Niño. The relationship inferred here between ENSO and NGP growing season droughts is not evident in anomaly maps from ‘permanent El Niño/La Niña’ simulations⁴⁴ and suggests that transient conditions in the intensity of both ENSO phases is required to cause severe NGP droughts.

The results from our new NGP proxy for growing season drought show that (1) Droughts with the severity of the 1930’s Dust Bowl occurred rarely throughout the Holocene; (2) The spatial pattern of severe growing season droughts in the NGP corresponds with increased moisture in the GOM that is constrained to a more southerly position; (3) Growing season droughts in the NGP appear to be linked to changes in ENSO through the modulation of cyclone formation. Testing the teleconnections inferred from our new proxy data require models to better simulate precipitation from remote water sources to the NGP and paleo-ENSO records capable of tracking changes in both phases of ENSO for the entire Holocene.

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Figure 1. NGP Lakes Regional Comparison

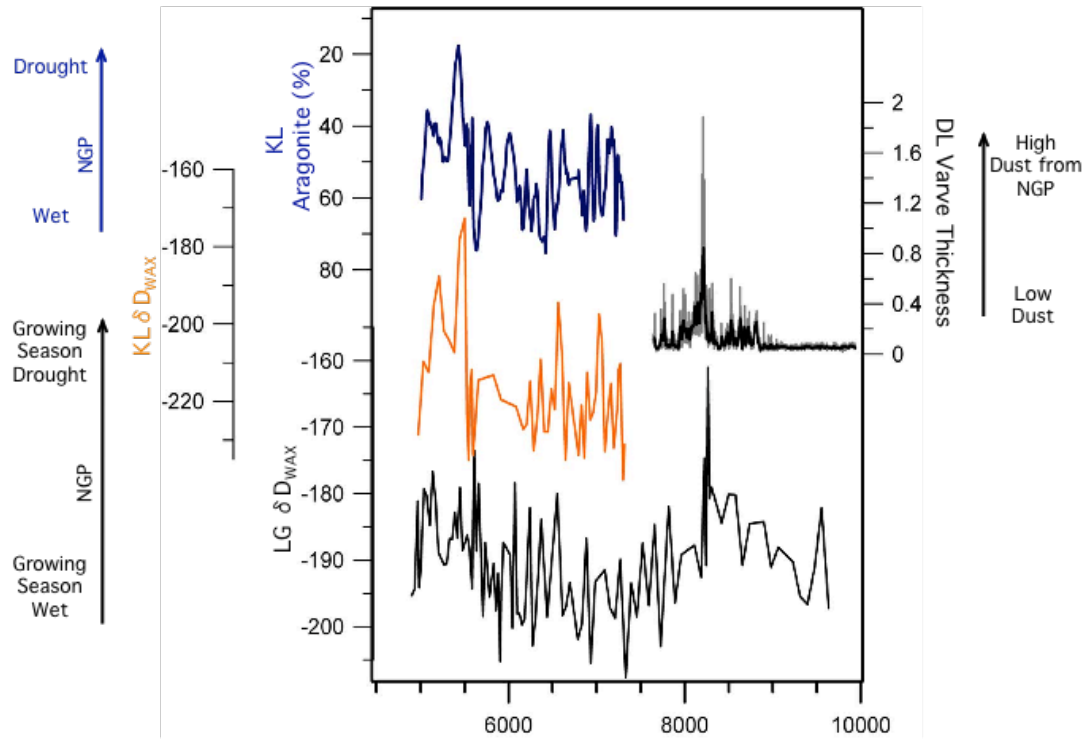


Figure 2. Holocene reconstruction with regional¹⁷ and global comparisons²³.

Average δD_{WAX} values (gray line) over the 1930s are -170 permil. Only eight events punctuate this threshold during the Holocene (8.2ka, 5.4ka, 4.8ka, 2.8ka, 610yrBP, 405yrBP, 167yrBP, and 130yrBP).

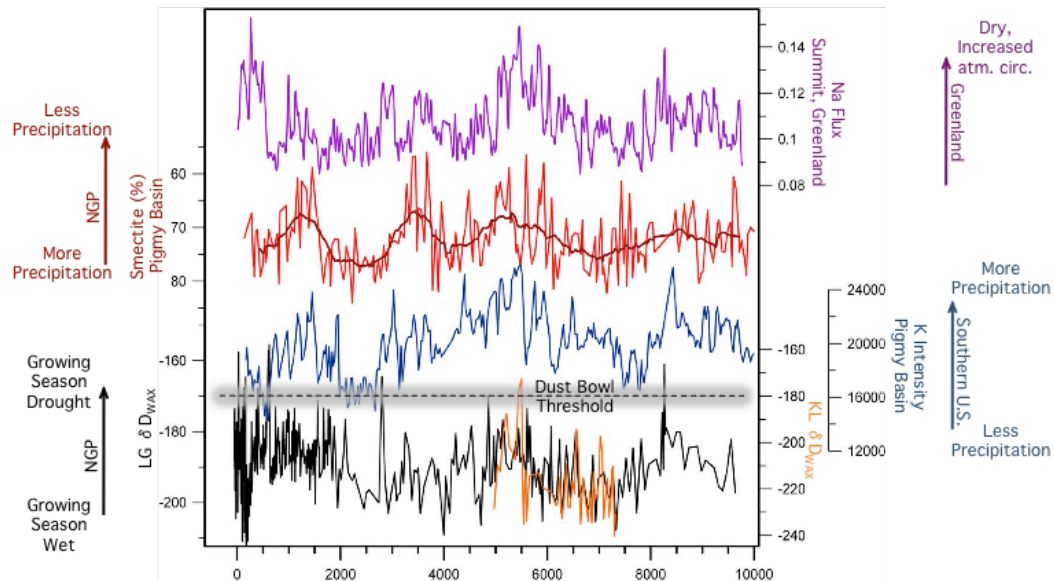


Figure 3. Global links in the last 4ka, including variability in the density of the Florida Current inferred from foram $\delta^{18}\text{O}$ (orange) and intensity

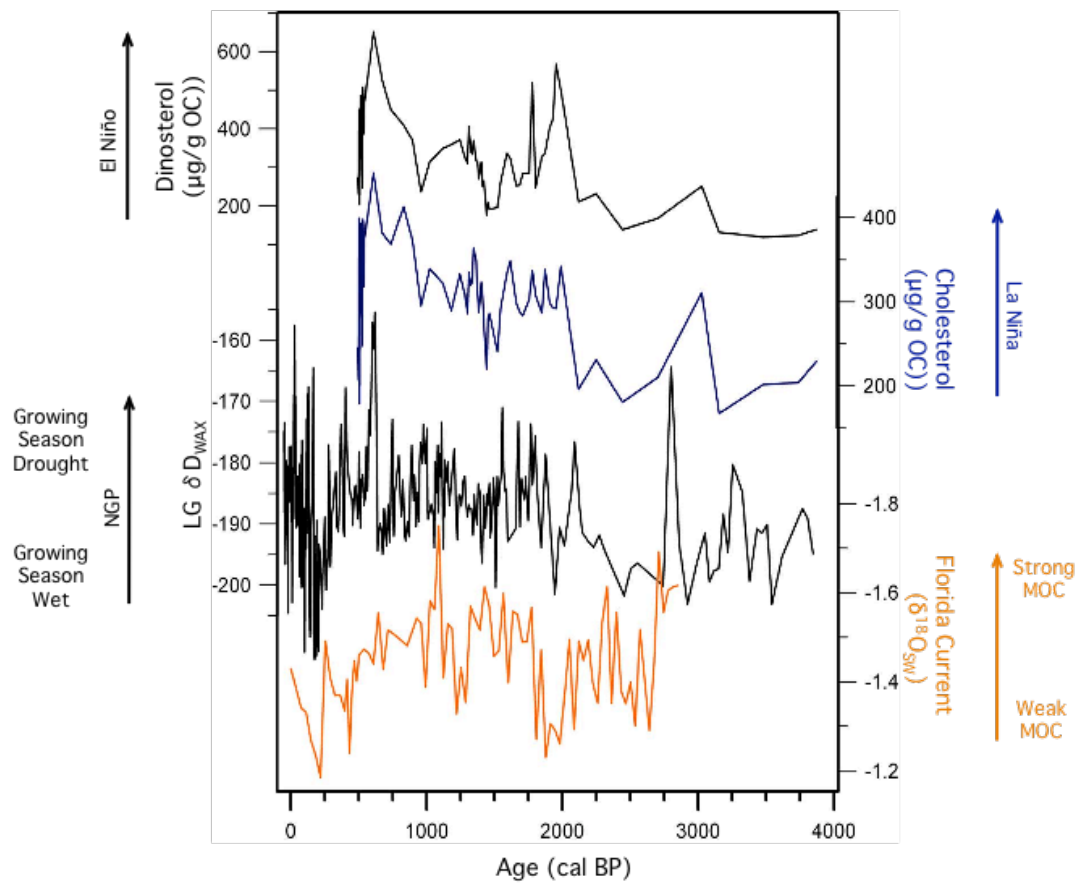
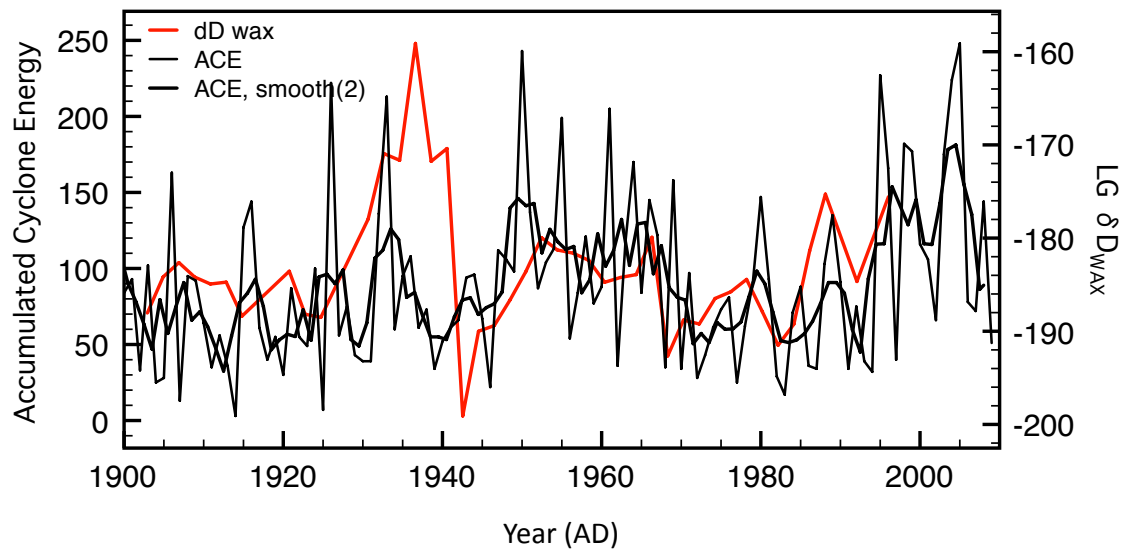
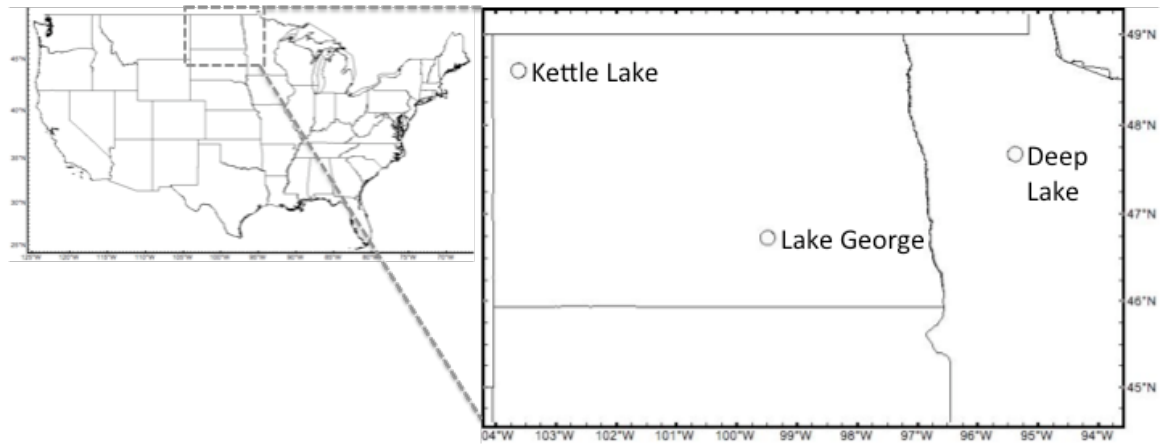


Figure 4. Accumulated Cyclone Energy (ACE) (with smoothed 10-point line) versus the freeze core δD_{WAX} record (red) from Lake George, ND. Note: the similarities between the 3-yr resolution δD_{WAX} and ACE records. The 1930s drought magnitude and length is greater than the three-peaks in ACE, which is likely the result of the drought's impact on sedimentation rates at Lake George.



SUPPLEMENTARY INFORMATION



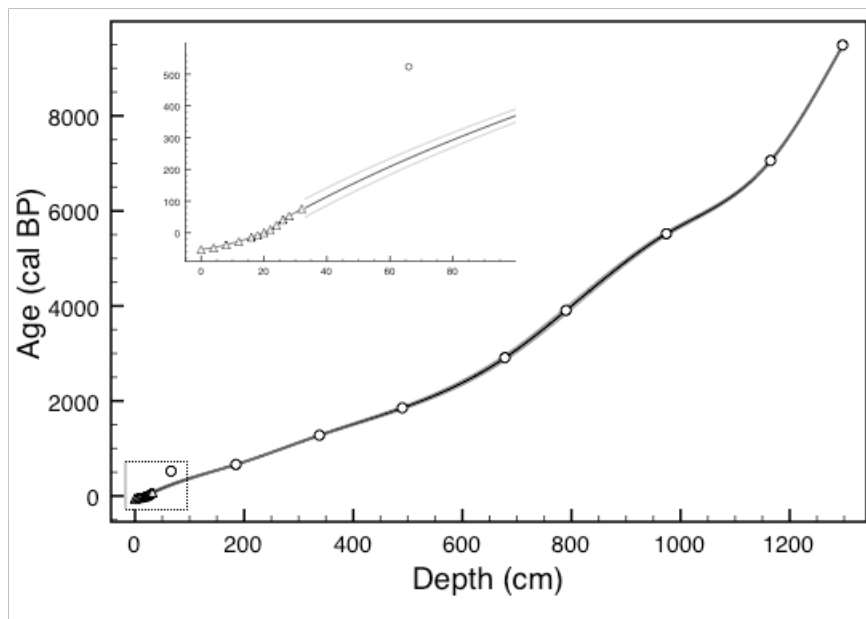
Supplementary Figure 1: Site map showing Lake George, ND in relation to Kettle Lake, ND and Deep Lake, MN.

Analytical Methods

Surface sediments (the top 0-1 cm) were collected from LG and other lakes in Supplementary F1 in the summer of 2007 with a HTH gravity surface corer. Terrestrial vegetation including: wheatgrass (*Pascopyrum smithii*) and blue grama grass (*Bouteloua gracilis*) were collected in June of 2009. A long core and freeze core were retrieved from a broad flat shelf at a depth of 14-m in February, 2009. Seismic data indicated that this location had a continuous and conformable sediment sequence. The long core was collected with a 7.5-cm diameter, Wright square-rod piston corer⁴⁵. The freeze core with a wedge-shaped coring device with an interior chamber that was cooled with dry-ice and isopropyl alcohol. Sediment and plant samples (~1 g dry mass) were freeze-dried, homogenized and extracted with dichloromethane (DCM):methanol (MeOH) (9:1, v/v) via an Accelerated Solvent Extractor ASE200 (Dionex) at 120°C and 1200 psi for three 15-min cycles. All samples were separated into neutral and acid fractions by elution through a LC-NH₂SPE column using DCM:isopropyl alcohol (2:1, v/v), followed by ether with 4% acetic acid (v/v)⁴⁶. Acid samples were methylated with 5% anhydrous HCl in MeOH at 60°C for 12-h. Hydroxyacids were recovered by eluting the methylated samples through silica-gel columns with *n*-hexane followed by collection of fatty acid methyl esters (FAME) with DCM. A hydroxymethylbenzene internal standard (0.73 µg µl⁻¹) was added to FAME samples, which were run on a GC-FID and GC-MS for detection, quantification and identification of *n*-acids. The samples were then run on an HP 6890 GC interfaced to a Finnigan DeltaPlus XL stable isotope spectrometer through a high-temperature pyrolysis reactor⁴⁶. See Huang et al. 2002⁴⁶ or Hou et al. 2008¹⁰ for detailed instrumental techniques and precision practices. All δD_{WAX} values reported here were calibrated to external fatty acid methylester standards C₁₈, C₂₂, C₂₄, C₂₆, C₂₈ to account for drift in instrument.

Age model and sampling

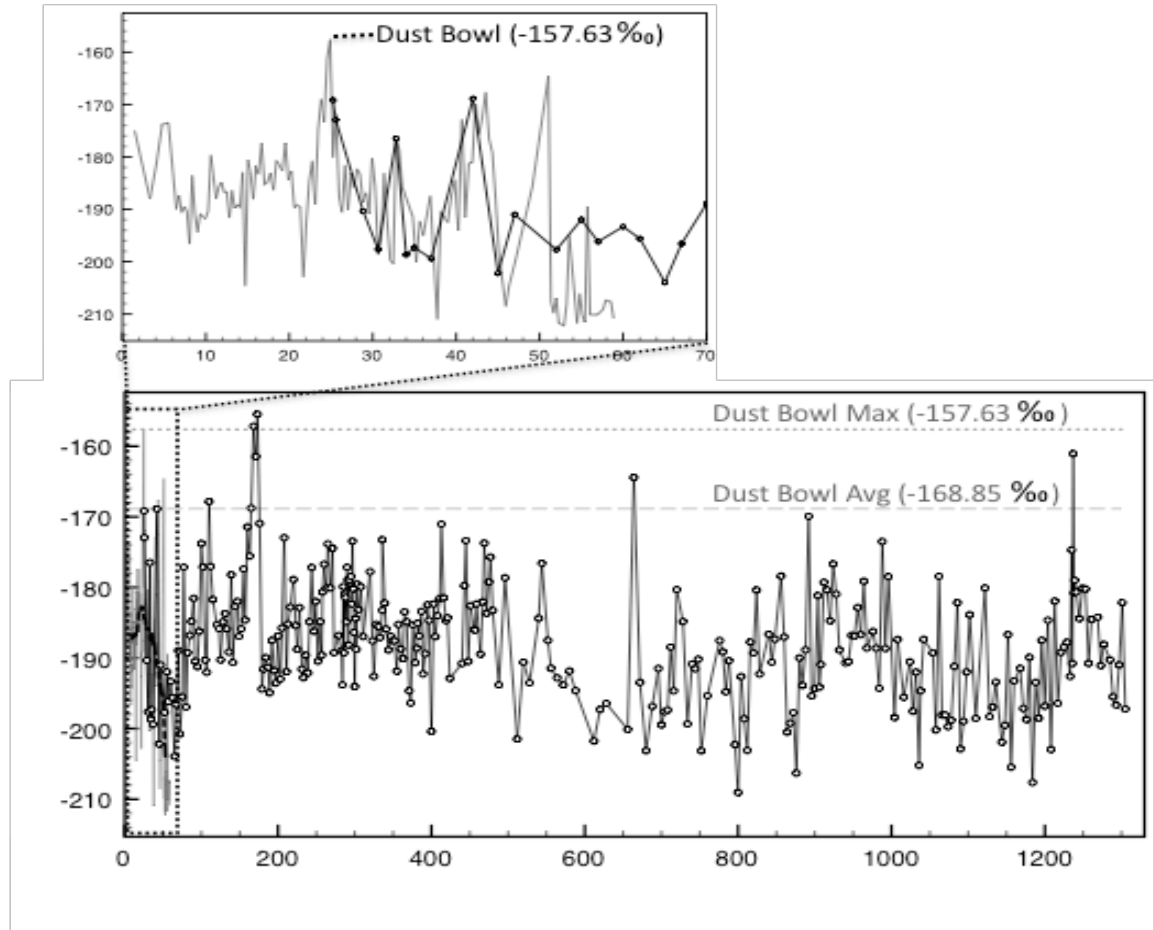
The long core extends 13-m below the water-sediment interface, while the freeze core extends 80-cm below the water-sediment interface. Nine AMS radiocarbon ages on charred plant material and a ^{210}Pb profile provide chronological control. These data are shown in Supplementary T1. The age model was created using the mixed-effect regression method⁴⁷ and implemented in R version 2.11.0 ©2010 (See Supplementary F2). Sedimentation rates were $\sim 0.12 \text{ cm yr}^{-1}$ prior to 3ka and nearly doubled ($\sim 0.23 \text{ cm yr}^{-1}$) post-3 ka. The long core was sampled every 8-cm post-3ka and every 4-cm pre-3ka to yield an average sample spacing of 40-years. Higher resolution ($\sim$ every 2-cm) samples were analyzed for the past 1.8ka for sample spacing of ~ 6 -years. In the freeze core, individual laminae were not consistently visible, so the freeze core was sampled every 0.25-cm (about every 1-3 years).



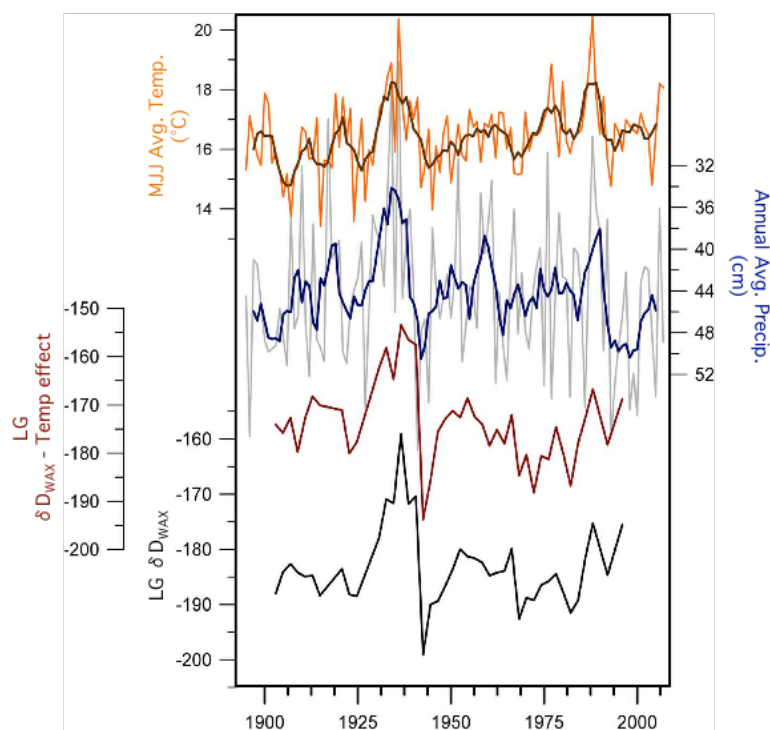
Supplementary Figure 2: Age model using a mixed-effect regression. Errors on the calibrated radiocarbon ages are within the size of the symbol (circle), and values are presented in Table 1. The standard deviation on the age model is shown by thin gray lines above and below the model (black). The inset zooms in to show the late-Holocene and ^{210}Pb data (triangles) used for the upper 32-cm of the core.

Splicing the Lake George freeze core and Lake George Core A

The LG freeze core captures the water-sediment interface and extends to 80-cm below the lake floor. LG Core A starts at 20-cm below the lake floor and extends 13-m below the lake floor. Data from LG freeze core and LG Core A were plotted via depth and a consistent 5-cm offset occurred among the overlapping upper eight data points from Core A and the freeze core. This offset most likely results from compression during piston coring or simply an offset between coring locations. Five centimeters was added to the upper data points in Core A to match the freeze core δD_{WAX} . The spliced cores are shown in Supplementary F3.



Supplementary Figure 3: Spliced long core and freeze core based on depth below lake floor. The δD_{WAX} record from the Lake George long-core (black) and freeze-core (gray) plotted versus depth (lower), showing the overlapping section zoomed in (upper). The maximum δD_{WAX} value for the Dust Bowl is plotted as a dashed line along with the δD_{WAX} value averaged across the Dust Bowl peaks (1930-1936AD). The latter represents the time incorporated into each single sample from the long core and is more representative comparison of the Dust Bowl drought with the full Holocene-length record.



Supplementary Figure 4. Freeze core δD_{WAX} record versus instrumental climate parameters. The LG freeze core δD_{WAX} uncorrected record (black) is plotted versus the δD_{WAX} record corrected (red) for the effect of surface air temperature using monthly May, June, and July averages and using the global relationship between $\delta^{18}O$ and temperature for the extratropics (Eq. 1) and substituting δD for $\delta^{18}O$, using the global meteoric water line equation (Eq. 2). See below:

Equation 1: $\delta^{18}O = 0.64T - 12.8$ (Jouzel et al. 1994, ref. 48)

Equation 2: $\delta D = 8\delta^{18}O + 10$ (Craig 1961, ref. 49)

Equation 3: $\delta D = 5.12T - 92.4$ (Eq. 2 substituted into Eq.1)

The corrected and uncorrected δD_{WAX} records are plotted against the annual average precipitation amount (blue) and monthly averaged May, June, July air temperature (orange) from the Jamestown, ND weather station. The chronology for the freeze core is based on ^{210}Pb ages (Table 1). Points were shifted within the error of chronology to line up with the instrumental data and accurately subtract the temperature effect.

The choice of C_{24} -acid

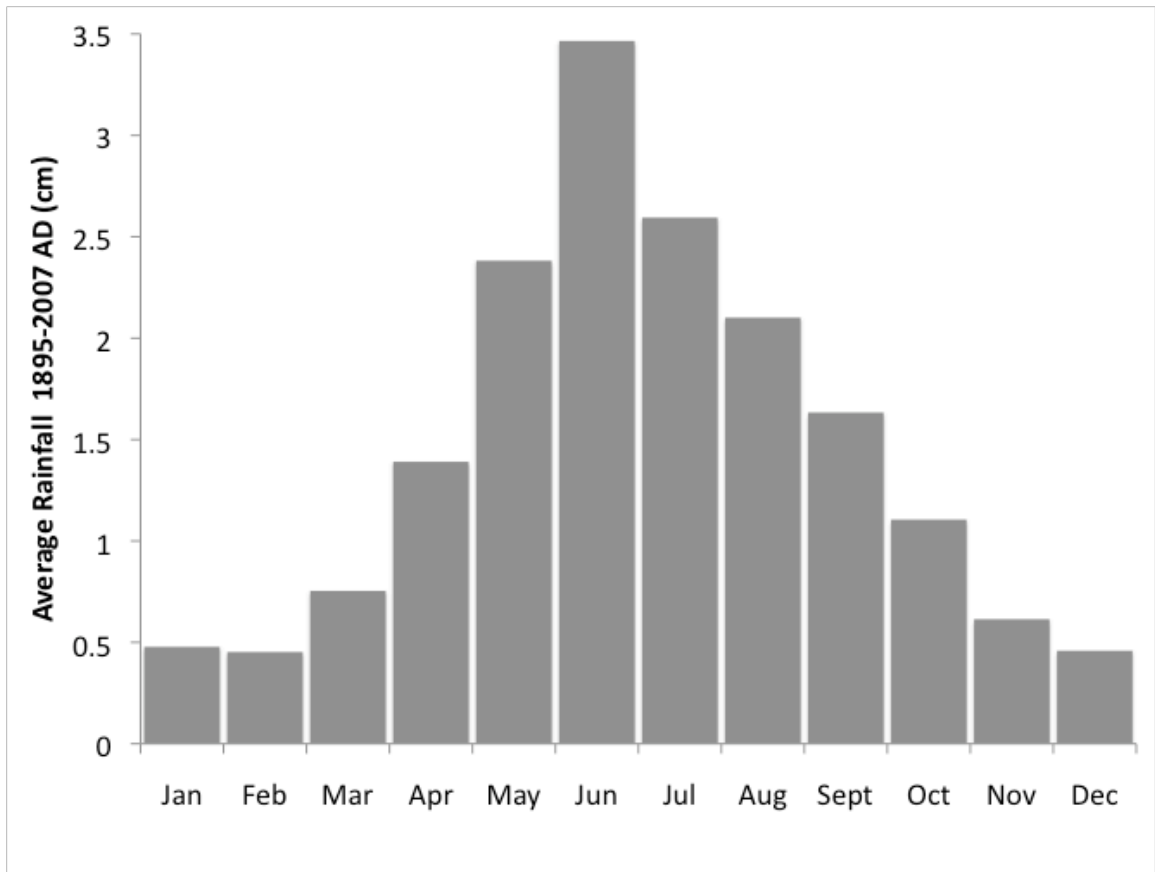
Long-chain fatty acid methyl-esters (FAMES) are produced by a number of different plants. Even-numbered FAMES, with chain numbers between 24 and 32 carbons, are produced by terrestrial vascular plants.⁵² Modern sampling of grass taxa ($n=5$) around Lake George shows that C_{24} makes up $68\pm 5\%$ of long chain acids relative to C_{24} , C_{26} , C_{28} , and C_{30} . The C_{24} acid also dominates the sedimentary acids ($>53\%$ for all 460 samples). Here we choose to use the C_{24} acid, because it is the dominant chain length produced by the dominant vegetation type.

Based on pollen and vegetation maps from the region (Laird et al 1996), LG was surrounded by grassland and steppe vegetation during the length of the reconstruction. While the composition of this prairie likely changed through time (contributions from C_3 versus C_4 grasses, forb content and shrubs), we make the assumption that grass remained the dominant vegetation type and that the H-isotope ratio of the C_{24} -acid is representative of grass taxa. Different taxa of vegetation can have different biosynthetic fractionation offsets, but these offsets predominantly occur between grasses and other forms of vegetation (i.e. trees, shrubs)¹⁰. The overall offset in δD_{WAX} value for trees is ~ 40 permil heavier than grasses¹⁰. If the contribution of leaf waxes to the Lake George sediment shifted through time (i.e. greater contribution from forested region when the ecotone was closer to the site in the Early Holocene) then we would expect to see a step-wise and permanent shift toward lower δD_{WAX} values from the Early Holocene toward the mid-Holocene, which is not the case.

Controls on δD_{WAX}

We considered a number of possible controls on δD_{WAX} values in the Northern Great Plains including: seasonality of precipitation, source of precipitation, and precipitation amount.

Seasonality and Source of Precipitation: Seasonality and the source of precipitation, are deeply intertwined. Summer (AMJJAS) precipitation is derived from the Gulf of Mexico (GOM) due to advection of moist air onto the continent and its subsequent transport in a low-level jet²⁵. In contrast, winter (DJF) precipitation is primarily derived from Pacific air masses that are modified as they traverse from across the continent²⁴. These two sources have distinct isotopic signatures, based on data from the closest Global Network of Isotopes in Precipitation station in Atikokan Ontario, Canada, about 400 km northeast of LG. Averages for the available years (1914-1971AD) show that Gulf derived precipitation (April to Sept.) has a δD value of -68 permil; whereas, Pacific derived precipitation has a δD value of -179 permil. These values are similar to the Online Isotopes in Precipitation Calculator (AMJJAS = -75, DJF = -168). These data indicate an offset in the range of 111 permil toward lower δD values in the winter. Based on the closest weather station data (NCDC for Jamestown, ND), winter precipitation constitutes $8 \pm 3\%$, (range of 3% to 20%) of the annual precipitation (measure from 1895 to 2007AD). Using a simple mass balance approach, the maximum effect of a change in winter contribution is 19 permil. However, we make the assumption that the winter precipitation does not contribute to the variability in the δD_{WAX} reconstruction, because it is not a significant source of moisture to the plants that produce the leaf waxes, and because there is no correlation or similarity in trends between δD_{WAX} and the instrumental winter precipitation. In addition to the low fraction of total rainfall, there are other reasons why winter precipitation is not a significant factor with respect to δD_{WAX} . The C_{24} -acids analyzed here are derived from terrestrial vascular plants, which only produce these epicuticular waxes during the growing season⁵⁰. Winter precipitation in this region falls after the ground is frozen by mid-October as snow and abruptly melts and runs off into surrounding rivers and lakes in early spring³⁸. While there likely is some stored soil moisture from the previous growing season that affects the water available for leaf wax production, winter precipitation is not a strong component of the water available to the plant during leaf wax production.



Supplementary Figure 5: Rainfall distribution from Jamestown, ND monthly averages from 1895-2007 AD (cm). Based on this distribution 78% of the annual precipitation falls between April and September.

Supplementary Table 1. ^{210}Pb measurements were made at the Science Museum of Minnesota in Dr. Engstrom's laboratory after methods in refs 51 and 52 on bulk freeze-dried sediment. Radiocarbon measurements were made on charred, terrestrial plant material at the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) and converted to calendar ages using Calib 5.0.2⁵³, and ages are reported in years before 1950 AD.

CORE	Depth (cm)	^{210}Pb age (yr AD)	S.D. (yr)	Calendar age (cal BP)	S.D. (yr)
LG Freeze	0	2008.3	1.31	-53.3	1.31
LG Freeze	4	2003.2	1.36	-48.2	1.36
LG Freeze	8	1994.2	1.55	-39.2	1.55
LG Freeze	12	1983.2	1.44	-28.2	1.44
LG Freeze	16	1970.4	1.69	-15.4	1.69
LG Freeze	18	1963.6	1.83	-8.6	1.83
LG Freeze	20	1956.1	2.13	-1.1	2.13
LG Freeze	22	1946.6	2.71	8.4	2.71
LG Freeze	24	1932.2	4.06	22.8	4.06
LG Freeze	26	1914.8	6.54	40.2	6.54
LG Freeze	28	1902.6	7.67	52.4	7.67
LG Freeze	32	1880.8	14.51	74.2	14.51
LG Freeze	36	1840.2	49.65	114.8	49.65
LG Freeze	37	1831.8	64.4	123.2	64.4

CORE	NOSAMS ID	Depth (cm)	^{14}C age (yr BP)	S.D. (yr)	Calendar age (cal BP)	S.D. (yr)
LG Core A	OS- 79421*	66	480	30	523.5	10.5
LG Core A	OS- 74739	185	675	30	664	11
LG Core A	OS- 74740	338	1320	30	1279	18
LG Core A	OS- 79422	490	1890	30	1854	34
LG Core A	OS- 74741	678	2800	55	2911	61
LG Core A	OS- 79423	790	3580	30	3907.5	74.5
LG Core A	OS- 74742	974	4770	50	5521	45
LG Core A	OS- 74861	1165	6150	40	7059	31
LG Core A	OS- 79424	1297	8450	50	9488	20

* This sample was excluded from the age model, because it forced a reversal in the mixed-effect regression model. There is no lithological evidence for discontinuous sedimentation during this period.

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CHAPTER 9

PYROGENIC POLYCYCLIC AROMATIC HYDROCARBONS RECORD FIRE HISTORY: A VALIDATION STUDY FROM YOSEMITE NATIONAL PARK, CA

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ABSTRACT

Understanding the natural mechanisms that control fire occurrence in terrestrial ecosystems requires long and continuous records of past fires. Proxies, such as sedimentary charcoal and tree-ring fire scars, have temporal or spatial limitations and do not directly detect fire intensity. We show in this study that polycyclic aromatic hydrocarbons (PAHs) produced during wildfires record local fire events and fire intensity. We demonstrate that high-performance liquid chromatography with fluorescence detector (HPLC-FLD) is better than gas chromatography-mass spectrometry (GC-MS) for detecting the low concentrations of sedimentary PAHs derived from natural fires. The HPLC-FLD is up to 60 times more sensitive than the GC-MS that is often used to detect PAHs as pollutants. Annual samples extracted from varved sediments from Swamp Lake in Yosemite National Park, California, are compared with the observational fire history record and show that PAH fluxes record fires within 0.5-km of the lake. The low molecular weight PAHs (e.g. fluoranthene, pyrene and benz[a]anthracene) are the best recorders of fire, whereas the high molecular weight PAHs likely record fire intensity. PAHs appear to resolve some of the issues inherent to other fire proxies such as secondary deposition of charcoal. This study advances our understanding of how PAHs can be used as biomarkers for fire events and poses new questions regarding the distribution of these compounds in the environment.

1. INTRODUCTION

Understanding wildfires and biomass burning are exceedingly important in the context global climate change, because they have direct effects on global carbon storage, atmospheric chemistry (input of CO₂, CH₄, NO_x), and ecosystem diversity (Gill et al. 1995; van der Werf et al. 2004). Even in the best-case global warming scenarios, fire frequency in the U.S. is expected increase (Scholze et al., 2006). While wildfires are important globally, increases in spatial extent, damage and management costs associated with fires have already been observed in the U.S. over the past decade. In particular, 2006 AD set the record for the largest area burned in U.S. history and cost the U.S. Federal Agency over \$2 billion (Costs of Wildfire Suppression, 2007; NICC, 2008). Anthropogenic activities such as fire suppression have left few natural fire ecosystems in many regions of the U.S., so land managers rely on reconstructions of fire history to restore natural ecosystems and to understand how climate influences natural fire regimes.

The most common methods for reconstructing fire history include sedimentary charcoal counts and tree-ring fire scar analysis. These methods have advanced the understanding of fire frequency, fire extent, and the timing of past fires in relation to mechanisms that control fire, such as climate (Clark, 1990; Niklasson and Drakenberg, 2001; Whitlock and Larsen, 2001; Prichard et al., 2009; Margolis and Balmat, 2009). Traditional

reconstruction methods have contributed greatly to the fundamental knowledge about fire occurrence and its relationship to climate; however, each method has spatial and temporal limitations, and neither directly detects fire intensity. Sedimentary charcoal analysis is a time intensive procedure that can require up to 5-cc of sediment collected consecutively along the core depending on the charcoal concentration (Whitlock and Larsen, 2001). For traditional lake coring techniques with a square-rod piston corer (2-inch diameter), this necessitates using up to a quarter of the core material. Physical processes of charcoal deposition and degradation can affect fire history interpretations. For instance, secondary deposition of charcoal can lead to ambiguous results, because charcoal from a single event can be transported into the lake several years later (Whitlock and Larsen, 2001). Redeposited charcoal can also mask smaller charcoal particles from regional fires. Degradation of individual pieces of charcoal post-deposition or site-specific influences of transport and erosion can break down larger charcoal pieces into smaller particles and can artificially alter the fire history (Whitlock and Larsen, 2001). Tree-ring fire scar analysis requires a large population of trees to ensure continuity and capture of all events and are limited temporally due to tree mortality. By using PAHs we assess whether it is possible to avoid some of the potential issues mentioned above, and add information, such as fire intensity, which cannot be detected by these other methods.

Biomass burning produces charcoal, as well as organic compounds known as polycyclic aromatic hydrocarbons (PAHs) (Ramdahl, 1983; Page et al., 1999; Yunker et al., 2002; McGrath et al., 2003, Yang et al., 2005). PAHs are categorized into three main groups based on their origin: biogenic, petrogenic, and pyrogenic. Biogenic PAHs are produced by organisms or through diagenesis (e.g. perylene, a 4-ring unsubstituted PAH)(Wells et al. 1995). Petrogenic PAHs are petroleum-based (e.g. from crude oil) and often have a branched or substituted structure derived from a parent PAH (e.g. C1-Chrysene or C2-Chrysene derived from Chrysene). Pyrogenic PAHs are produced during the combustion of plant material, such as fossil fuels or organic material during forest fires, and are predominantly unbranched, parent compounds, mostly 3- to 6-ring PAHs (Page et al., 1999). Preliminary work shows that the concentration of pyrogenic PAHs increases in sediments following forest fires and are distinguishable from petrogenic PAHs (Page et al., 1999). A few extant studies use PAHs as indicators of fire in the paleorecord in Triassic or Jurassic age sediments in conjunction with charcoal or pollen analysis (Killops and Massoud, 1992; van de Schootbrugge et al., 2009; Marynowski and Simoneit, 2009). While these studies show that the ratios of specific PAHs, such as Phenanthrene/Anthracene, indicate that the source of PAHs is pyrogenic (Yunker et al., 2002; van de Schootbrugge et al., 2009); validation of PAHs as a biomarker through comparison with

historical fire events has not been undertaken. Therefore, additional studies are necessary to determine the relationship between sedimentary PAHs and known fire events to evaluate how PAHs record the fire events.

Sedimentary PAHs have several characteristics that make them attractive biomarkers, including: (1) high resistance to diagenesis (Johnsen et al., 2005), which may help improve the temporal constraints on tree-ring analysis; (2) their structure is related to the temperature of the burn event with a more condensed structure (increasing number of rings) related to a higher burn temperature (McGrath et al., 2003), which could provide information on fire intensity; and (3) PAHs are produced during a broader temperature range than charcoal (charcoal: 200-600 °C, PAHs: 200-900 °C or hotter) (Conedera et al., 2009; Lu et al., 2009) and could record higher temperature events than those recorded by charcoal. In addition to these important characteristics, PAHs are quantifiable with modern analytical instruments, and suitable for correlations with fire parameters such as total area burned, fire proximity to the lake, and fire intensity. As aerosols, PAHs potentially can provide a more regional record of fire while individual PAH distributions can provide information about the type of material that was combusted (Yang et al., 2007; Burns et al., 1997, Lu et al., 2009).

The main concern with using pyrogenic PAHs is detection limits, because PAHs are typically studied as pollutants (Notar et al., 2001; Oros et al., 2007;

Liu et al., 2007) in much higher concentrations than those produced naturally by forest fires. In high concentrations, PAHs are detected using gas chromatography-mass spectrometry (GC-MS) (Burns et al., 1997; Gabos et al., 2001). However, high-performance liquid chromatography with fluorescence detector (HPLC-FLD) also detects these compounds at much greater sensitivities. The HPLC-FLD's selectivity and sensitivity is particularly important for detecting and quantifying PAHs from high-resolution records where only a small volume of sediment is available. Vergnoux et al. (*in press*) report PAH analysis by HPLC-FLD from near surface soils; however, there is so far no report of using HPLC-FLD to analyze PAHs in aquatic sediment cores to reconstruct natural fires at longer time scales.

Here we assess the use of known pyrogenic PAHs as an indicator of local and regional fire events and as a recorder of fire intensity using freeze core samples that were collected from Swamp Lake, Yosemite National Park, California, U.S. (Figure 1). We develop a new method for PAH measurement by HPLC-FLD and compare the sedimentary PAH patterns with the well-documented fire history record of Yosemite National Park since 1930 AD. We apply the newly developed PAH biomarker to a second core that extends from 1334 and 1435 AD, and use the PAH concentrations to infer fire events

adjacent to the lake.

2. METHODS

2.1. Site Description

Swamp Lake is located at 1554-m elevation in the northwest corner of Yosemite National Park (YNP), California (37.57°N, 119.49°W) (Figure 1). This site was chosen for its isolated location, which reduces the risk of PAH input from sources other than forest fires. The surface area of Swamp Lake is about 8 hectares and its maximum depth is 20-m with no inlet and only a shallow, marshy outlet (Smith and Anderson, 1992). The forest surrounding the lake is dominated by white fir (*Abies concolor*), ponderosa pine (*Pinus ponderosa*), Jeffrey pine (*Pinus jeffreyi*), sugar pine (*Pinus lambertiana*), black oak (*Quercus kelloggii*), and incense cedar (*Calocedrus decurrens*) (Smith and Anderson, 1992).

From 1891 until 1968 AD, YNP practiced total fire suppression. After 1968 AD, the park performed prescribed burns to restore near natural conditions (van Wagtendonk and Lutz, 2007). YNP is an advantageous site for this study, because the National Park Service has kept detailed GIS records including: area burned and location, on all fires that have occurred in the park since 1930 AD.

2.2. Sample Collection

Two freeze cores were collected by R. Scott Anderson and Lydia Roach in October 2006 AD (Core SL0601T) and September 2007 AD (Core SL0708) from Swamp Lake. The 22-cm SL0601T core contains the surface sediments, while the 62-cm SL0708 core overlaps the base of SL0601T and extends the record back through time. Both cores are varved (Roach and Cayan, 2007; Cayan and Charles, 2010).

2.3. Sediment Sampling and Age Control

SL0601T was sampled in a -20 °C cold room with a razor blade, with care taken to follow annual laminations. The lower portion of SL0708 (36.5-62 cm) was sampled for PAH analyses and was sampled similar to SL0601T, but at 2-mm intervals. The dry mass of each sample was recorded prior to extraction. The SL0601T core was dated by varve-counting and the assigned ages were confirmed using spectral analysis. The chronology for SL0708 was calculated by determining the sedimentation rate through linear interpolation between 1872 and 1350 AD (based on fixed dates for an earthquake and a tephra layer). The chronology is independently verified by varve-counting and spectral analysis. SL0601T samples date from 1898-2006 AD, while the estimated chronology for SL0708 is 1325-1432 AD.

2.4. Organic Geochemical Sample Preparation

Samples were transferred into Teflon extraction vessels and extracted in 20-mL of the 9:1 (*v/v*) azeotrope of dichloromethane(DCM):methanol at 100 °C in a Mars Express microwave-assisted solvent-extraction system (Mars 5, CEM corp.) for 30-minutes with stirring. Samples were separated using Solid Phase Extraction by Lydia Roach in the laboratory of Alex Sessions, California Institute of Technology. The elution scheme was: F1- *n*-hexanes, F2- 4:1 *n*-hexanes:DCM, F3- 9:1 DCM:acetone, F4- 2% formic acid in DCM. PAHs were captured in the F1 and F2 fractions.

F1 and F2 fractions from 71 depth levels from SL0601T and F2 fractions from 37 depth levels from SL0708 were prepared for and analyzed with reverse-phase high-performance liquid chromatography. Each sample was dissolved in 1.0-ml of acetonitrile (ACN), shaken vigorously by hand, and sonicated for 40 seconds. There was no change in chromatography when a sample was dissolved in 20% water and 80% ACN, so 100% ACN was used to allow for easier evaporation. Each sample was filtered with a syringe filter (Whatman 4mm Single Use Filter, 0.45µm PTFE membrane) to a 2-ml vial. Samples were refrigerated at 5 °C and brought to room temperature before any preparation or analysis. Samples run on GC-MS were evaporated with N₂ gas and dissolved in DCM prior to analysis.

2.5. HPLC Method Development and Sample Analysis

The HPLC-FLD analyses were performed with an Agilent 1200 Series Chromatograph with a binary pump interfaced to a diode array detector (DAD) and fluorescence detector in series and an autosampler. A method for DAD was developed with a 24-PAH standard mix (Quebec Ministry of Environment PAH Mix, H-OME-01) to assess the separation and elution order of the PAH compounds. Once the compounds were identified on DAD, the method was developed on FLD to optimize the signal for each compound while maintaining a suitable chromatogram. Note that the PAH, acenaphthylene, does not fluoresce, but could be quantified by DAD in future studies. The original HPLC conditions were developed based on the Agilent method (Henderson et al., 2008). The FLD configuration was developed based on previously published studies of PAHs in sediments using HPLC-FLD (Liu et al., 2008; Rey-Salgueiro et al., 2009). For the FLD, if too many excitation and emittance wavelengths were applied in a short time period, a staircase baseline tended to develop. The peaks and their retention times were identified with the standard. The PMT gain on the FLD settings was adjusted to obtain the highest peak signal, while maintaining a Gaussian shape. Retention time for each PAH between runs was very consistent (<2% deviation) and was used to identify individual PAH peaks for the samples. Two methods were developed because the original column was later replaced with a new column with different specifications (Table 1).

The data acquisition and analysis on the HPLC-FLD was done with ChemStation for LC 3Dsystems software (Rev. B.04.01SP1 [647]). An external calibration curve was developed for each compound, using the 16-PAH standard mixture solution from AccuStandard, M-610-QC, diluted to various concentrations (1 ng/ml to 0.00025 ng/ml). The external calibration for retene was based on an individual compound standard (Sigma-Aldrich, Technical grade) since retene was not a part of the mixed PAH standard. The calibration curves for all compounds were constructed in duplicate with relative standard deviation lower than 3%. The abundances of the selected compounds were calculated by comparisons of peak areas on the calibration curve with the peak areas of the individual PAHs obtained from the HPLC-FLD chromatograms. Peak identification was carried out by comparison of retention times with standards.

Analyses for the F2 samples of SL0601T and SL0708 were performed according to 'Method 1' conditions in Table 1. The SL0601T F1 samples were analyzed using 'Method 2', which was developed subsequently using a new column with different specifications (Table 1). The two methods were similar and optimized the signal for each compound while maintaining a suitable chromatogram (Figure 2).

2.6. GC-MS Analyses

In order to confirm the main compounds indentified by HPLC-FLD, gas chromatography-mass spectrometry (GC-MS) analyses were performed using an Agilent 6890 Series Gas Chromatograph with a split/splitless injector and an autosampler interfaced to an Agilent 5973N (quadrupole mass analyzer) mass spectrometer on selected samples that contain relatively high concentrations of PAHs. Separation of the compounds was achieved using a fused-silica capillary column (HP-5MS, 30 m x 0.25 mm i.d. x 0.25 μ m film thickness) and injecting 1 μ L. The oven temperature was programmed from 60°C (isothermal for 1 min) to 100°C at a rate of 15°C/min, then to 310°C at a rate of 6°C/min. The final temperature was held for 15-min. The carrier gas was Helium at 1.0-mL/min. The MSD Transfer Line Heater temperature was kept at 300°C. The ion source was kept at 230°C and quadrupole mass analyzer at 150°C. The mass spectrometer was operated in the electron impact mode at 70 eV ionization energy. Mass spectra were recorded in full-scan mode at 50 to 500 daltons. Also, mass spectra were obtained in selected ion monitoring (SIM) mode to improve instrument sensitivity for low concentration compounds (mass-to-charge ratio m/z 165 and 166 for fluorene, m/z 178 for phenanthrene and anthracene, m/z 202 for fluoranthene and pyrene, m/z 219 and 234 for retene, m/z 228 for benz[a]anthracene and chrysene, m/z 252 and 253 for benzo[b]fluoranthene, benzo[k]fluoranthene,

and benzo[a]pyrene, m/z 276 and 277 for benzo[g,h,i]perylene, m/z 276 and 278 for indeno[1,2,3-cd]pyrene, and m/z 278 and 279 for dibenzo[a,h]anthracene. MSD ChemStation software (ver. E. 02.00.493) was used for data acquisition and analysis. The PAHs were identified by matching the retention times and fragmentation profiles against corresponding standards and those in the NIST/EPA/NIH Mass Spectra Library version 2.0d.

2.7. Climate Data

The National Park Service Fire History for YNP from 1930-2008 AD was acquired from the NPS Data Store online. The records include total area burned, longitude and latitude, fire dates, and other relevant information for all fires in that time span, although not all fires have equally detailed records. Using ARCGis, a 200-cell Distance Raster was created to calculate the distance from the center of a fire to the Swamp Lake centroid (37.57°N, 119.49°W). Annual data was manually separated and viewed as yearly GIS maps for analysis of fire characteristics and occurrences. From these data the number of fires per year and the total area burned per year were determined. The maximum, minimum, and average distance from Swamp Lake and area burned were calculated for each year (Supplementary T1). Years that have fires within 2-km of the lake were dubbed, ‘fire years’. Precipitation data for YNP was retrieved from the NOAA.gov site.

3. RESULTS

3.1. HPLC-FLD versus GC-MS for PAH Analyses

To optimize identification and quantification of sedimentary PAHs, we compared the use of HPLC-FLD to that of GC-MS. The HPLC-FLD was more sensitive to PAHs than the GC-MS in full-scan mode or selected ion monitoring (SIM) mode. For example, for the GC-MS in full-scan mode, only retene was detected and no other PAHs for the 2001 AD sample. On the HPLC-FLD, the retene peak was very high (500 LU), and 10 other known PAHs were detected and quantified. By comparing injection mass and the signal-to-noise ratio, the HPLC-FLD was about 60-times more sensitive to retene than GC-MS in full-scan mode and about 5-times more sensitive than GC-MS in SIM mode. The concentrations of other PAHs in the samples were much lower than retene; however, the sensitivity difference between the instruments for other PAHs appeared comparable to that of retene.

3.2. Fractions 1 and 2

The extracted sediment samples were eluted by solid phase extraction into four fractions. PAHs were detected only in the first (F1- *n*-hexanes) and second fractions (F2- 4:1 *n*-hexanes:DCM). Pyrene, chrysene, retene, and benzo[a]pyrene were detected in almost all of the F1 fractions; phenanthrene, anthracene, and fluoranthene were detected in most of the F1 fractions, while

fluorene was only detected in some, and naphthalene and acenaphthene were only detected in a few samples. Benz[a]anthracene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene were not detected in the first fraction.

In the second fraction, phenanthrene, fluoranthene, pyrene, chrysene, retene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene were detected in almost all of the samples, benz[a]anthracene, benzo[a]pyrene, and dibenzo[a,h]anthracene were detected in most samples, and naphthalene, acenaphthene, and fluorene were only detected in some of the samples.

3.3. PAH Data Analysis

Unless otherwise noted, further data discussion of the SL0601T samples combines the concentrations of compounds found in the F1 and F2 fractions as determined by HPLC-FLD. The PAH fluxes in the SL0601T samples varied in relation to fire events (Figure 3). All PAHs had a distinct, high peak in flux in 1967 AD. The low molecular weight (LMW) PAHs (3- and 4-ring PAHs) had a distinct, high peak in 1996 AD, as well. Less distinct peaks in PAHs occurred in 1953 and 1988 AD. In these four years fires occurred within 0.5-km of the lake. Some years with fires within 2-km of the lake are recorded with peaks in PAH flux (e.g. 1958 AD). Individual PAH

concentrations have varied over time in relation to fire parameters such as fire proximity, area burned, and number of fires per year, and are discussed below.

The SL0708 samples had several peaks in PAH concentration through time (Figure 4). Because a constant sedimentation rate was estimated for this time period, the concentrations were not converted to flux. Around 1395 AD, both fluoranthene and pyrene had high peaks in concentration. Other periods with peaks in PAH flux occurred at about 1340, 1360, 1380, and 1435 AD. The SL0601T F2 samples were three- to five-times more concentrated than the SL0708 F2 samples for all PAHs except for benzo[k]fluoranthene. Retene, however, was on average 17-times more concentrated in the SL0601T F2 samples than the SL0708 F2 samples. Analysis of the F1 samples from this time period would likely provide a more complete picture of PAH concentration, especially for the LMW PAHs, which were found in this fraction from the SL0601T core.

3.4. PAH Ratios Indicate a Pyrogenic Source

Previous studies have used ratios between different PAHs to assess whether the PAH source was petrogenic or pyrogenic. For example, Yunker et al. (2002) show that PAH samples with an Anthracene:(Anthracene + Phenanthrene) ratio value greater than 0.10 are associated with pyrogenic

sources. This ratio was above 0.10 for all SL0601T samples in which both PAHs were detected and for all but one sample for SL0708. Thus, this ratio supports that these samples have a pyrogenic signature and indicates that the PAHs were from plant combustion (i.e. forest fires).

3.5. Fire History Data

From 1930 to 2005 AD, as recorded by the National Park Service, there were five fires within 0.5-km of Swamp Lake, six fires between 0.5 to 1-km, and 11 fires between 1 and 2-km. In 1996 AD there was a very large fire that burned around the full perimeter of Swamp Lake (Figure 1). Based on the National Park Service records, the number of fires per year in YNP has increased from 1930 to 2005 AD. The total area burned per year in YNP has increased since 1930 AD, with an average area burned per year of 14.7-km² between 1930 and 2005 AD and 28.3-km² since 1970 (Figure 3).

4. DISCUSSION

4.1. HPLC-FLD Analysis

The HPLC-FLD is more sensitive for detection of sedimentary pyrogenic PAHs than the GC-MS. Based on the signal-to-noise ratio for retene, the sensitivity of HPLC-FLD is about 60-times greater than GC-MS in full-scan mode and 5-times greater than GC-MS in SIM mode. We hypothesize that other PAHs should have a comparable limit of detection to that of retene;

however, due to low concentrations they were not detected by GC-MS in full-scan mode. The HPLC-FLD method developed in this study allowed us to detect and quantify 16 known PAHs in a single run, because the HPLC-FLD specifically selects for these compounds (Table 1 and Figure 2). Other PAHs were present in these samples, which once identified, could be quantified and provide more information in the future.

The high selectivity of the HPLC-FLD is important for assessing how PAHs record fire because pyrogenic PAH concentrations tend to be much lower than pollutant PAHs. In addition, constructing a nearly annual record required using very little sediment per sample. In the SL0601T core, for example, yearly horizons were ~0.3 cm thick, and on average, 80-mg dry mass of sediment was extracted per sample. Thus, it was not possible to use a greater injection volume because the total injection was limited by the amount of available sample.

4.2. PAH Relationship to Known Fire Events

The 16 PAHs detected and quantified in the SL0601T samples from 1930 to 2005 AD show that PAHs do record fires within 0.5-km of the lake with increased LMW PAH fluxes (Figure 3). During data analysis we assessed the correlation of individual PAHs and several PAH ratios with the known fire events and found that the LMW PAH flux best captured the known fires. We

chose to use the flux rather than concentration of PAHs because flux takes into account the annual sedimentation rate. A high sedimentation rate might otherwise dilute the apparent concentration of PAHs in a given sample. Increased concentrations of PAHs do occur during known fires events, however, the PAH flux provides a more accurate relationship to known fire events.

The LMW and HMW PAHs show different signals. The LMW PAHs fluoranthene, pyrene and benz[a]anthracene showed the most distinct peaks during known fire events, especially those nearest the lake (≤ 0.5 -km) as indicated by high peaks in flux (i.e. the 1996 and 1967 AD fire events). We chose these three PAHs to represent the LMW PAHs by empirical evidence of correspondence to known fire events. However, because previous studies show that combustion of different organic material (e.g. wood types) can have different PAH profiles (Yang et al. 2007), we suggest that more research is necessary from individual sites to determine what pyrogenic PAHs are the best indicators of fire in different forest regimes. Although these three PAHs were the best recorders for fires adjacent to the lake, the other PAHs provide useful information by amplifying key fire events. For example, the 1967 AD fire is recorded by a distinct peak in flux for all the PAHs. The pervasive pattern among many PAHs highlights the occurrence of a single fire event and may be more accurate than relying on a single compound. We also chose

three HMW PAHs, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene, to combine for comparison of LMW and HMW PAH signals (Figure 3).

Of the five fire events within 0.5-km of Swamp Lake since 1930 AD, the 1996 and 1967 AD fires were recorded by distinct peaks in LMW PAH flux. The HMW PAHs recorded the 1967 AD fire, but did not detect the 1996 AD fire. All of the individual PAHs had a distinct peak in flux in 1967 AD. We hypothesize that the different response of LMW and HMW PAHs may be attributed to differences in burn temperature. HMW PAHs with a greater number of rings require higher burn temperatures (McGrath et al., 2003); therefore, the lack of HMW PAHs in the sedimentary record during the 1996 AD fire may indicate that the event was not hot enough to produce as many HMW PAHs. The peaks in LMW PAH flux associated with the 1996 and 1967 AD fires are distinct and suggest that PAHs do not have secondary deposition in the years following these fires. If there was secondary PAH deposition, we would expect to see broad peaks in flux or tailing of peaks. Therefore, based on these fire years, it is possible that PAHs could resolve ambiguity resulting from secondary deposition of charcoal particles that can occur up to several years following a fire (Whitlock and Larsen, 2001).

The three other fire events within 0.5-km of the lake were in 1988, 1953, and 1944 AD; in 1953 AD there were actually two fires that burned within this distance. Although the peaks in flux for 1988 and 1953 AD were not as high as those for the 1967 and 1996 AD, peaks are still distinguishable above the background for LMW PAH flux (Figure 3). The 1944 AD fire is only evident in retene. This fire may not have been recorded with a high amplitude peak if the transport mechanisms reduced the amount of PAHs reaching the lake sediments (e.g. unfavorable wind direction or transport of the PAHs in the fire plume up and away from the burn area).

Several fires occurred within 1 and 2-km of the lake in the late 1930s, early 1960s, and late 1970s. Peaks in LMW or HMW PAH flux are not as discernable during these times, which suggests that PAHs primarily detect fires that are within 0.5-km of the lake site. We evaluated whether variations in individual and combined PAH fluxes through time may relate to other fire parameters, such as total area burned, the number of fires burned per year, or annual precipitation. On average, 51 fires occurred annually from 1930 to 2005 AD, with a maximum of 146 fires during 1987 AD. The PAH fluxes best detect those fires within 0.5-km and show little relationship to more distant fire parameters. Notably, increased annual precipitation values occur during most fire years except 1988 AD and 1944 AD. The converse is not true, not all increased annual precipitation years have fires within 0.5-km of the lake.

Rainfall associated with fire events may help scavenge PAH particles from the atmosphere and increase the likelihood of deposition in the lake. It is less plausible that rainfall causes runoff that washes PAHs into the lake, because PAH fluxes only correspond with enhanced rainfall during years with fires within 0.5-km of the lake. The low rainfall in 1944 AD may explain the lack of increase in PAH flux for the 1944 AD fire.

4.3. Comparison of PAH Record to Charcoal

PAH fluxes were analyzed from 1898-1929 AD, years that predate the National Park Service fire records for Yosemite. A charcoal-inferred fire record from Swamp Lake shows that between 1910 AD and 1925 AD there were two peaks in sedimentary charcoal (R. S. Anderson, pers. comm. *unpublished data*, Figure 5). Two peaks also occur in LMW PAH flux during this time, specifically at 1908 and 1917 AD. In addition, the period from about 1920 to 1930 AD, which predates the National Park Service fire records, had consistently high LMW PAH fluxes that correspond to elevated charcoal levels, as well, and may reflect increased fires within a 0.5-km of the lake. The annual precipitation record for YNP does not have annual resolution at this time, so it is difficult to assess the impact of rainfall on charcoal and PAH deposition. A small peak in the charcoal record also recorded the 1953 AD fire, but there was no charcoal peak associated with the 1967 AD fire, which was a prominent peak in the LMW PAH flux. One

possibility is that the 1967 AD fire did not occur in the drainage basin of Swamp Lake, and thus charcoal was not deposited in the lake, but the PAHs as aerosols did reach the lake. Because the PAHs did record the 1967 AD fire that did not appear in the charcoal record, PAHs could complement existing fire reconstructions and help provide a more complete picture of fires history for a given site.

4.4. Detection of Fires from the Thirteenth and Fourteenth Centuries

The previous sections show that PAH flux indicates when fires occurred nearby the lake (within 0.5-km), which suggests that PAHs can serve as indicators of fires in the paleorecord. We analyzed the LMW and HMW PAHs in a core from Swamp Lake that extends from 1325-1432 AD. We interpret the peaks in LMW PAH concentration at 1341, 1359, 1378, 1394, 1401, 1420, 1432 AD as fire events within 0.5-km of Swamp Lake (Figure 4). While the fires in 1341, 1359, 1378, 1401, 1420, and 1432 AD were accompanied by a peak in HMW PAH concentration, the peak at 1394 AD has no corresponding peak in HMW PAHs. Like the 1996 AD fire event, the 1395 AD fire event appears to have burned at a lower temperature and produced few or no HMW PAHs. The fire years inferred from the LMW PAH concentrations occurred at ~20-years intervals, except the 1401 AD fire. This is consistent with the fire return interval for the dominant vegetation-type, incense cedar, inferred from pollen around Swamp Lake at the time (Smith and Anderson, 2002). The fire

return interval for incense cedar forests ranges from 15 to 40 years, with an average of 27 years, based on a study conducted in Sequoia National Park, CA (Knapp et al., 2005). This fire return interval agrees with the interval between inferred fire events from our PAH record from about 1325 to 1432 AD.

4.5. Source of PAHs

The anthracene/phenanthrene ratio indicates that for both cores the dominant PAH source was from plant combustion and PAHs are recording forest fires. Samples with a ratio greater than 0.10 are associated with pyrogenic sources (Yunker et al., 2002). For SL0601T, all samples in which both phenanthrene and anthracene were detected have a pyrogenic signature. For SL0708, only one sample had a ratio lower than the 0.10 threshold, which may be due to incomplete data since the F1 samples were not analyzed. Anthracene and phenanthrene were both found in abundance in the F1 samples of SL0601T.

Retene recorded the 1967 AD fire very clearly, and with more improved quantification may provide a record for other nearby fires as well. Because retene is related specifically to the combustion of coniferous wood (Ramdahl, 1983), in future work retene should be investigated in more detail, especially for regions with conifers as an important part of the vegetation history.

Comparison of PAH profiles in sediments after wildfires in a variety of vegetation settings could help to discern how the burning of different vegetation affects the relative distribution of PAHs (Burns et al., 1997, Lu et al., 2009).

5. CONCLUSIONS

Previous studies of PAHs in sediments often used GC-MS, but the HPLC-FLD's selectivity for and sensitivity to PAHs make it an ideal detector for analyzing low concentrations of natural PAHs produced by regional fires, especially at nearly annual resolution where sampling material is limited. The sensitivity of the HPLC-FLD to retene was about 60 times greater than the GC-MS in full-scan mode and 5 times greater than the GC-MS in SIM mode.

Based on a comparison of the National Park fire records with the PAH samples from 1930 to 2005 AD, LMW PAH flux records fires within 0.5-km of the lake. Of the five fire events within 0.5-km of the lake in YNP since 1930 AD, the 1967 AD and 1996 AD fires were recorded the best with distinct peaks in PAH flux for all PAHs in 1967 AD and all LMW PAHs in 1996 AD. That only LMW PAHs recorded the 1996 AD fire suggests that this fire burned at a lower temperature, so it did not produce as many HMW PAHs. Fluoranthene, pyrene and benz[a]anthracene were the best recorders of fire,

but patterns from many PAHs in combination emphasized the occurrence of a fire event. The relationship among rainfall, fire years, and PAH flux suggest that rainfall during the fire season may help scavenge PAHs from the atmosphere and aid in their deposition into the lake.

Two peaks in PAH flux occurred at about 1908 AD and 1917 AD and were likely two fire events that contributed to the high charcoal counts during that time. The 1953 AD fire was also recorded slightly in the charcoal record, but the 1967 fire, which was very well-recorded by PAHs was not recorded by this charcoal record. That the 1996 and 1967 fires were recorded with such distinct peaks in PAH flux suggests that PAHs do not have secondary deposition in the years following a fire. Future work should include paired studies of sedimentary PAHs and sedimentary charcoal to determine if PAHs can resolve the issue of secondary deposition of charcoal particles. The anthracene/phenanthrene ratio indicates that for both cores the dominant PAH source was from plant combustion and that PAHs are recording forest fires. Thus, PAHs can serve as indicators of fires farther in the past.

We infer that several fires occurred within 0.5-km of Swamp Lake between 1324 and 1432 AD. Around 1394 AD, both fluoranthene and pyrene had high peaks in concentration, which strongly suggests a fire nearby the lake, possibly a low temperature burn as indicated by the lack of peak in HMW

PAHs. In addition, there may have been nearby fires around 1341, 1359, 1378, 1401, 1420, 1432 AD. This interval between fire events is consistent with the fire return interval for incense cedars, which were the dominant vegetation at this time (Smith and Anderson, 1992; Knapp et al., 2005).

By capturing distinct events, PAHs may provide details for more complete paleofire reconstructions. Further analysis of PAH fluxes in relation to fire parameters such as fire proximity, burn temperature, vegetation type burned, area burned, and the number of fires per year is necessary to facilitate the correlation of these parameters with PAHs. By comparing PAH fluxes with known fire events we can determine how PAHs record regional fire in the paleorecord.

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Table 1. HPLC-FLD Method Conditions. Analyses for the F2 samples of SL0601T and SL0708 were performed according to 'Method 1' conditions. The SL0601T F1 samples were analyzed using 'Method 2', which was developed subsequently using a new column with different specifications.

Method 1					Method 2				
Column:	Zorbax EclipsePAH 2.1 x 100 mm, 3.5 µm				Column:	Zorbax EclipsePAH 2.1 x 50 mm, 1.8 µm			
FLD settings:	Time	Ex	Em	PMT	FLD settings:	Time	Ex	Em	PMT
Zero all	4.00	224	330	12	Zero all	0.00	224	330	12
baselines	8.00	276	330	12	baselines	3.40	276	330	12
	9.70	250	368	12		4.70	250	368	12
	11.55	270	470	15		5.80	270	470	15
	12.75	240	386	14		6.26	240	386	14
	14.50	270	390	15		7.50	270	390	15
	22.00	300	470	15		9.10	255	420	15
Flow:	0.420 ml/min					11.70	300	470	15
Mobile phase:	A = Water; B = Acetonitrile				Flow:	0.417 ml/min			
Gradient:	Time (min)	%B	Flow		Mobile phase:	A = Water; B = Acetonitrile			
	0.00	40	0.420		Gradient:	Time (min)	%B	Flow	
	0.53	40	0.420			0.00	45	0.417	
	19.85	100	0.420			10.00	100	0.417	
	21.00	100	0.500			15.00	100	0.417	
	26.00	100	0.500			16.00	100	0.300	
	27.00	100	0.420			21.00	100	0.300	
	28.00	40	0.420			22.00	45	0.417	
	Stoptime = 30.00					Stoptime = 22.00			
Temp.:	25.0°C				Temp.:	25.0°C			
Injection:	5.00 µL				Injection:	5.00 µL			
Samples:	SL0601T and SL0708 F2s				Samples:	SL0601T F1's 1-71			

Table 2. Summary of Yosemite National Park fires since 1930 AD

Year (AD)	Area Burned				Center Distance to Swamp Lake			Total Number of Fires
	Total (1000 m ²)	Mean (1000 m ²)	Maximum (1000 m ²)	Minimum (m ²)	Mean (km)	Maximum (km)	Minimum (km)	
1930	3.6	1.2	3.5	18	40.7	41.6	39.4	3
1931	19.1	2.7	8	45	28	44.4	7	7
1932 ^c	78.4	3.4	21.3	19	20.4	54.8	1.5	23
1933	430.1	43	255.7	19	30	50.5	3.6	10
1934	7086.4	337.4	6856	19	27.9	54.2	7.3	21
1935	13	1.3	5	40	26.1	41.8	2.9	10
1936	60.9	2.5	16.1	40	24.4	53.1	4.8	24
1937 ^c	41.2	3.7	20.1	201	14.7	38	1.6	11
1938 ^c	19.1	1.2	16.1	19	21.1	44	1.4	16
1939 ^c	19.7	0.6	6.1	40	22.2	52.1	1.5	32
1940	12.2	3	8	482	26	36.6	10	4
1941	3.1	0.4	1	121	21.2	31.7	9.1	7
1942	8	0.9	3.2	40	37.7	45.1	3.8	9
1943	58	4.1	28.2	40	19	52.3	4.6	14
1944 ^c	18.2	1.1	8	40	20.2	38.9	0.4	16
1945	20.7	1.5	12.1	201	27.3	43	3.3	14
1946	54	2.7	20.1	40	24.9	49.3	5.9	20
1947	60.9	6.1	36.2	804	14.9	31	6	10
1948	49032.6	12258.1	49032.2	80	29.5	41.3	17.1	4
1949	274.2	6.2	180.2	80	27.7	52	6.1	44
1950 ^c	3373.3	80.3	2728.4	9	27.7	53.1	2	42
1951	25.3	2.1	8	201	17.3	52	1.8	12
1952	143.2	2.6	63.4	40	28.3	53.7	3.8	55
1953 ^c	15759.3	238.8	11406.9	40	26.6	52.1	1.5	66
1954	1	1	1	1006	42.7	42.7	42.7	1
1955	2.3	0.5	1.2	40	29.8	43	10.6	5
1956	111.5	2.1	36.2	80	25.3	52.1	3.1	52
1957	87.5	8	70.1	40	26.2	43.1	3.4	11
1958 ^c	81.8	1.6	10.1	40	20.7	54	2.3	52
1959	836.6	8.6	700.6	40	30	54.1	5.8	97
1960	7528.5	96.5	5956.3	121	21	55.9	2.6	78
1961 ^c	986.6	13	695.8	40	25.8	55.6	2.1	76
1962 ^c	67.8	1	6	121	27	51.2	1.7	60
1963	80.5	2	27.2	40	29.9	51	3.8	40
1964	0.5	0.3	0.3	201	35.9	43.6	28.2	2
1965	60.6	0.8	20.1	40	22	51.1	2.6	76
1966	860	22.6	591.5	40	31.8	53.5	2.3	38
1967 ^c	188	1.6	72.5	40	27.4	51.1	0.6	121
1968 ^c	12265.2	255.5	6844.2	40	24.8	51.6	1.8	48
1969	31.5	0.6	4	40	29	54.2	8.6	49
1970	3122.6	89.2	1789.4	40	30.1	49.3	5.5	35
1971	2596	60.4	772.2	40	30.8	51.9	3	43

Table 2. (cont.)

Year (AD)	Area Burned				Center Distance to Swamp Lake			Total Number of Fires
	Total (1000 m ²)	Mean (1000 m ²)	Maximum (1000 m ²)	Minimum (m ²)	Mean (km)	Maximum (km)	Minimum (km)	
1972	767.2	11.6	412	40	23.7	51.5	0.7	66
1973	1514.6	23.7	635.3	40	32.1	57.3	2.5	64
1974 ^b	16206.9	195.3	15060.7	40	25.7	53.4	3.1	83
1975	14865.1	285.9	3870	40	34	52.2	9.1	52
1976 ^c	9357.4	80.7	2721.2	19	27.1	54	1.5	117
1977 ^c	1027.5	17.1	317.9	40	24.2	48.8	1	60
1978 ^c	31130.8	432.4	20742.7	40	24.7	49.5	1.5	72
1979	20364.7	1357.6	19325	40	37	52.2	20.1	15
1980 ^c	32517.4	663.6	8303.6	40	29.2	50.9	2	49
1981	21302.7	300	8797.3	40	28.1	51.8	2.4	71
1982 ^c	456.2	41.5	448	80	22.1	48.7	1.5	11
1983	18288.8	703.4	11962.8	40	28.9	52.9	7	26
1984 ^a	6514.1	141.6	2115.3	40	25.6	52.5	4.2	64
1985 ^{a,c}	30886.7	454.2	6409.1	121	26.9	56.6	1.3	103
1986 ^a	18895.9	787.3	8225.9	402	30.2	53.1	3.4	57
1987 ^{a,c}	38665.9	311.8	14972	40	28.9	55.3	1.4	146
1988 ^{a,c}	49581.6	481.4	13873.1	40	31.1	54.9	0.3	132
1989 ^a	7373.5	117	6674.6	40	25.4	53.3	3.4	109
1990 ^a	105541.9	917.8	71911.9	40	25	53.6	2.2	131
1991 ^{a,c}	26802.4	326.9	12445.9	40	30.7	52.9	3.2	117
1992 ^{a,c}	6782.9	58.5	2095.6	304	23.5	53.3	0.8	136
1993 ^a	6436.6	378.6	4729.9	405	32	53.2	6.6	62
1994 ^a	25596	673.6	10848.4	405	31.7	54.9	4	68
1995	5407	85.8	2468.5	110	34.1	53.1	17.3	63
1996 ^c	252811.7	3241.2	239384.7	88	28.9	53.1	2.3	81
1997 ^a	17351.3	244.4	9934.1	363	29.7	53.3	2.7	74
1998 ^a	11182.6	211	8768.9	28	29.9	51.9	3.4	54
1999 ^a	72006.2	1043.6	35856.5	341	30	53.1	6.4	75
2000 ^a	1659.9	23.7	890.2	386	29.7	52.2	7.8	71
2001 ^a	29782.7	408	29272.2	402	31.2	51.8	7.1	85
2002	26329.9	461.9	13603.1	375	31.6	52.2	10.1	57
2003	50667.8	684.7	22558.7	405	28.5	52.8	5.1	74
2004 ^b	33677.4	635.4	20369.3	403	n/a	n/a	n/a	53
2005 ^b	19770.2	366.1	6990.1	407	30	52.4	5	56

^aGIS data were not available for one or more fires; therefore, these fires were not included in area calculations.

^bLatitude and longitude coordinates were not available for one or more fires; therefore these fires were not included in distance calculations. (1974, 1 fire; 2004, all fires; 2005, 15 fires)

^c"Fire Years": Years that have fires within 2 km of the lake.

Table 3. The PAH flux for each sample from core SL0601T. Individual PAH flux for each sample from core SL0601T determined from HPLC-FLD analysis. All years are in AD based on the chronology of the core. The SL0601T results are for the combined values of fractions 1 and 2, except for compounds specified with an asterisk, which were only quantifiable in the F2 fraction. The PAH abbreviations are as follows (in order of elution on HPLC): Na (Naphthalene), Ayl (Acenaphthylene), F (Fluorene), Phe (Phenanthrene), An (Anthracene), Fl (Fluoranthene), Py (Pyrene), BaA (Benz[a]anthracene), Ch (Chrysene), Ret (Retene), BbF (Benzo[b]fluoranthene), BkF (Benzo[k]fluoranthene), BaP (Benzo[a]pyrene), DA (Dibenzo[a,h]anthracene), Bghi (Benzo[g,h,i]perylene), IP (Indeno[1,2,3-cd]pyrene). (See next page)

Table 3.

Sample	Year	Na	Ayl	F	Phe	An	Fl	Py	BaA*	Ch	Ret	BbF	BkF*	BaP	DA*	Bghi*	IP*
SL2	2005				0.130	0.078	0.061	0.052	0.027	0.107	21.901	0.031	0.016	0.059		0.017	0.180
SL3	2004	0.023		0.058	0.184	0.060	0.060	0.056	0.023	0.157	24.572	0.029	0.010	0.060		0.013	0.113
SL4	2003	0.055			0.143		0.046	0.023	0.013	1.422	45.481	0.038	0.010	0.014		0.014	0.143
SL5	2002	0.006	0.011		0.044	0.021	0.025	0.017	0.006	0.133	14.020	0.016	0.003	0.021	0.031	0.004	0.024
SL6	2001				0.024	0.008	0.035	0.029	0.015	0.151	17.129	0.021	0.006	0.041	0.080	0.009	0.053
SL7	2000		0.005		0.048	0.204	0.014	0.020		0.075	7.957	0.024	0.003	0.025	0.095		0.029
SL8	1999				0.044	0.096	0.018	0.017		0.072	6.892	0.030	0.003	0.008	0.035	0.006	0.036
SL9	1998			0.004	0.049	0.007	0.012	0.018	0.006	0.043	8.383	0.019	0.002	0.021	0.082	0.013	0.036
SL10	1997		0.048	0.034	0.026	0.120	0.240	2.951	0.029	0.067	1.320	0.011	0.002	0.010	0.014	0.006	0.013
SL11	1995		0.011	0.008	0.036	0.048	0.012	0.015	0.003	0.027	2.373	0.010	0.002	0.030		0.006	0.031
SL12	1993	0.012	0.034	0.023	0.094	0.069	0.030	0.025		0.033	1.694	0.027	0.002	0.040		0.010	0.053
SL13	1992	0.003	0.014	0.016	0.038	0.023	0.040	0.014		0.032	1.874	0.030	0.003	0.032			
SL14	1991		0.016	0.008		0.056	0.012	0.018		0.037	3.097	0.030	0.002	0.096			
SL15	1990		0.047	0.022	0.097	0.230	0.051	0.052		0.091	5.988	0.060	0.009	0.093		0.027	
SL16	1988	0.001	0.007	0.005	0.020	0.023	0.012	0.009		0.018	1.016	0.015	0.002	0.013		0.005	
SL17	1987		0.050	0.032	0.117	0.136	0.086	0.060		0.103	7.122	0.064	0.008	0.046		0.031	
SL18	1986	0.038	0.043	0.020	0.096	0.079	0.054	0.040		0.074	13.027	0.050	0.006	0.050	0.065	0.026	
SL19	1985				0.002	0.037	0.007	0.008		0.012	0.574			0.067			
SL20	1984				0.060	0.044	0.025	0.023	0.004	0.021	0.744	0.023	0.002	0.046	0.016	0.013	0.038
SL21	1983			0.003	0.038	0.036	0.013	0.015		0.047	12.859	0.017	0.001		0.013	0.009	0.021
SL22	1982		0.006	0.016	0.013	0.066	0.019	0.017		0.028	4.671	0.031	0.003	0.091	0.031	0.014	0.038
SL23	1980	0.012		0.020	0.069	0.032	0.032	0.031	0.010	0.071	8.175	0.057	0.006	0.315	0.037	0.022	0.069
SL24	1979			0.013	0.023	0.010	0.020	0.016	0.007	0.027	3.350	0.027	0.007	0.174	0.018	0.015	0.044
SL25	1978			0.002	0.006	0.003	0.004	0.004	0.002	0.005	0.348	0.005	0.001	0.040	0.014	0.003	0.012
SL26	1977			0.005	0.013		0.020	0.014	0.006	0.011	0.384	0.011	0.002	0.061	0.013	0.007	0.023
SL27	1976				0.012		0.009	0.007	0.003	0.011	0.265	0.011	0.002	0.066	0.019	0.008	0.021
SL28	1974				0.014	0.004	0.023	0.009	0.003	0.013	0.613	0.010	0.001	0.077	0.013	0.009	0.021
SL29	1972			0.003	0.020	0.009	0.016	0.015	0.003	0.018	0.869	0.015	0.002	0.119	0.031	0.014	0.029
SL30	1970			0.003	0.016	0.012	0.023	0.012	0.001	0.016	3.413	0.008	0.002	0.097	0.020	0.010	0.031
SL31	1968				0.036		0.015	0.022	0.004	0.027	0.466	0.024	0.007	0.078	0.064	0.024	0.040
SL32	1967			0.061	0.634	0.149	0.249	0.345	0.023	0.202	26.573	0.160	0.079	1.576	0.458	0.183	0.393
SL33	1966			0.003	0.024	0.007	0.017	0.019	0.002	0.016	0.372	0.014	0.003	0.115	0.042	0.018	0.034
SL34	1965			0.003	0.041	0.007	0.030	0.035		0.020	1.103	0.033	0.004	0.116	0.043	0.023	0.040
SL35	1964	0.001		0.003	0.022	0.009	0.019	0.021	0.003	0.016	1.144	0.022	0.003	0.121	0.058	0.028	0.038
SL36	1963	0.002		0.001	0.012	0.006	0.009	0.010	0.002	0.008	0.888	0.013	0.002	0.062	0.027	0.013	0.021
SL37	1961				0.021	0.010	0.019	0.021	0.003	0.012	0.314	0.015	0.003	0.086	0.033	0.021	0.034
SL38	1959			0.003	0.029	0.006	0.026	0.044	0.003	0.018	0.629	0.062	0.007	0.146	0.056	0.044	0.069
SL39	1958				0.011		0.012	0.014	0.002	0.006	0.049	0.013	0.002	0.006	0.022	0.019	0.030
SL40	1956			0.004	0.016	0.026	0.018	0.018	0.004	0.014	0.678	0.069	0.004	0.074	0.055	0.036	0.055
SL41	1955				0.002	0.045	0.022	0.012		0.010	0.242	0.059	0.002	0.056	0.056	0.027	0.038
SL42	1954			0.001	0.011	0.003	0.010	0.011	0.002	0.008	1.002	0.041	0.004	0.102	0.059	0.015	0.025
SL43	1952			0.001	0.013	0.004	0.015	0.019	0.002	0.009	0.636	0.037	0.002	0.094	0.031	0.034	0.029
SL44	1950	0.003		0.003	0.023	0.007	0.049	0.027	0.005	0.017	0.311	0.080	0.004	0.232	0.053	0.033	0.050
SL45	1949			0.002	0.011	0.004	0.012	0.013	0.002	0.008	0.272	0.034	0.002	0.165	0.022	0.016	0.029
SL46	1947			0.002	0.012	0.010	0.011	0.012	0.002	0.008	0.473	0.030	0.002	0.196	0.023	0.015	0.029
SL47	1946				0.012		0.012	0.011	0.002	0.006	0.103	0.040		0.064	0.039	0.016	0.075
SL48	1943			0.001	0.009		0.013	0.002	0.001	0.009	0.675	0.016	0.003	0.073	0.034	0.035	0.037
SL49	1941		0.001	0.003	0.009		0.015	0.010	0.002	0.007	0.123	0.034	0.003	0.082	0.042	0.024	0.036
SL50	1939				0.013		0.013	0.014	0.002	0.009	0.177	0.043	0.003	0.059	0.054	0.032	0.046
SL51	1937			0.008	0.019	0.008	0.017	0.026	0.003	0.011	1.618	0.058	0.004	0.117	0.048	0.034	0.045
SL52	1935			0.003	0.012	0.002	0.009	0.012	0.002	0.008	0.806		0.003	0.063	0.041	0.018	0.036
SL53	1933			0.001	0.006		0.003	0.007	0.001	0.004	0.052	0.033	0.002	0.034	0.024	0.011	0.045
SL54	1931			0.003	0.022	0.004	0.011	0.017	0.002	0.011	0.525	0.034	0.006	0.070	0.068	0.046	0.075
SL55	1929			0.002	0.052		0.033	0.046	0.005	0.026	2.616	0.076	0.014	0.127	0.150	0.098	0.377
SL56	1927	0.015		0.006	0.048	0.008	0.021	0.024	0.007	0.022	0.486	0.070	0.016	0.095	0.125	0.141	0.212
SL57	1925	0.001		0.005	0.028	0.005	0.024	0.033	0.008	0.023	0.209	0.081	0.019	0.086	0.146	0.150	0.254
SL58	1923			0.006	0.048		0.025	0.029	0.005	0.019	0.071	0.065	0.015	0.084	0.100	0.096	0.162
SL59	1921			0.003	0.045	0.007	0.016	0.033	0.004	0.013	0.070	0.044	0.010	0.061	0.067	0.064	0.110
SL60	1919	0.001		0.002	0.022	0.012	0.014	0.005	0.004	0.012	0.098	0.041	0.009	0.060	0.059	0.054	0.106
SL61	1917			0.003	0.029	0.007	0.028	0.055	0.010	0.024	0.392	0.052	0.018	0.096	0.164	0.081	0.193
SL62	1915			0.002	0.022	0.005	0.013	0.017	0.002	0.012	0.102	0.014	0.009	0.032	0.039	0.020	0.046
SL63	1913			0.002	0.019	0.005	0.020	0.016	0.004	0.016	0.201	0.013	0.004	0.036	0.034	0.017	0.032
SL64	1912			0.001	0.010	0.003	0.007	0.007	0.001	0.004	0.053	0.019	0.002	0.018	0.025	0.012	0.030
SL65	1910			0.002	0.026	0.009	0.013	0.010	0.002	0.008	0.101	0.028	0.005	0.026	0.050	0.019	0.061

Table 3 (continued)

Sample	Year	Na	Ayl	F	Phe	An	Fl	Py	BaA*	Ch	Ret	BbF	BkF*	BaP	DA*	Bghi*	IP*
SL66	1908			0.003	0.033	0.006	0.010	0.016	0.001	0.007	0.287	0.028	0.004	0.033	0.043	0.016	0.042
SL67	1906	0.001		0.002	0.011	0.001	0.007	0.004	0.001	0.005	0.062	0.021	0.002	0.013	0.021	0.008	0.020
SL68	1904	0.002		0.003	0.018	0.002	0.006	0.005	0.001	0.005	0.087	0.022	0.002	0.010	0.025	0.007	0.022
SL69	1902			0.002	0.026	0.003	0.008	0.006	0.002	0.007	0.091	0.032	0.003	0.015	0.038	0.012	0.037
SL70	1900	0.001		0.002	0.013	0.003	0.006	0.005	0.001	0.004	0.070	0.021	0.002	0.011	0.058	0.007	0.020
SL71	1898			0.002	0.012	0.002	0.005	0.004	0.001	0.004	0.065	0.022	0.002	0.010	0.024	0.007	0.019
Mean		0.010	0.022	0.008	0.043	0.033	0.027	0.066	0.005	0.051	3.777	0.034	0.006	0.093	0.055	0.029	0.066
Minimum		0.001	0.001	0.001	0.002	0.001	0.003	0.002	0.001	0.004	0.049	0.005	0.001	0.006	0.013	0.003	0.012
Maximum		0.055	0.050	0.061	0.634	0.230	0.249	2.951	0.029	1.422	45.481	0.160	0.079	1.576	0.458	0.183	0.393

Figure 1. A) **GIS Map of Yosemite National Park Boundary.** The blue dot is Swamp Lake. Red marks the area burned by 1996 fires and pink by 1968 fires, two years with fires within 1 km of the lake. B) **United States Map with Yosemite National Park Marked in California.** Adapted from the web image on the John A. Dutton e-Education Institute website.

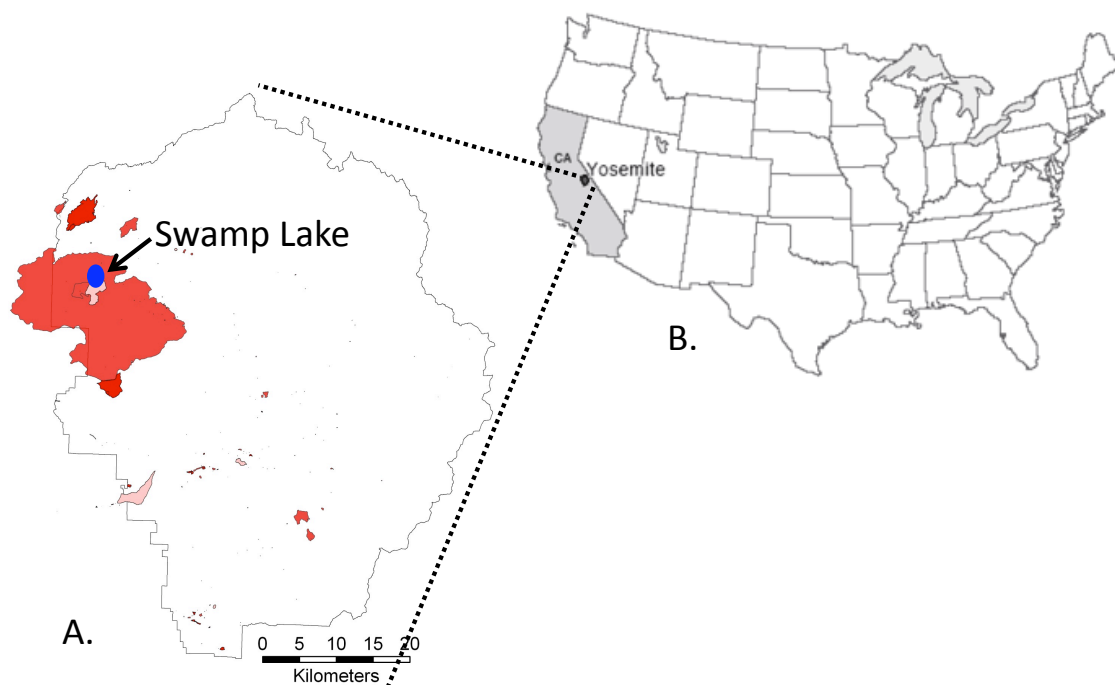


Figure 2. **Chromatograms of 16-PAH Standard on HPLC-FLD.**

A) Method 1 for SL0601T and SL0708 F2 Samples. B) Method 2 for

SL0601T F1 Samples. A different column was used in each method (see Table 1), but the two methods are similar and equally appropriate for PAH analysis. The 16 commonly analyzed PAHs are: 1) Naphthalene, 2)

Acenaphthene, 3) Fluorene, 4) Phenanthrene, 5) Anthracene, 6)

Fluoranthene, 7) Pyrene, 8) Benz[a]anthracene, 9) Chrysene, 10)

Benzo[b]fluoranthene, 11) Benzo[k]fluoranthene, 12) Benzo[a]pyrene,

13) Dibenz[a,h]anthracene, 14) Benzo[g,h,i]perylene, 15) Indeno[1,2,3-

cd]pyrene. Acenaphthylene does not fluoresce, but elutes after naphthalene.

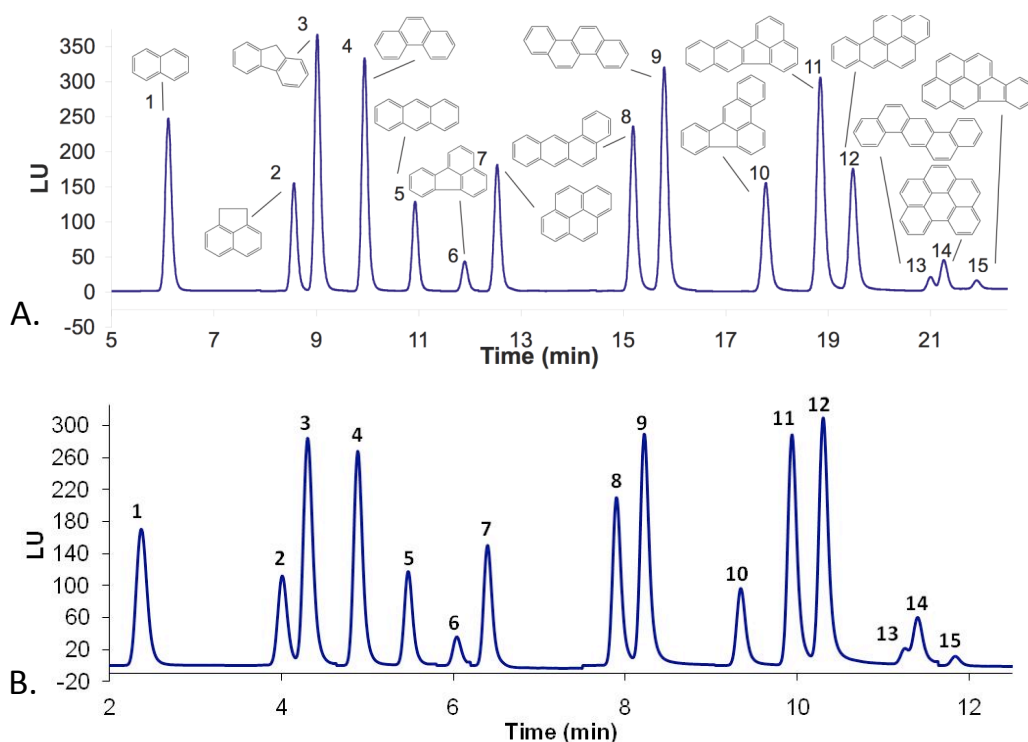


Figure 3. Σ LMW and Σ HMW PAH Flux through Time Compared with Fire and Precipitation. PAH flux is on a logarithmic scale. The LMW PAHs summed were fluoranthene, pyrene, and benz[a]anthracene. The HMW PAHs summed were dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene. The PAH data were from the SL0601T core. The National Park Service fire records extend from 1930-2005 AD; there is no observational fire history before 1930 AD.

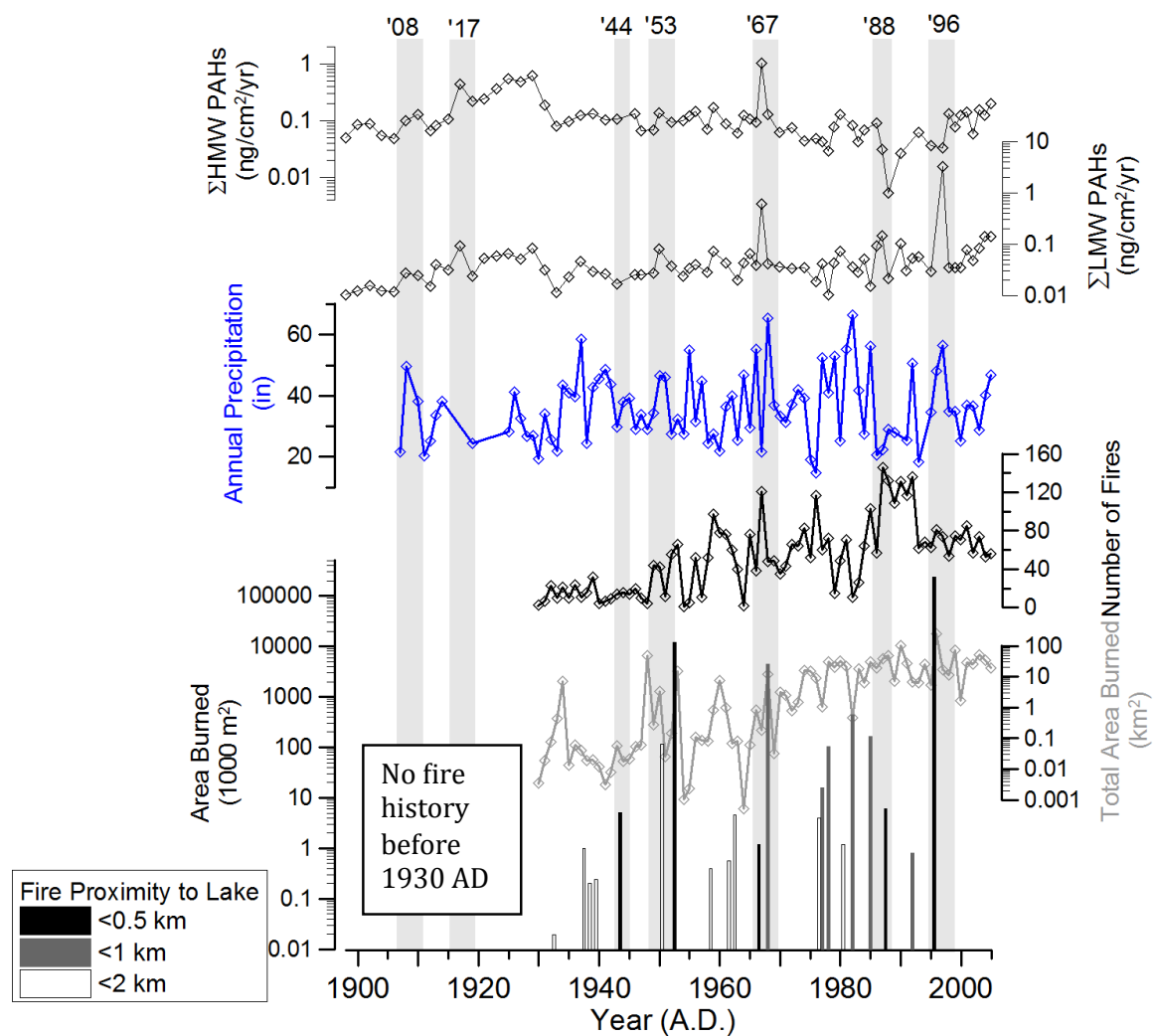


Figure 4. **Σ LMW and Σ HMW PAH Concentrations from SL0708 Core through Time.** Note that PAH concentrations are on a logarithmic scale and from the F2 fraction only. The low molecular weight PAHs summed were fluoranthene, pyrene, and benz[a]anthracene. The high molecular weight PAHs summed were dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene. The PAH data were from the SL0708 core.

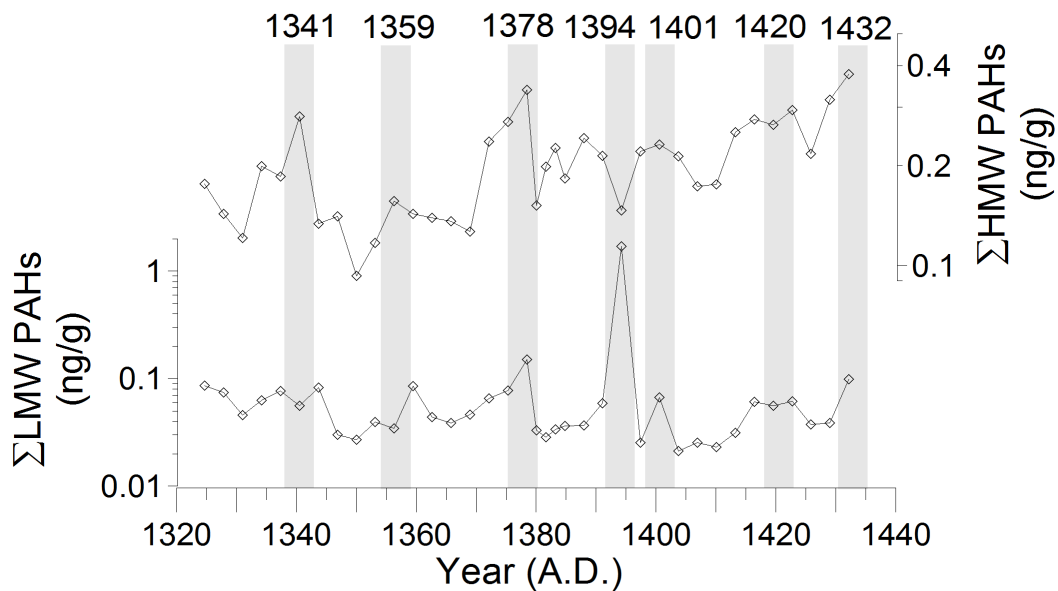
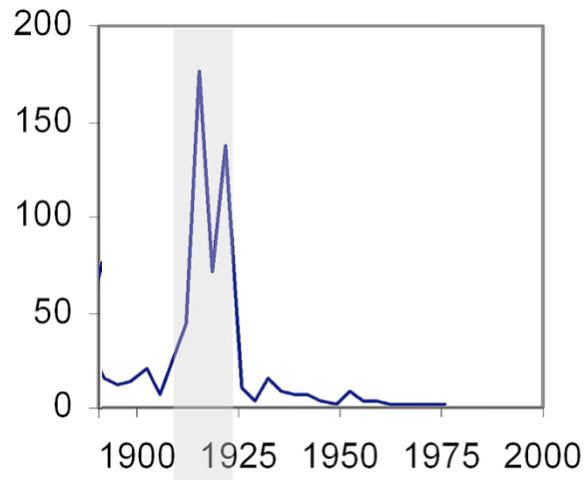


Figure 5. Swamp Lake, CA Recent Charcoal Record from R. Scott Anderson (unpublished). Y-axis is charcoal counts and X-axis is in years (AD).



CHAPTER 10

THE CASE OF LOBSTER SHELL DISEASE: INCORPORATING SCIENCE STANDARDS INTO PROBLEM-BASED LEARNING MODULES

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In press:

Science and Children

Did you ever wonder if lobsters have noses? Our students did, and from that curiosity grew knowledge. At the end of our eight-week project, Michael, a sixth-grade student, was able to describe how microbes affect lobster shell disease: “Microbes play an important role in harming lobsters, because they’re probably an invasive species ... and lobsters have no immunity to them.”

We, two graduate student visitors to an elementary school as part of an outreach fellowship combined content-driven and inquiry-based lessons into the framework of problem-based learning (PBL) in 3rd through 6th grade classrooms. This method included activities that directly addressed science standards while allowing students to delve into scientific questions and become comfortable with the scientific process and lingo. Although structured, the lessons were not simply a series of instructions with questions included as inquiry. Instead, students generated their own questions through guided research and hands-on labs that were used to develop subsequent lessons. Students were exposed to the required content through their own curiosity, making them more engaged while they simultaneously discovered the connections between science and the world around them.

Module Framework

Engaging students while delivering standards-based science content is a continual challenge in science teaching. Traditional ‘cookbook’ labs instruct students in a highly structured format and hands-on activities are often mistaken as full inquiry. Whereas, full scientific inquiry should encourage students to make predictions, design experiments, test methodology, and use experimental observations to determine the validity of their initial predictions. These true inquiry-based methods have led to increased student engagement, and research has shown that these methods also improve students’ understanding of science (AAAS 1993; NRC 2007). PBL is one such inquiry method that has been employed at the high school, undergraduate, and elementary school levels with much success (Michaels, Shouse, and Schweingruber 2007; Marsteller et al. 2009;). Integrating PBL into the science curriculum has allowed us to shift away from previously used ‘cookbook labs’ and disconnected minilessons and toward engaging our students in full inquiry.

After attending a workshop on PBL for high school biology at the Annual National Science Foundation GK–12 Meeting in 2009, we adapted and implemented PBL in the 3rd to 6th grade science curriculum. We left the workshop with three main ideas for an elementary setting: (1) use problems, puzzles, and mysteries to engage students in science content, (2) provide lesson continuity by participating in a long-term project, and (3) develop clear

end products. These end products do not necessarily require the students to solve the overall puzzle and can have a variety of formats that appeal to all learning styles (e.g., drawing, performing, or writing).

Through discussions at the workshop, we became aware that a common criticism of PBL is that by design it can be too open-ended to incorporate content-based curriculum well. We found that the structure that we used to develop and implement this module successfully countered that perceived shortcoming.

We define PBL as inquiry-based learning in which teachers act as facilitators for student-driven investigation that is centered on a current, local problem. Implementing PBL can be done by adhering to the following steps: identify a current, local problem; list the related state science standards; and identify some related hands-on minilessons that will allow the students to encounter the content. Next, divide the investigation of the problem into topics to be explored by groups of three to four students. Introduce the module with an informative news or scientific article, discuss the project with the students, and as a class determine what the culminating product or activity should be. Throughout the module, the student groups regularly present their findings to the class as a whole and the end product combines learned content from every group. Figure 1 provides a flow diagram describing this process.

Unit Development

The PBL module was centered on the current, local problem of lobsters in Narragansett Bay infected by lobster shell disease. After finding the article *Rampant Lobster Disease Mystifies Scientists* (Lord 2005), we examined Rhode Island's Grade Span Expectations (GSEs) to develop a focus question that could address the largest number of standards and set the goals to guide the direction of the project. The GSEs in life sciences fall into broader categories that concern organism structure for survival, cycles and energy flow in ecosystems, how groups of organisms change through time, and how humans are both similar and unique among Earth's organisms. With this in mind we developed our focus question: How does a single species affect an ecosystem, and how does that affect our lives? We also used the GSEs to choose specific activities relevant to the individual groups. See Table 1 for specific examples of how the GSEs were used to develop relevant focus questions and activities within the PBL module.

Introduction

In the first week's activity, we introduced the students to the problem by reading the news article in class, and then we assigned students into groups and had them identify "what we know" after reading the article, "what we don't know," and "how we can find answers." The "what we don't know"

responses comprised problems with simple solutions (e.g., What does DEM stand for? Department of Environmental Management) to more complex inquiries (e.g., How does the disease kill lobsters?).

The students then identified which questions could be easily answered versus those that would require more research to solve. They took class time to answer the easy questions, and we provided resources the following meeting period to solve the more difficult questions.

First Activity

In the second week, each group was assigned to tackle one of the five possible causes of lobster shell disease: (1) an oil spill, (2) a food web scenario, (3) toxins in the environment, (4) warming water currents, and (5) foreign microbes.

Groups can be chosen in different ways. For this activity we wanted the self-directed students to excel, so groups were based on the level of challenge.

This allowed for more teacher involvement with the groups that needed more time. Alternatively, we have had success choosing students with leadership qualities and distributing them throughout the groups. The choice of topic for each group came directly from the inquiries the students developed in the initial activity. Resources were provided throughout the project from the local and school libraries, the internet, and sources that we brought to the

classroom. We found that reading of intermediate level material worked well if the students were sent home with the article in an envelope with a packet of sticky notes. This allowed students to ask a wide range of questions at their comfort level. For example, one fourth-grader asked, “Do lobsters have noses?” whereas a more advanced reader asked, “What is being done to restore lobster populations to the area?”

Each of these groups encountered the relevant state standards throughout their experimental discoveries, but in different ways. For example, the foreign microbe group addressed the effect of warmer temperatures on bacterial growth through experimentation with growing yeast; whereas the warm water group addressed the relationship as to where and when warm temperatures occur in the environment by experiments with warm and cold (dyed) water. (Note: when groups performed different experiments simultaneously, we found that choosing groups wisely as suggested above, preparing comprehensive and detailed instructions, using parent volunteers, and discussing appropriate group work behavior were key to maintaining order.) Group sharing allowed the students to demonstrate their methods and what they learned. To facilitate this process the students were asked to answer specific questions relating to their experiment on an index card (See example lesson plan titled, “How Pollution Gets from Land to the Ocean”). A succinct, one-sentence answer to each allowed for a brief and informed

presentation of the material, and allowed students that typically were not comfortable presenting to feel more comfortable (see Table 2 for more tips).

Culminating Activities

Last, the students participated in two culminating activities. The first was the dissection of a lobster as a class (Figure 2), in which the students compared and contrasted human and lobster anatomy. We checked for shellfish allergies before this activity. Every person in the room wore a pair of gloves and was debriefed with safety information, such as, ‘food is never tasted in science labs’. Safety goggles were unnecessary because the lobster was fully cooked and only fingers were used as tools. The dissected lobster was divided into stations where students performed a hands-on investigation of claw, tail, external anatomy, digestive tract, and the function of the legs. For the second activity, the students created a newsletter in which they showed what they had learned and summarized their findings as a group (Figure 3). It is clear from the end product that the students learned a large amount of science and were influenced in their learning by the expertise of the groups outside of their own. Most of the groups independently came to the conclusion that lobster shell disease was probably a combination of some or all of the causes that the class as a whole was exploring.

Assessment is Key

Assessment of student progress is essential throughout the course of the project, not only to determine what methods work best and ensure that students grasped the concepts, but also to continually incorporate student inquiries into the lesson material. For example, on research-intensive days, the students ended with a 10-minute reflection on what they learned and what questions remained. We collected notebooks at the end of these days and used these questions to develop the next week's lesson plan. For culminating activities such as the newsletter, students were individually responsible for preparing their article and then met as a group to incorporate everyone's ideas into the final product. The classroom teachers formally evaluated the students throughout the course of the project. Some of the assessments included daily entrance and exit tickets (a brief writing exercise issued and collected at the beginning or end of the class) to assess the students' prior knowledge and what they learned, pretests and post-tests (true/false, multiple-choice questions), and constructed responses for minilabs or articles. Constructed responses included writing time devoted to answering the smaller focus questions and were graded using a rubric (Figure 4). Students were also asked to create a written narrative about lobster shell disease from different perspectives. For example, the students wrote a letter from a lobsterman whose livelihood was affected by the disease and another letter to one of the scientists that we learned about in our

reading. As a fun evaluation, we created a Jeopardy–style game at the end of the project with questions stemming from our state standards.

Built on Foundations

We have successfully incorporated scientific standards into the PBL method and adapted it to elementary science lessons. In year two, we carried out a second PBL with the previous year’s fourth-grade students. This second PBL focused on fish kills in the Narragansett Bay and built on the foundations from the previous year. The second PBL allowed for deeper analysis of organisms and their relationship to the ecosystem and culminated in a dogfish dissection and a play written, performed, and staged by the students. After participating in these successive projects, fifth-grader Milan, said “We didn’t really think of this as work, we really thought of it as fun and using our imaginations.” In addition to enjoying the projects, the students became more engaged in science on a day-to-day basis. The partner teachers have benefited by seeing the scientific, critical thinking process at work, while we have benefited by learning new pedagogical approaches to teaching science. The PBL method actively engages students in solving real-world problems using a scientific approach, and we enthusiastically encourage its use. Because not everyone has an estuary in their backyard, we suggest combing the local papers for compelling problems in your own town or city!

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Connections to the Standards

This article relates to the following National Science Education Standards (NRC 1996):

NRC Standard A – K-6: Abilities to do scientific inquiry.
NRC Standard A – K-6: Understanding about scientific inquiry
NRC Standard C – K-4: Organisms and environments
NRC Standard C – 5-8: Diversity and adaptations of organisms

National Research Council (NRC). 1996. National science education standards. Washington, DC: National Academies Press.

Activity	Focus Question	Standards Addressed
Marine Food Web – <i>Students are assigned organisms and pass a ball of yarn to other organisms they have a relationship to.</i>	<i>Where do our lobsters sit in the food web?</i> <i>What is important for balance in a food web?</i>	Identifying relationships between organisms within an ecosystem.
		Explaining the way plants and animals in a habitat depend on each other.
		Explaining how the balance of the ecosystem can be disturbed
Lobster dissection – <i>Students participate in classroom lobster dissection. Examine body systems at various stations.</i>	<i>What are the different parts of the lobster important for?</i> <i>Which are affected by shell disease?</i>	Recording and analyzing and sort observations/data about the external features of an organism.
		Recognizing that organisms have different features and behaviors for meeting their needs to survive.
Human/Lobster comparison – <i>Students study, draw and compare the anatomies of humans and lobsters.</i>	<i>What part of the lobster is being affected by shell disease?</i> <i>What is a similar disease in humans?</i>	Comparing and analyzing external features and characteristics of humans and other animals.
		Identifying the biotic factors (e.g., microbes, parasites, food availability, aging process) that have an effect on human body systems.
Ecosystem balance – <i>Students observe bubbling that shows potatoes can change hydrogen peroxide to water.</i>	<i>Can living things affect their environment?</i>	Explaining how the balance of the ecosystem can be disturbed.

Table 1: Specific examples of activities and focus questions show how Rhode Island science standards were incorporated into the PBL module (Rhode Island Department of Education).

What works:	Be Aware of:
Hand out post-it notes with Articles	The need to know your students
Have alternative activities (i.e. How to draw a lobster)	Group dynamics
Use parent volunteers if possible	The need to vary activities
Bring in visuals	The need to have alternate activities for different learning styles
Design lessons based on students' interests and questions	Transitions from one activity to the next
Use note cards for group sharing	Make attainable short-term student goals
Assign tasks to specific group members (ex. reader, recorder, etc.)	
Entrance/exit tickets	
End research intensive days with: •A quiet reflection •Group reflection/discussion •Drawing •Sharing	

Table 2: Advice for implementing a PBL at the elementary level gathered from our personal experiences and lessons-learned.

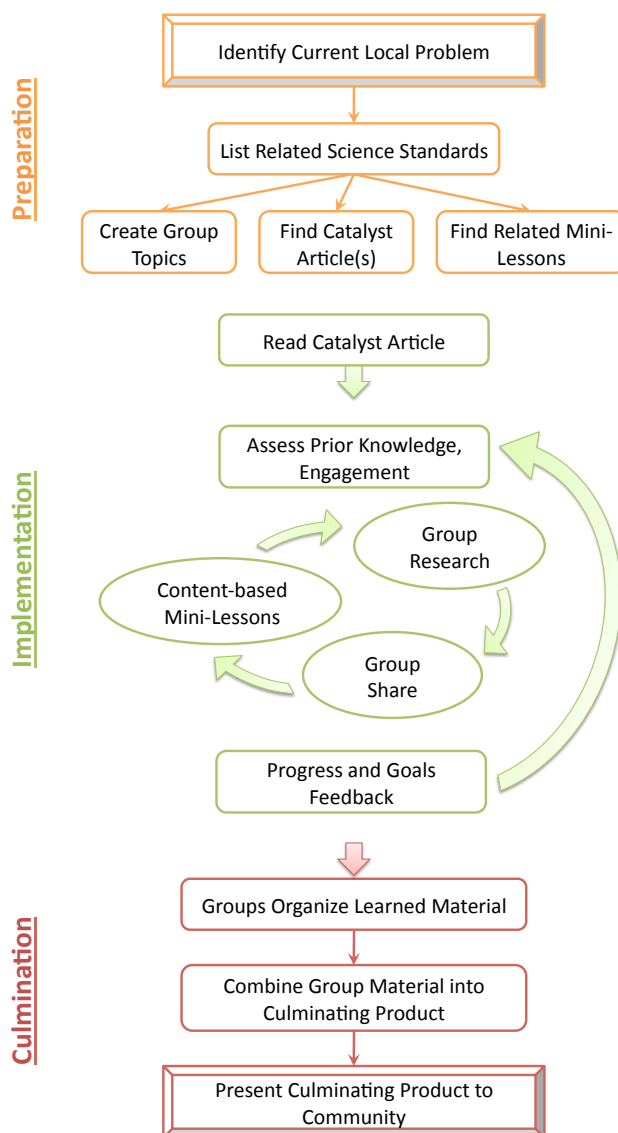


Figure 1: The PBL model at the elementary level begins with identifying a (preferably local and current) problem of interest that can be related to the science standards. The facilitator then prepares for the module by identifying topics for research groups, finding mini-lessons that directly address the standards, and finding an appropriate article or other resource that will spur student interest and enthusiasm. The implementation of the PBL consists of a flow of activities from student driven research to self-directed mini-lessons and labs and includes discussion for facilitator feedback and group share. Assessment occurs in the progress and feedback stage of the PBL, as well as, in grading the culminating products. Finally, groups synthesize what they have learned and bring their expert knowledge to the culmination product, which could vary from a play to a descriptive report or interview.



Figure 2: Fifth grade students at Vartan Gregorian elementary school explore the differences between the crusher and pincer claws of a lobster and hypothesize for what each claw is used.

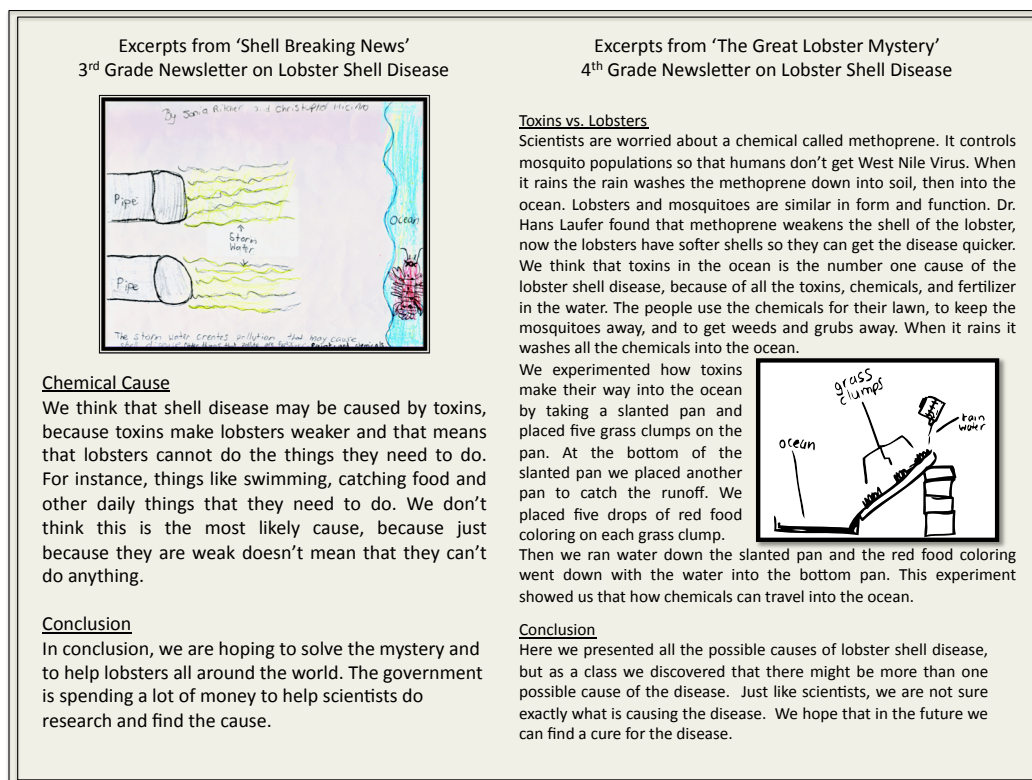


Figure 3. Shows examples of text and illustrations from the student-written newsletter that culminated the Lobster Shell Disease PBL. In the left panel is an example from a 3rd grade class at Vartan Gregorian Elementary (VG) and in the right panel is an example from a 4th grade class at VG.

Figure 4.

Rubric for Grading Lobster Shell Disease Newsletter Submissions

Score	Description
4	Newsletter provides a thorough explanation of the cause investigated by the group. Response includes relevant information on three of the five causes investigated by all groups.
3	Response provides an explanation of the cause investigated by the group. Response includes relevant information on two of the five causes investigated by all groups.
2	Response provides a partial explanation of the cause investigated by the group. Response includes limited information about one cause other than their own.
1	Response is vague or minimal.
0	Response is totally incorrect or irrelevant.

Causes of Lobster Shell Disease:

- Oil Spill
- Warm water
- Toxins
- Food chain
- Microbes

SUPPLEMENTARY INFORMATION: *Example Mini-Lesson Plan*

How Does Pollution Get from Land to the Ocean?

Toxins can be transported to the ocean in many ways. In the past, people dumped their trash directly into the ocean. Pollutants can also get to the ocean from rivers, the air, or through runoff. Runoff is rainwater that flows through the land and into the ocean.

Materials:

Long, shallow baking pan
Cookie sheet
Soil
Trowel
Red food coloring
Tap water
2-liter pitcher
6 blocks

Procedure:

1. Place the baking pan on the floor.
2. Place one end of the cookie sheet on the long edge of the pan. Use the blocks to raise the other end of the cookie sheet about 4 inches above the rim of the pan.
3. Cover the surface of the cookie sheet with soil.
4. With permission, use the trowel to dig up 5 small grass clumps. Set the grass on the soil-covered sheet.
NOTE: The grass clumps can be replaced in the ground when the experiment is done.
5. Squeeze 5-6 drops of food coloring at the base of each clump of grass.
6. Pour a shallow layer of water in the baking pan (about 1 inch).
7. Fill the pitcher with water.
8. Hold the pitcher at the raised end of the cookie sheet and slowly pour the water across the soil at the raised end of the sheet.

9. Observe the color of the water washing into the pan of water.

Read the following information:

Although we hear a lot about oil spills and trash as pollutants, because they are very visible - runoff is actually one of the most harmful sources of pollution to the bay and the ocean. Runoff can flow through farms, highways, city streets, lawns and mines. It can pick up the following pollutants and carry them to the ocean: fertilizers, pesticides, salt, oil, and all kinds of chemicals.

Use your results to answer the following questions in your notebook:

1. Assume that the red food coloring from your experiment is fertilizer. Where did the fertilizer end up?
2. If fertilizer helps grass or other plants to grow, what will it do to algae (plankton) in the bay?
3. When algae grow too quickly, it is called a bloom. The algae then die and are eaten by bacteria that use up all the oxygen in the water. What happens to fish, lobster and other animals in the water if bacteria use up the oxygen?
4. Based on your homework reading and the article on Lobster Shell Disease, what are alkylphenols found in and could they be transported via runoff?

EPILOGUE

EPILOGUE

The research presented in this dissertation was performed and compiled over the course of five years. As such, some chapters, which are already in the publication process are updated by subtle nuances in subsequent chapters. In particular I would like to clarify the following points:

1. In Chapter 1, the survey of sites from the semi-arid grasslands of the United States identified two clusters of long-chain alkenone lipid profiles. In the *Geochimica Cosmochimica et Acta* publication (Chapter1), we speculate that these two profiles indicate the presence of two different haptophyte taxa. Based on subsequent enrichment and culture studies (Chapters 4 and 5), there is the possibility that these two different profiles are instead the result of different life stages of the same haptophyte. Additional investigations are necessary to resolve this issue.
2. Both Chapters 7 and 8 present paleoreconstructions of from Lake George, North Dakota. The temperature record presented in Chapter 7 uses a different chronology than the summer drought record in Chapter 8, which uses a more recently obtained freeze core ^{210}Pb chronology in the mixed-effect regression model. The main differences between these two age models is whether the model is fit through calibrated age (9488 ± 20) at 1297cm dblf. Because the first (Chapter 7)

model does not go through this point, the resultant basal ages are up to 200 years younger than those presented in Chapter 8. Overall, there are few changes that occur in the alkenone-inferred temperature in this section of the core, and do not affect the interpretations of the data.

3. We now recognize that outlier data points in the latest calibration (Chapter 7) and in the alkenone-inferred temperature record (Chapter 7) have LCA profiles that include both the methyl and ethyl homologues of $C_{38:3}$ and do indicate that these points are likely produced by a different species or life stages of the haptophyte algae.

APPENDIX A. Depth sampling and corresponding age for Lake George, Core A,

Drives 1 to 13

Drive	Depth in Drive (cm)	Depth below lake floor (cm)	Age (yr BP)	Drive	Depth in Drive (cm)	Depth below lake floor (cm)	Age (yr BP)	Drive	Depth in Drive (cm)	Depth below lake floor (cm)	Age (yr BP)	Drive	Depth in Drive (cm)	Depth below lake floor (cm)	Age (yr BP)
1	0	20	32.6	3	24	244	891.1	5	8	448	1699.0	9	92	932	5267.1
1	2	22	34.6	3	27	247	903.3	5	11	451	1718.0	10	0	940	5298.4
1	3	23	56.8	3	29	249	911.5	5	16	456	1721.9	10	4	944	5329.1
1	5	25	70.1	3	32	252	923.9	5	13	457	1729.5	10	8	948	5359.5
1	7	27	87.1	3	35	255	936.3	5	19	459	1748.8	10	12	952	5389.3
1	10	30	88.2	3	37	257	944.5	5	24	464	1760.4	10	16	956	5418.7
1	12	32	90.4	3	38	258	948.7	5	27	467	1768.2	10	20	960	5447.6
1	17	37	98.7	3	40	260	956.9	5	29	469	1779.9	10	24	964	5476.0
1	22	42	123.9	3	43	263	969.4	5	32	472	1791.7	10	28	968	5531.2
1	25	45	138.6	3	45	265	977.7	5	35	475	1799.7	10	36	976	5558.0
1	27	47	148.3	3	48	268	990.1	5	37	477	1811.6	10	40	980	5584.4
1	32	52	172.0	3	51	271	1002.6	5	40	480	1843.8	10	44	984	5610.3
1	35	55	185.9	3	53	273	1010.9	5	48	488	1876.6	10	48	988	5635.8
1	37	57	195.0	3	59	279	1035.9	5	56	496	1944.4	10	52	992	5661.0
1	40	60	208.5	3	63	283	1052.6	5	72	512	1979.4	10	56	996	5710.6
1	42	62	217.3	3	64	284	1056.8	5	80	520	2015.3	10	64	1004	5735.1
1	45	65	230.4	3	65	285	1060.9	5	88	528	2070.7	10	68	1008	5783.7
1	47	67	239.0	3	66	286	1065.1	6	0	540	2089.7	10	76	1016	5832.1
1	50	70	251.7	3	67	287	1069.2	6	4	544	2128.3	10	84	1024	5856.3
1	52	72	260.1	3	68	288	1073.4	6	12	552	2148.0	10	88	1028	5880.6
1	55	75	272.5	3	69	289	1077.6	6	16	556	2188.3	10	92	1032	5905.0
1	57	77	280.7	3	70	290	1081.7	6	24	564	2229.6	10	96	1036	5917.3
1	60	80	292.7	3	71	291	1085.9	6	32	572	2272.1	11	0	1038	5942.1
1	62	82	300.7	3	72	292	1090.0	6	40	580	2315.8	11	4	1042	6018.0
1	65	85	312.5	3	73	293	1094.2	6	48	588	2454.8	11	16	1054	6044.0
1	67	87	320.3	3	75	295	1102.5	6	72	612	2503.9	11	20	1058	6070.4
1	70	90	331.8	3	76	296	1106.6	6	80	620	2554.4	11	24	1062	6097.4
1	72	92	339.4	3	77	297	1110.8	6	88	628	2743.8	11	28	1066	6124.9
1	75	95	350.7	3	78	298	1114.9	7	16	656	2801.6	11	32	1070	6152.9
1	77	97	358.1	3	79	299	1119.0	7	24	664	2861.2	11	36	1074	6181.6
1	80	100	369.2	3	80	300	1123.2	7	32	672	2922.7	11	40	1078	6211.0
1	82	102	376.5	3	81	301	1127.3	7	40	680	2985.9	11	44	1082	6241.1
1	85	105	387.3	3	82	302	1131.4	7	48	688	3050.8	11	48	1086	6271.9
1	87	107	394.5	3	83	303	1135.6	7	56	696	3083.9	11	52	1090	6303.6
1	90	110	405.2	3	85	305	1143.8	7	60	700	3117.4	11	56	1094	6336.1
1	92	112	412.2	3	88	308	1156.1	7	64	704	3151.2	11	60	1098	6369.5
1	95	115	422.8	3	91	311	1168.4	7	68	708	3185.4	11	64	1102	6439.3
2	0	120	440.1	4	0	320	1205.1	7	72	712	3219.9	11	72	1110	6551.9
2	3	123	450.4	4	3	323	1217.2	7	76	716	3254.8	11	84	1122	6612.1
2	5	125	457.3	4	5	325	1225.3	7	80	720	3325.4	11	92	1128	6653.8
2	8	128	467.5	4	8	328	1237.3	7	88	728	3379.2	12	4	1132	6696.9
2	11	131	477.7	4	11	331	1249.3	7	94	734	3433.5	12	8	1136	6787.0
2	13	133	484.4	4	13	333	1257.3	8	0	740	3470.0	12	16	1144	6834.2
2	16	136	494.6	4	16	336	1269.2	8	4	744	3506.8	12	20	1148	6883.0
2	19	139	504.7	4	16	336	1281.0	8	8	748	3543.7	12	24	1152	6933.2
2	21	141	511.4	4	19	339	1288.8	8	12	752	3618.2	12	28	1156	6985.0
2	24	144	521.5	4	21	341	1300.5	8	20	760	3769.2	12	32	1160	7093.7
2	27	147	531.5	4	24	344	1312.1	8	36	776	3807.2	12	40	1168	7150.5
2	29	149	538.2	4	27	347	1319.8	8	40	780	3845.4	12	44	1172	7208.9
2	32	152	548.3	4	29	349	1331.4	8	44	784	3883.6	12	48	1176	7268.9
2	35	155	558.4	4	32	352	1342.8	8	48	788	3960.2	12	52	1180	7330.4
2	37	157	565.2	4	35	355	1350.5	8	56	796	3998.6	12	56	1184	7393.3
2	40	160	575.3	4	37	357	1361.8	8	60	800	4037.0	12	60	1188	7457.7
2	43	163	585.5	4	40	360	1373.2	8	64	804	4075.4	12	64	1192	7523.4
2	45	165	592.3	4	43	363	1380.7	8	68	808	4113.9	12	68	1196	7590.5
2	48	168	602.6	4	45	365	1392.0	8	72	812	4152.3	12	72	1200	7658.7
2	51	171	612.9	4	48	368	1403.2	8	76	816	4190.7	12	76	1204	7728.2
2	53	173	619.8	4	51	371	1410.7	8	80	820	4229.1	12	80	1208	7816.7
2	56	176	630.3	4	53	373	1421.8	8	84	824	4267.4	13	0	1213	7888.7
2	59	179	640.8	4	56	376	1432.9	8	88	828	4381.9	13	4	1217	7961.7
2	61	181	647.9	4	59	379	1440.4	9	0	840	4419.9	13	8	1221	8035.7
2	64	184	658.6	4	61	381	1444.1	9	4	844	4457.7	13	12	1225	8110.7
2	67	187	669.4	3	62	382	1451.4	9	8	848	4533.1	13	16	1229	8186.5
2	69	189	676.7	4	64	384	1462.5	9	16	856	4570.5	13	20	1233	8224.8
2	72	192	687.7	4	67	387	1469.9	9	20	860	4607.8	13	22	1235	8243.9
2	75	195	698.7	4	69	389	1480.9	9	24	864	4644.9	13	23	1236	8263.2
2	77	197	706.2	4	72	392	1491.9	9	28	868	4681.8	13	24	1237	8282.5
2	80	200	717.4	4	75	395	1499.3	9	32	872	4718.5	13	25	1238	8301.8
2	83	203	728.8	4	77	397	1510.3	9	36	876	4755.0	13	26	1239	8340.6
2	85	205	736.4	4	80	400	1521.3	9	40	880	4791.2	13	28	1241	8418.8
2	88	208	747.8	4	83	403	1528.6	9	44	884	4827.2	13	32	1245	8497.6
2	91	211	759.4	4	85	405	1539.6	9	48	888	4863.0	13	36	1249	8577.1
2	93	213	767.2	4	88	408	1550.6	9	52	892	4898.5	13	40	1253	8657.1
2	96	216	778.8	4	91	411	1557.9	9	56	896	4933.6	13	44	1257	8737.7
3	0	220	794.6	4	93	413	1568.9	9	60	900	4968.5	13	48	1261	8900.2
3	3	223	806.4	4	96	416	1580.0	9	64	904	4985.9	13	56	1269	8982.0
3	5	225	814.4	4	99	419	1587.3	9	66	906	5003.1	13	60	1273	9064.1
3	8	228	826.3	4	101	421	1598.4	9	68	908	5037.4	13	64	1277	9229.1
3	11	231	838.3	4	104	424	1657.7	9	72	912	5071.3	13	72	1285	9311.8
3	13	233	846.4	5	0	440	1668.9	9	76	916	5104.9	13	76	1289	9394.7
3	16	236	858.5	5	3	443	1676.4	9	80	920	5138.1	13	80	1293	9477.6
3	19	239	870.7	5	5	445	1687.7	9	84	924	5171.0	13	84	1297	9553.8
3	21	241	878.8					9	88	928	5203.4	13	88	1301	9635.0

APPENDIX B. Long-chain alkenone concentration data for alkenone-containing lakes in Canada

	Std	Std	Sed												
	(ug/ml)	Response	mass												
			(g)	C ₃₇	C _{37:4}	C _{37:3}	C _{37:2}	C _{38:4}	C _{38:3}	C _{38:2}	UK38	UK37	UK37'	LG	%
														Calib	37:4
Redberry	70.05	3.93	5.51	199.3	46.3	13.2	2.2	11.9	11.0	2.4	-0.37	-0.71	0.14	7.5	75.1
Dead Moose	70.05	5.61	2.90	2357.8	396.9	124.2	25.9	71.8	109.2	34.8	-0.17	-0.68	0.17	9.2	72.6
Charron	70.05	6.28	3.13	484.8	91.9	37.2	7.1	18.1	26.6	9.4	-0.16	-0.62	0.16	12.0	67.5
Rabbit	70.05	124.70	9.42	2.7	30.5	11.9	2.5	6.3	7.4	2.3	-0.25	-0.62	0.18	12.0	67.8
Antelop	70.05	57.09	5.55	120.7	359.4	149.5	36.8	70.9	107.1	42.6	-0.13	-0.59	0.20	13.5	65.9
L. Man	70.05	10.45	3.11	1232.6	369.1	165.6	37.1	64.0	101.9	43.3	-0.10	-0.58	0.18	14.0	64.6
Waldsea	70.05	3.15	2.99	5110.3	423.0	226.0	38.2	88.2	127.2	46.9	-0.16	-0.56	0.14	15.0	61.6
Fishing	70.05	175.69	2.79	71.8	301.1	180.8	20.7	217.5	164.5	28.5	-0.46	-0.56	0.10	15.1	59.9
Lenore	70.05	136.00	5.15	6.9	40.7	25.2	2.8	19.2	15.9	1.1	-0.50	-0.55	0.10	15.4	59.3
Shannon	70.05	200.30	5.22	50.6	434.2	289.1	32.4	319.0	283.1	68.2	-0.37	-0.53	0.10	16.4	57.5
Humboldt	70.05	184.67	3.70	21.3	107.5	87.5	13.1	43.2	49.5	17.0	-0.24	-0.45	0.13	20.1	51.7
Snake Hold	70.05	131.57	2.68	3.3	8.3	7.3	0.9	2.4	5.0	1.3	-0.13	-0.45	0.11	20.5	50.3
Success	70.05	103.38	1.15	584.2	472.2	450.2	71.5	159.2	450.3	###	-0.05	-0.40	0.14	22.6	47.5

Lakes in Canada, Sampled in August of 2008. Received from P. Leavitt in Sept 2009.

APPENDIX C. Environmental data and calculations for alkenone-containing lakes in Canada

	Latitu de (deg N)	Longitu de (deg W)	Strat.	Vol. (m3 x 106)	Surf. Area (km2)	Elev. (m)	Zmax (m)	Salinity (g L-1)	Cond.(µS cm- 1)	Temp °C	Strat.	pH	HC03 (mg L- 1)	DOC	Cl	SO4	Na	K	Ca	Mg	TKN (µg L- 1)	TDP	dD	dO	SO4:CO3
Redberry	52.43	107.09	T	532	61	502	16	12	17480	19.20	T	9	412	39	273	17584	230	230	18	3020	1973	35	-65	-6	43
Dead Moose	52.19	105.10	TM	75	11	539	30	19	26250	18.60	TM	9	135	41	7136	14673	5950	303	48	1900	2607	62	-75	-7	109
Charron	52.24	104.20	N	14	4	556	5	7	10640	18.50	N	9	802	39	424	8689	1490	210	46	1630	2491	131	-67	-5	11
Rabbit	52.36	107.00	N	9	5	504	5	6	8530	19.30	N	9	108	36	157	7296	134	134	33	1230	2214	57	-81	-7	67
Antelop	50.17	108.24	N	63	14	701	5	11	17060	21.40	N	9	914	68	524	12478	3670	265	14	1650	4062	25	-79	-7	14
L. Man	52.09	105.60	N	71	19	544	6	2	2383	19.40	N	9	368	21	176	1271	199	64	104	240	1474	111	nd	-8	3
Waldsea	52.17	105.12	TM	37	5	533	13	18	22650	18.30	TM	9	402	31	4973	20214	317	317	325	3360	2027	45	-100	-7	50
Fishing	51.50	103.30	N	176	32	529	20	2	3589	19.10	N	9	461	19	64	2366	344	76	98	406	1187	16	-68	-7	5
Lenore	51.45	105.30	T	40	13	493	4	51	66800	20.20	T	9	168	159	19009	49690	905	905	48	10950	6337	437	-86	-5	297
Shannon	52.38	105.26	N	3	1	549	7	2	3986	18.90	N	9	323	23	51	2936	110	110	59	640	1465	40	-67	-6	9
Humboldt	52.30	104.59	N	1	1	537	10	3	1056	19.80	N	8	251	35	215	4670	748	169	40	900	2233	28	-85	-8	19
Snake Hold	50.30	108.28	ND	nd	nd	858	3	53	70600	21.40	ND	5	136	402	14043	77307	18400	934	87	12400	2067	2067	-86	-6	567
Success	50.29	108.01	TM	3	1	715	20	28	26130	19.00	TM	10	1771	53	1286	20182	406	406	5	2400	2951	84	-83	-6	11

Environmental data from Latitude to dO are from:
Pham, S.V., Leavitt, P.R., McGowan, S., Wissel, B., and Wassenaar, L.I. (2009) Spatial and temporal variability of prairie lake hydrology as revealed using stable isotopes of hydrogen and oxygen. *Limnology and Oceanography* **54**(1), 101-118.

APPENDIX D. ^{210}Pb Age model data for Brush Lake, MT – short core

Sample	Depth (cm)	Excess Pb (dpm/gr)	Ln(excess ^{210}Pb)	^{210}Pb age (year AD)	Error
BR07A 0-02cm	1	18.90	2.94	2002	5.14%
BR07A 02-04cm	3	16.67	2.81	1991	3.29%
BR07A 04-06cm	5	15.11	2.72	1980	3.13%
BR07A 06-08cm	7	9.76	2.28	1969	4.33%
BR07A 08-10cm	9	6.91	1.93	1958	5.28%
BR07A 10-12cm	11	2.04	0.72	1947	9.40%
BR07A 12-16cm	14				
BR07A 16-20cm	18	2.08	0.73	1908	6.74%
BR07A 20-24cm	22	0.45	-0.80	1886	10.58%
BR07A 24-28cm	26	-0.44			23.86%
BR07A 28-32cm	30				
BR07A 32-36cm	34	-0.29			16.13%
BR07A 36-40cm	38				
BR07A 40-44cm	42	-0.44			25.42%
BR07A 44-48cm	46				
BR07A 48-52cm	50				
BR07A 52-56cm	54	-0.62			23.38%
BR07A 56-60cm	58				

APPENDIX E. Age control for Brush Lake, MT using Calib 5.0

CAMS#	Depth (cm)	¹⁴ C age	SD	cal (lower)	cal (50% median)	cal (upper)	
113589	10	1880	35	1721	1826	1890	
109291	40	2300	40	2156	2321	2361	
113590	79.5	2405	35	2345	2434	2696	
109292	161	3030	40	3081	3247	3356	
109293	391	4080	40	4438	4583	4811	
109294	693	6205	40	7000	7096	7246	
113591	736	6645	35	7462	7529	7581	
113592	4674	8380	35	9302	9421	9481	slump
113593	4727	8990	35	9939	10191	10236	slump
116256	4752	7575	40	x	x	x	slump
113594	4792	7295	35	8023	8103	8175	
113595	4897	8660	35	9542	9602	9687	
113596	5046	10555	35	12396	12598	12732	
109295	5150	10675	40	12665	12753	12821	

APPENDIX F. Brush Lake, MT – Compound specific dD acids

Age	dD C24	Age	dD C26	Age	dD C28
1778	x	1778	x	1778	x
1895	x	1895	x	1895	x
2088	x	2088	x	2088	x
2186	x	2186	x	2186	x
2234	-231.74	2234	-207.99	2234	-210.28
2282	x	2282	x	2282	x
2328	-195.73	2328	-193.13	2328	-211.33
2375	x	2375	x	2375	x
2420	-187.72	2420	-164.63	2420	-210.54
2465	x	2465	-196.94	2465	-202.90
2509	-197.83	2509	-222.28	2509	-223.86
2553	-220.24	2553	-200.85	2553	-219.80
2596	-215.11	2596	-219.30	2596	-220.45
2639	x	2639	-177.30	2639	-187.21
2681	-212.28	2681	-216.07	2681	-230.55
2764	-205.83	2764	-203.87	2764	-228.36
2804	-184.54	2804	-164.29	2804	-220.40
2844	-203.71	2844	-190.76	2844	-225.71
2884	-191.78	2884	-211.14	2884	-220.35
2923	-228.05	2923	-213.88	2923	-224.61
2962	-209.09	2962	-201.59	2962	-220.91
3000	-196.99	3000	-212.10	3000	-218.25
3045	x	3045	x	3045	x
3075	-181.39	3075	-175.34	3075	-218.12
3148	-193.37	3148	-194.65	3148	-213.92
3185	-201.53	3185	-217.46	3185	-220.00
3220	-213.95	3220	-220.61	3220	-232.26
3256	x	3256	x	3256	x
3291	-215.93	3291	-200.62	3291	-185.47
3332	x	3332	x	3332	x
3460	-215.23	3460	-237.09	3460	-233.07
3526	-219.24	3526	-218.50	3526	-234.35
3591	x	3591	x	3591	x
3654	-199.05	3654	-202.66	3654	-231.98
3686	-204.98	3686	-211.75	3686	-231.30
3717	-194.81	3717	-204.86	3717	-219.93
3748	-191.31	3748	-202.65	3748	-213.97
3779	-206.77	3779	-230.80	3779	-230.47
3810	-216.42	3810	-214.02	3810	-228.21
3840	x	3840	x	3840	x
3870	-201.65	3870	-215.47	3870	-227.88
3900	x	3900	x	3900	x
3930	-208.18	3930	-216.05	3930	-218.68
3990	-219.19	3990	-222.70	3990	-217.56
4020	-202.43	4020	-203.17	4020	-180.15
4049	-207.81	4049	-206.56	4049	-213.45
4079	-222.47	4079	-205.43	4079	-220.32
4108	-198.87	4108	-210.15	4108	-210.77
4137	-212.83	4137	-215.71	4137	-223.37
4167	-194.38	4167	-212.40	4167	-222.94
4196	x	4196	x	4196	x
4225	-207.95	4225	-214.31	4225	-224.98
4254	-201.15	4254	-207.79	4254	-188.62
4283	-186.56	4283	-182.24	4283	-220.01
4312	x	4312	x	4312	x
4342	-187.53	4342	-183.83	4342	-225.95
4429	-219.41	4429	-204.38	4429	-238.40
4488	-239.40	4488	-220.84	4488	-241.82
4517	-194.59	4517	-208.03	4517	-212.74
4535	-202.37	4535	-200.49	4535	x
4547	x	4547	x	4547	x
4576	-192.95	4576	-216.90	4576	-219.13
4594	-183.96	4594	-208.90	4594	-220.32
4606	x	4606	x	4606	x
4624	-190.52	4624	-204.33	4624	-217.22
4636	-181.03	4636	-207.62	4636	-188.85
4654	-185.59	4654	-208.01	4654	x
4666	x	4666	x	4666	x
4684	-194.38	4684	-210.93	4684	-228.58
4696	-183.69	4696	-214.52	4696	-208.19
4714	-192.63	4714	-203.74	4714	x
4726	-205.14	4726	-223.99	4726	-229.62
4745	-190.40	4745	-210.28	4745	-214.46
4757	-195.42	4757	-213.60	4757	-220.66
4775	-185.03	4775	-210.51	4775	-195.87
4788	-233.34	4788	-238.42	4788	-248.62
4806	-199.44	4806	-213.13	4806	-224.96
4818	-180.39	4818	-208.53	4818	-206.67
4837	-191.80	4837	-212.23	4837	-229.75
4849	-201.87	4849	-241.66	4849	-255.11
4868	-201.22	4868	-220.30	4868	-227.82
4881	-183.37	4881	-215.02	4881	-216.49
4899	-194.72	4899	-219.80	4899	-226.90
4912	x	4912	x	4912	x
4944	-197.82	4944	-209.52	4944	-221.20
4976	-239.31	4976	-260.29	4976	-263.82
5008	-184.21	5008	-206.86	5008	-213.12
5040	-233.67	5040	-260.82	5040	-257.18
5073	-189.37	5073	-214.32	5073	-223.60
5139	-202.27	5139	-216.09	5139	-226.02
5173	-244.12	5173	-262.11	5173	-265.49
5207	-193.07	5207	-202.17	5207	-216.66
5276	-198.53	5276	-213.60	5276	-225.64
5311	-256.10	5311	-267.13	5311	-271.97
5346	-202.29	5346	-204.11	5346	-215.83

APPENDIX F. (cont.)

Age	dD C24	Age	dD C26	Age	dD C28
5418	-206.00	5418	-209.07	5418	-210.33
5491	-203.75	5491	-210.01	5491	-221.37
5566	-200.09	5566	-209.73	5566	-225.38
5604	x	5604	x	5604	x
5643	-212.14	5643	-205.90	5643	-227.59
5682	-209.59	5682	-209.62	5682	-223.76
5721	-216.75	5721	-219.90	5721	x
5761	-206.34	5761	-208.19	5761	-225.51
5802	-227.90	5802	-215.04	5802	x
5843	-215.56	5843	-218.01	5843	-230.98
5885	-213.59	5885	-218.28	5885	-220.36
5927	-212.46	5927	-224.11	5927	-223.57
5969	-215.94	5969	-209.41	5969	-233.10
5995	-212.11	5995	-219.87	5995	-218.75
6056	-211.85	6056	-216.30	6056	-229.32
6083	-214.88	6083	-222.15	6083	-237.49
6173	-210.53	6173	-219.04	6173	-223.75
6360	-183.40	6360	-212.11	6360	x
7066	-220.88	7066	-221.04	7066	-227.01
7427	-194.23	7427	-204.20	7427	-231.95
8096	-219.31	8096	-215.61	8096	-217.95
8224	x	8224	x	8224	x
8339	-207.41	8339	-217.45	8339	-221.90
8367	x	8367	x	8367	x
8438	-208.75	8438	-205.42	8438	-211.39
8481	-220.07	8481	-225.26	8481	-206.74
8510	x	8510	x	8510	x
8581	-210.73	8581	-207.83	8581	-207.22
8624	-207.66	8624	-218.40	8624	-235.40
8653	x	8653	x	8653	x
8724	-211.49	8724	-225.81	8724	-228.12
8767	-197.54	8767	-203.49	8767	-224.12
8795	x	8795	x	8795	x
8867	-201.58	8867	-208.88	8867	-209.82
8938	x	8938	x	8938	x
9010	-190.41	9010	-204.88	9010	-214.65
9052	-193.14	9052	-212.95	9052	-218.89
9081	x	9081	x	9081	x
9124	-193.73	9124	-203.14	9124	x
9152	-193.98	9152	-213.87	9152	-220.24
9195	-214.79	9195	-216.17	9195	-223.90
9224	x	9224	x	9224	x
9267	-212.78	9267	-216.38	9267	-222.72
9338	-209.48	9338	-213.46	9338	-221.25
9366	x	9366	x	9366	x
9438	-212.60	9438	-209.17	9438	-225.68
9481	-206.30	9481	-219.09	9481	-208.19
9509	x	9509	x	9509	x
9581	-222.83	9581	-214.42	9581	-225.08
9632	-209.95	9632	-212.79	9632	-218.31
9672	x	9672	x	9672	x
9732	-193.26	9732	-206.97	9732	x
9873	-284.35	9873	-273.59	9873	-261.33
10073	-246.52	10073	-233.43	10073	-233.58
10233	-197.67	10233	-207.32	10233	x
10273	-250.05	10273	-233.82	10273	-228.12
10474	-236.73	10474	-246.92	10474	-236.75
10674	x	10674	x	10674	x
10734	-210.80	10734	-214.44	10734	x
10875	-227.75	10875	x	10875	x
10875	-221.15	10875	-222.77	10875	x
10935	-216.07	10935	-215.92	10935	x
10995	-213.10	10995	-220.24	10995	x
11075	x	11075	x	11075	x
11075	-203.19	11075	-218.22	11075	x
11135	-192.04	11135	-216.91	11135	x
11175	-210.73	11175	-220.36	11175	x
11275	-237.99	11275	-232.85	11275	-240.74
11315	-198.02	11315	-216.42	11315	x
11476	x	11476	x	11476	x
11576	-233.57	11576	-233.74	11576	x
11596	-196.18	11596	-219.04	11596	x
11596	-198.61	11596	-218.75	11596	x
11616	-199.89	11616	-217.31	11616	x
11676	x	11676	x	11676	x
11696	-214.97	11696	x	11696	x
11716	-208.67	11716	-218.48	11716	x
11756	-205.86	11756	-214.61	11756	x
11776	-204.76	11776	-218.35	11776	x
11796	-217.39	11796	x	11796	x
11816	-225.71	11816	-217.66	11816	x
11836	-221.12	11836	-222.13	11836	x
11857	-231.59	11857	-232.64	11857	x
11877	-248.30	11877	-250.46	11877	-241.37
11897	-224.40	11897	-218.24	11897	x
12057	-206.39	12057	-194.17	12057	x
12077	-248.20	12077	-259.93	12077	-245.95
12097	-212.07	12097	-198.81	12097	x
12117	-199.82	12117	-206.86	12117	x
12257	-212.14	12257	-196.88	12257	x
12277	-252.35	12277	-249.95	12277	-249.22
12317	x	12317	x	12317	x
12337	-255.70	12337	-259.61	12337	-261.42
12358	-218.32	12358	-228.78	12358	x
12378	-190.66	12378	-153.58	12378	x

APPENDIX G. Long-chain alkenone data for Brush Lake, MT

Sample ID	Age (cal BP)	[C ₃₇] (ug/g sed)	UK'37	UK37	% C _{37:4}	C ₃₇ :C ₃₈
30	2088	0.47	0.20	-0.57	64.45	5.42
45	2234	0.79	0.16	-0.51	57.89	3.67
60	2375	0.74	0.19	-0.49	57.02	4.43
80	2553	26.58	0.19	-0.59	65.44	7.36
110	2804	3.49	0.18	-0.50	57.54	4.12
115	2844	1.86	0.29	-0.15	33.77	8.69
120	2884	0.21	0.24	-0.56	64.76	1.96
125	2923	40.97	0.20	-0.58	65.12	9.77
130	2962	5.33	0.24	-0.63	70.12	1.23
135	3000	7.29	0.20	-0.60	66.65	10.44
141	3045	2.34	0.15	-0.63	67.65	5.48
160	3185	1.91	0.23	-0.64	70.78	3.26
165	3220	12.37	0.19	-0.58	64.73	12.96
170	3256	1.95	0.14	-0.62	66.62	9.07
185	3359	0.00	nd	nd	nd	nd
190	3393	0.93	0.11	-0.60	64.23	1.80
195	3427	36.68	0.20	-0.63	69.39	2.08
200	3460	12.05	0.19	-0.53	60.58	7.22
210	3526	2.96	0.15	-0.68	71.80	3.97
220	3591	1.34	0.18	-0.56	62.72	6.40
225	3623	0.00	nd	nd	nd	nd
230	3654	1.37	0.15	-0.57	62.75	5.09
235	3686	4.69	0.18	-0.53	60.07	2.00
240	3717	29.63	0.18	-0.60	66.45	5.10
245	3748	0.00	nd	nd	nd	nd
250	3779	3.65	0.16	-0.53	59.50	4.61
255	3810	186.66	0.18	-0.68	72.59	3.23
260	3840	5.11	0.15	-0.53	59.29	1.22
265	3870	0.00	nd	nd	nd	nd
270	3900	2.47	0.16	-0.58	64.08	6.67
275	3930	3.13	0.14	-0.72	75.53	1.73
280	3960	4.04	0.17	-0.70	73.85	1.63
285	3990	185.52	0.17	-0.74	78.02	3.11
290	4020	0.33	0.20	-0.44	53.90	3.98
295	4049	0.00	nd	nd	nd	nd
300	4079	0.65	0.09	-0.53	56.88	nd
305	4108	0.00	nd	nd	nd	nd
315	4167	0.00	nd	nd	nd	nd
320	4196	5.80	0.16	-0.57	63.18	6.54
325	4225	0.00	nd	nd	nd	nd
330	4254	0.28	0.20	-0.52	59.92	2.20
335	4283	0.00	nd	nd	nd	nd
340	4312	0.49	0.20	-0.56	63.06	2.07
345	4342	6.26	0.21	-0.39	49.64	2.20
350	4371	0.36	0.21	-0.49	57.38	3.14
355	4400	0.00	nd	nd	nd	nd
360	4429	0.33	0.19	-0.43	52.45	3.59

APPENDIX G. (cont.)

Sample ID	Age (cal BP)	[C ₃₇] (ug/g sed)	UK'37	UK37	% C _{37:4}	C ₃₇ :C ₃₈
365	4458	1.99	0.16	-0.67	71.63	1.61
370	4488	5.59	0.19	-0.63	68.89	3.17
375	4517	51.38	0.21	-0.55	62.63	2.12
378	4535	105.46	0.21	-0.57	64.37	2.50
380	4547	61.11	0.17	-0.65	69.81	6.42
385	4576	47.33	0.19	-0.62	68.18	2.03
388	4594	0.00	nd	nd	nd	nd
390	4606	0.62	0.19	-0.46	54.73	3.57
393	4624	2.11	0.08	-0.59	62.24	1.35
395	4636	2.71	0.13	-0.71	74.26	2.11
398	4654	2.07	0.13	-0.54	58.83	1.59
403	4684	7.77	0.07	-0.84	84.60	2.68
405	4696	4.70	0.12	-0.77	79.48	2.94
408	4714	1.97	nd	-0.81	80.90	2.37
410	4726	4.99	0.17	-0.73	76.85	5.70
413	4745	103.47	0.17	-0.75	78.69	3.04
415	4757	7.81	0.13	-0.76	78.66	2.79
418	4775	15.82	0.10	-0.81	82.42	3.09
420	4788	11.37	0.14	-0.80	82.00	2.05
423	4806	76.79	0.17	-0.79	82.17	2.85
425	4818	6.11	0.14	-0.80	82.83	2.87
428	4837	90.88	0.17	-0.78	81.01	2.82
430	4849	14.40	0.19	-0.74	77.69	5.96
433	4868	41.58	0.18	-0.75	78.68	2.53
435	4881	3.62	0.14	-0.79	81.62	2.50
438	4899	114.60	0.17	-0.75	79.10	3.20
440	4912	0.67	0.22	-0.52	60.43	nd
445	4944	9.42	0.16	-0.69	73.16	2.51
455	5008	9.69	0.18	-0.76	79.73	3.02
465	5073	5.39	0.19	-0.71	nd	nd
475	5139	10.82	0.18	-0.77	75.37	2.65
485	5207	67.70	0.19	-0.74	80.37	3.08
495	5276	3.25	0.06	-0.75	77.91	2.69
505	5346	3.58	0.07	-0.77	76.88	2.40
515	5418	84.41	0.19	-0.64	78.89	2.07
525	5491	12.43	0.16	-0.68	69.50	3.19
535	5566	41.78	0.41	-0.82	72.29	3.77
545	5643	1.55	0.21	-0.69	87.16	2.42
550	5682	0.94	0.23	-0.52	74.82	nd
555	5721	4.34	0.11	-0.76	61.06	nd
565	5802	4.89	0.14	-0.68	78.20	nd
570	5843	0.10	0.40	-0.42	72.40	2.50
575	5885	109.18	0.18	-0.58	58.33	nd
583	5952	3.72	0.18	-0.66	64.33	2.50
585	5969	5.10	0.10	-0.67	71.38	3.56
588	5995	4.15	0.10	-0.69	71.95	1.77
595	6056	31.94	0.20	-0.63	69.20	2.72

APPENDIX G. (cont.)

Sample ID	Age (cal BP)	[C ₃₇] (ug/g sed)	UK'37	UK37	% C _{37:4}	C ₃₇ :C ₃₈
595	6056	1.42	0.21	-0.69	74.51	2.77
598	6083	19.82	0.16	-0.63	68.38	3.08
605	6145	3.91	0.14	-0.70	73.95	2.34
608	6173	45.88	0.19	-0.64	69.76	2.72
615	6237	6.05	0.08	-0.78	79.80	3.52
618	6265	9.47	0.14	-0.74	77.02	3.21
620	6284	0.35	0.30	-0.42	55.32	nd
628	6360	5.60	0.08	-0.77	78.53	2.04
630	6379	0.47	0.29	-0.44	56.95	4.79
635	6428	0.00	nd	nd	nd	nd
638	6457	0.00	nd	nd	nd	nd
640	6477	0.15	0.27	-0.60	68.57	nd
645	6537	10.63	0.08	-0.77	78.74	2.68
648	6567	7.60	0.17	-0.71	75.04	2.53
655	6639	2.06	0.10	-0.73	75.12	1.96
658	6670	37.26	nd	-0.73	76.71	2.89
665	6744	4.89	0.07	-0.82	83.14	2.32
668	6776	2.29	nd	-0.77	76.65	1.50
675	6852	4.19	0.10	-0.78	80.21	2.52
678	6885	3.28	0.15	-0.73	76.39	2.39
685	6963	0.00	nd	nd	nd	nd
688	6997	5.90	0.21	-0.74	78.82	2.88
690	7009	89.58	0.18	-0.79	82.57	6.13
695	7077	0.00	nd	nd	nd	nd
698	7112	4.19	0.17	-0.74	77.69	2.01
700	7124	5.69	0.17	-0.80	83.32	6.44
705	7195	18.19	0.15	-0.72	75.57	3.16
708	7231	5.77	0.19	-0.77	80.90	2.19
715	7315	5.17	0.22	-0.76	79.95	2.99
718	7352	4.52	0.14	-0.79	81.42	2.73
720	7364	0.25	0.23	-0.56	64.14	nd
725	7439	25.44	0.23	-0.66	72.30	2.42
728	7477	9.41	0.14	-0.80	82.05	2.89
735	7567	0.00	nd	nd	nd	nd
738	7606	4.53	0.23	-0.77	81.21	2.45
4791	8096	3.71	0.12	-0.53	58.44	nd
4793	8139	50.10	0.17	-0.79	81.72	3.00
4795	8167	6.14	0.20	-0.68	73.41	2.63
4795	8167	19.55	0.21	-0.65	70.63	2.57
4798	8210	101.21	0.15	-0.63	67.76	2.35
4800	8239	96.72	0.15	-0.70	74.26	2.50
4803	8281	4.73	0.20	-0.70	75.02	2.65
4805	8310	10.37	0.17	-0.70	74.39	2.57
4808	8353	110.98	0.11	-0.68	70.96	2.53
4810	8381	192.79	0.14	-0.71	74.40	2.47
4813	8424	168.98	0.17	-0.71	75.33	3.03
4815	8453	103.79	0.17	-0.69	73.85	2.58

APPENDIX G. (cont.)

Sample ID	Age (cal BP)	[C ₃₇] (ug/g sed)	UK'37	UK37	% C _{37:4}	C ₃₇ :C ₃₈
4818	8496	14.07	0.18	-0.55	61.66	2.51
4818	8496	7.53	0.19	-0.55	62.46	2.25
4820	8524	41.09	0.18	-0.62	67.71	3.09
4823	8553	38.95	0.15	-0.40	48.06	2.79
4825	8581	74.50	0.15	-0.15	26.22	1.80
4828	8634	26.08	0.15	-0.17	28.06	1.97
4830	8653	28.18	0.17	-0.07	20.26	2.26
4833	8695	59.39	0.18	-0.79	82.16	2.71
4835	8710	321.88	0.14	-0.50	55.92	1.66
4838	8738	40.11	0.14	-0.47	53.15	1.87
4840	8795	0.00	nd	nd	nd	nd
4843	8781	132.74	0.18	-0.72	76.58	2.50
4845	8867	116.29	0.18	-0.73	76.93	2.84
4848	8910	224.13	0.18	-0.79	82.43	2.94
4850	8938	0.00	0.09	-0.63	65.93	nd
4853	8981	13.06	0.13	-0.83	84.91	3.22
4855	9010	13.94	0.16	-0.18	29.78	0.85
4858	9052	36.66	0.20	-0.64	69.69	2.55
4860	9081	18.03	0.19	-0.50	58.13	1.07
4863	9124	2.99	0.15	-0.19	29.76	1.36
4865	9152	14.58	0.13	-0.11	21.20	0.64
4868	9195	12.64	0.17	-0.26	36.75	1.49
4870	9224	36.35	0.13	-0.21	30.49	0.77
4873	9267	37.31	0.04	-0.50	52.09	1.67
4875	9295	35.03	0.19	-0.44	53.12	2.54
4878	9338	15.36	0.15	-0.76	79.06	2.24
4880	9366	39.74	0.19	-0.46	54.11	nd
4883	9409	16.11	0.20	-0.30	41.40	2.97
4885	9438	43.81	0.15	-0.72	75.77	2.33
4888	9481	49.15	0.13	-0.27	35.77	0.97
4890	9509	38.10	0.38	-0.64	73.61	1.46
4893	9552	5.83	0.18	-0.39	48.67	1.63
4895	9581	7.47	0.20	-0.46	55.29	2.28
4898	9632	0.00	nd	nd	nd	nd
4900	9672	8.14	0.16	-0.43	51.07	2.17
4903	9732	265.00	0.13	-0.80	82.19	2.35
4905	9772	358.85	0.16	-0.81	83.90	2.98
4908	9832	499.28	0.13	-0.83	85.18	2.65
4910	9873	167.66	0.11	-0.65	68.79	nd
4913	9933	46.30	0.14	-0.51	56.80	1.84
4915	9973	168.26	0.15	-0.81	83.39	3.39
4918	10033	236.34	0.17	-0.79	82.07	2.48
4920	10073	115.71	0.11	-0.62	66.04	nd
4923	10133	92.23	0.17	-0.57	63.30	1.98
4925	10173	98.36	0.15	-0.62	67.05	2.43
4928	10233	44.70	0.17	-0.61	66.79	2.72
4930	10273	12.47	0.09	-0.57	60.78	nd

APPENDIX G. (cont.)

Sample ID	Age (cal BP)	[C ₃₇] (ug/g sed)	UK'37	UK37	% C _{37:4}	C ₃₇ :C ₃₈
4933	10333	53.58	0.15	-0.70	73.89	2.71
4935	10374	14.70	0.14	-0.44	51.40	2.73
4940	10474	110.40	0.13	-0.78	80.86	2.23
4943	10534	50.06	0.13	-0.80	82.33	2.58
4945	10574	17.42	0.11	-0.40	45.71	2.98
4948	10634	8.61	0.13	-0.62	66.39	2.64
4950	10674	14.73	0.17	-0.57	63.42	1.87
4953	10734	117.97	0.15	-0.76	79.14	2.25
4955	10774	57.80	0.05	-0.31	34.51	0.77
4958	10834	12.43	0.13	-0.60	64.87	1.54
4960	10875	29.18	0.15	-0.50	56.08	1.56
4963	10935	9.35	0.11	-0.54	58.65	1.30
4965	10975	146.53	0.05	-0.38	41.49	0.73
4966	10995	216.60	0.11	-0.16	24.72	nd
4968	11035	46.85	0.12	-0.31	37.85	0.96
4970	11075	33.47	0.18	-0.75	79.08	3.09
4973	11135	55.17	0.13	0.10	2.82	0.58
4975	11175	81.60	0.14	-0.60	64.78	1.80
4978	11235	77.45	0.15	-0.55	61.00	1.37
4983	11335	323.45	0.10	-0.72	74.26	2.35
4985	11376	537.46	0.10	-0.73	75.13	2.42
4988	11436	358.50	0.11	-0.65	68.56	2.06
4990	11476	468.61	0.11	-0.55	59.49	nd
4993	11536	0.78	0.13	-0.71	74.34	2.21
4995	11576	526.65	0.10	-0.59	62.45	nd
4996	11596	221.15	0.10	-0.63	66.26	nd
4997	11616	163.73	0.10	-0.63	66.14	nd
4998	11636	374.75	0.15	-0.79	81.68	nd
5000	11676	58.55	0.09	-0.59	61.94	nd
5001	11696	59.35	0.09	-0.64	66.99	nd
5002	11716	74.06	0.08	-0.64	66.90	nd
5004	11756	416.86	0.11	-0.65	68.06	nd
5005	11776	140.82	0.10	-0.68	70.76	nd
5006	11796	335.08	0.14	-0.82	84.13	nd
5007	11816	278.94	0.18	-0.79	81.83	nd
5008	11836	5.92	0.13	-0.82	83.66	nd
5009	11857	42.67	0.09	-0.60	63.74	nd
5010	11877	13.67	0.18	-0.67	72.39	nd
5011	11897	17.14	0.14	-0.52	57.92	nd
5012	11917	126.37	0.11	-0.45	50.26	nd
5014	11957	1.26	0.13	-0.43	49.38	nd
5017	12017	0.11	nd	nd	nd	nd
5018	12037	0.10	0.12	-0.01	11.40	nd
5019	12057	0.00	nd	nd	nd	nd
5020	12077	67.66	0.13	-0.68	71.85	nd
5021	12097	2.71	0.10	-0.17	25.11	nd
5022	12117	0.32	0.15	-0.16	27.10	nd
5023	12137	0.00	nd	nd	nd	nd
5024	12157	0.00	nd	nd	nd	nd
5025	12177	0.00	nd	nd	nd	nd
5026	12197	0.00	nd	nd	nd	nd
5027	12217	0.00	nd	nd	nd	nd
5028	12237	0.00	nd	nd	nd	nd
5029	12257	0.00	nd	nd	nd	nd
5030	12277	0.00	nd	nd	nd	nd
5032	12317	0.00	nd	nd	nd	nd
5033	12337	0.00	nd	nd	nd	nd
5034	12358	0.00	nd	nd	nd	nd
5035	12378	0.00	nd	nd	nd	nd

APPENDIX H. Carbon stable isotopes for Brush Lake, MT

Age (cal BP)	C ₂₄	C ₂₆	C ₂₈	C ₃₀
1778	-26.9	-27.2	-29.3	-27.8
1895	-21.6	-21.1	-25.9	-23.9
2186	-24	-23.6	-25.3	-25.6
2282	-28.3	-27.8	-29.5	-28.7
2375	-29	-29	-28.4	-28.9
2465	-27.5	-26.6	-27.5	-27.4
2639	-26.7	-29	-33.5	-29
2962	-24.4	-24.9	-25.6	-26.1
3045	-25.8	-25.9	-27.3	-27.6
3112	-28.9	-27.9	-28.6	-29.7
3332	-25.9	-26.1	-27.5	-26.7
3460	-30.5	-28	-30.2	-28.2
3526	-27.2	-27.8	-29.4	-28.6
3654	-28.2	-27.8	-28.8	-29.2
3779	-29.1	-26.7	-27	-26.8
3840	-28.8	-28.9	-27.8	-29.3
3900	-26.9	-26.1	-26.6	-26.6
3960	-25.7	-25.4	-26.2	-26.5
4079	-28.6	-26.9	-27.9	-27.6
4196	-30.2	-28.4	-30.6	-28.6
4254	-26.4	-28.2	-36.3	-29.2
4312	-28.3	-29.5	-29.9	-27.5
4371	-27.2	-28.2	-28.3	-28.5
4429	-27.3	-27.5	-27.9	-28.5
4488	-27.4	-27.8	-28.1	-28.4
4547	-31.3	-29.9	-28.3	-27
4606	-29	-26.6	-27.3	-27.7
4666	-28.1	-26.8	-27.2	-27.2
4788	-23.1	-23.5	-25.1	-26.1
4849	-28.9	-25.9	-26.3	-29.2
4912	-27.3	-28.2	-27.5	-28.7
4976	-29.5	-25.8	-27.3	-28.4
5040	-27.6	-25.4	-26.9	-27.7
5311	-26.3	-25.5	-26.6	-27.1
5604	-25.2	-26.3	-26.4	-27.4
6012	-24.6	-25.4	-26.3	-26.3
6100	-25.7	-26.3	-27.3	-27.2

APPENDIX I. Acid leaf wax chain length concentrations for Kettle Lake, ND

Depth (cm)	Age (cal BP)	C ₁₆ (ug/g sed)	C ₁₈ (ug/g sed)	C ₂₀ (ug/g sed)	C ₂₂ (ug/g sed)	C ₂₄ (ug/g sed)	C ₂₆ (ug/g sed)	C ₂₈ (ug/g sed)	C ₃₀ (ug/g sed)	C ₃₂ (ug/g sed)
2080	4974	71.51	64.61	267.23	157.90	66.90	21.97	0.00	0.00	42.01
2085	5033	218.84	163.25	456.89	1788.12	1658.56	2622.72	1139.98	491.16	73.78
2090	5091	106.23	110.01	265.50	1163.62	852.11	1102.53	390.03	164.05	20.01
2095	5150	79.61	89.19	341.77	195.16	936.15	891.28	244.48	103.92	25.80
2100	5208	106.02	93.57	295.62	1755.85	1121.67	1431.27	515.53	204.95	87.60
2105	5267	18.47	17.24	57.25	280.51	213.17	356.04	147.34	73.62	4.42
2110	5326	99.38	52.34	146.63	848.69	666.24	1151.00	563.54	296.03	26.96
2115	5384	40.58	31.60	81.08	450.02	309.94	356.10	167.38	68.62	9.36
2120	5443	65.48	114.41	43.97	694.71	387.88	443.26	166.35	59.39	24.85
2125	5501	29.26	26.46	90.25	581.06	481.17	527.46	255.57	105.48	7.77
2130	5527	34.72	31.35	85.79	376.36	331.57	461.01	310.47	148.56	8.18
2135	5545	60.78	45.52	127.77	588.93	795.12	1521.31	1013.44	18.67	7.89
2140	5563	23.03	24.33	85.25	388.21	366.88	438.31	268.12	119.10	4.74
2145	5580	74.01	52.12	118.00	508.62	558.86	784.32	391.47	167.30	6.43
2150	5598	53.91	46.13	119.03	442.47	436.30	877.27	553.22	330.23	6.52
2155	5656	24.19	27.99	78.78	290.24	314.80	572.52	366.56	183.71	4.49
2160	5742	53.39	53.33	98.43	361.24	341.50	545.28	294.59	125.88	5.84
2165	5828	28.84	30.80	84.53	281.97	265.95	360.89	240.51	117.16	7.66
2170	5914	35.47	28.10	74.82	304.85	312.39	560.64	414.11	218.06	4.03
2175	5999	57.91	40.25	113.74	421.98	465.65	976.67	518.21	269.78	7.39
2180	6085	37.24	38.34	107.65	544.74	580.93	734.67	379.85	161.93	6.86
2185	6125	3.61	0.00	2.84	8.55	7.19	8.11	4.07	2.00	0.64
2190	6165	33.65	32.28	77.89	318.13	349.55	394.06	169.29	56.15	8.76
2195	6205	37.68	39.17	102.92	535.85	576.02	895.62	475.06	195.09	3.60
2200	6245	43.75	58.35	141.56	397.10	356.04	371.86	190.76	80.55	4.61
2205	6285	24.83	27.78	84.79	434.68	448.14	527.71	241.29	92.48	4.20
2210	6325	28.72	28.41	80.02	497.57	455.42	363.28	187.43	65.61	10.75
2215	6365	31.85	33.21	88.00	484.26	425.55	743.09	295.15	123.41	5.51
2220	6405	111.49	69.12	247.99	943.02	872.38	2233.80	1150.03	556.40	0.00
2225	6445	32.57	40.14	99.99	427.31	432.23	474.51	183.41	54.92	16.34
2230	6485	41.97	47.95	143.05	877.05	484.88	588.80	253.03	103.01	19.12
2235	6525	50.94	54.16	25.05	1139.14	960.23	1029.52	526.91	214.70	20.88
2240	6565	61.21	45.04	160.48	648.97	703.55	1376.45	669.12	288.74	14.52
2245	6605	39.66	31.72	129.72	872.90	589.93	1047.67	363.52	159.64	18.61
2250	6645	56.07	46.99	154.79	691.73	711.69	1288.92	528.78	252.99	20.26
2255	6685	19.41	24.08	100.38	386.04	296.88	388.61	188.48	73.62	6.98
2260	6793	56.91	34.89	151.10	108.66	811.35	1991.52	919.25	532.00	11.20
2265	6826	39.06	39.98	139.24	692.56	625.08	766.43	335.80	121.56	11.53
2270	6860	20.39	25.22	86.66	460.41	447.61	503.94	251.00	99.71	7.97
2275	6893	24.73	24.52	76.04	324.33	284.98	303.61	162.04	53.82	10.93
2280	6926	45.38	45.73	141.82	479.14	473.61	934.32	409.11	181.58	15.16
2285	6960	34.08	36.70	113.80	623.30	526.17	516.46	253.36	88.01	17.72
2290	6993	25.32	27.06	121.50	390.91	294.15	695.66	310.71	165.18	3.76
2295	7027	31.90	31.01	95.54	511.08	349.39	669.29	232.57	87.30	13.69
2300	7060	18.64	21.83	70.75	262.86	39.79	513.56	255.37	117.76	3.28
2305	7093	41.49	37.83	80.22	286.54	315.22	732.28	379.35	187.39	7.02
2310	7127	34.82	34.93	103.28	511.33	465.14	828.54	342.72	153.06	8.77
2315	7160	28.42	30.12	87.61	444.93	424.52	517.58	226.32	80.62	12.51
2320	7194	32.25	31.70	109.75	470.18	434.47	958.66	500.29	234.94	5.96
2325	7217	18.16	0.00	14.56	70.19	41.80	32.95	11.22	0.00	0.00
2330	7234	34.17	39.59	136.96	586.09	586.46	918.58	448.23	189.41	11.38
2335	7250	47.93	45.94	130.27	567.03	433.82	807.52	382.34	178.64	15.63
2340	7267	21.14	26.56	101.93	572.33	436.56	446.57	265.68	117.60	8.13
2345	7284	24.31	27.30	68.35	312.12	295.75	437.26	213.04	82.08	9.92
2350	7301	10.75	10.50	29.43	130.73	157.49	369.71	181.81	96.09	2.44
2355	7317	28.59	29.54	87.34	369.94	425.12	741.70	379.51	153.84	11.08
2360	8737	45.76	41.27	97.47	440.38	538.56	1052.71	408.97	177.78	29.32
2365	10372	13.39	19.34	52.71	224.28	265.79	444.72	201.73	74.45	3.76
2650	8737	55.57	59.54	169.37	792.49	666.31	728.37	458.19	195.14	41.84
2900	10372	107.01	163.06	485.48	1361.74	1913.83	2308.52	836.76	160.81	128.41

APPENDIX J. Acid leaf wax hydrogen stable isotopes for Kettle Lake, ND

Depth (cm)	NEW Age	C ₂₂	C ₂₄	C ₂₆	C ₂₈	C ₃₀
2080	4974	-209.09	-228.6	-229.08	-231.24	-206.13
2085	5032.6	-202.59	-209.69	-223.84	-219.58	-224.95
2090	5091.2	-194.17	-212.44	-219.92	-208.37	-222.57
2095	5149.8	-179.19	-195.23	-208.21	-188.73	-219.55
2100	5208.4	-201.35	-187.67	-219.66	-205.25	-224.44
2105	5267	-195.71	-201.83	-225.25	-229.69	-224.78
2110	5325.5	-206.35	-204.23	-234.11	-231.13	-227.18
2115	5384.1	-194.27	-207.31	-230.06	-227.78	-215.23
2120	5442.7	-174.32	-178.16	-226.07	-215.08	nd
2125	5501.3	nd	-172.68	-208.51	-200.86	nd
2130	5527.2	-213.12	-223.37	-235.96	-237.45	-226.65
2135	5544.9	-208.76	-235.19	-250.81	-252.95	-234.23
2140	5562.5	-206.05	-216.2	-233.54	-235.49	-226.6
2145	5580.2	-209.77	-211.78	-236.81	-223.77	-233.1
2150	5597.9	-211.67	-233.88	-244.88	-245.84	-234.56
2155	5656.4	-203.3	-214.5	-227.67	-244.7	-229.02
2160	5742.1	nd	nd	nd	-234.4	nd
2165	5827.9	-201.98	-213.18	-219.04	-227.31	-211.25
2170	5913.6	-200.85	-219.53	-234.76	-242.55	-213.19
2175	5999.3	nd	nd	nd	-238.83	nd
2180	6085	-220.9	-221.35	-245.64	-253.53	-226.13
2185	6125	nd	nd	nd	nd	nd
2190	6165	-208.62	-227.24	-228.02	-220.36	-215.55
2195	6205	-178.16	-225.94	-229.91	-230.67	-211.77
2200	6245	-167.01	-214.86	-235.47	-244.02	-216.99
2205	6285	-187.15	-232.74	-230.71	-234.06	-226.18
2210	6325	-186.81	-224.37	-220.25	-218.28	-194.11
2215	6365	-197.63	-209.13	-224.35	-213.83	-218.67
2220	6405	-217.3	-227.91	-237.59	-258.52	-234.39
2225	6445	-217.98	-227.91	-224.63	-224.21	-210.05
2230	6485	-205.91	-216.8	-227.82	-234.23	-220.44
2235	6525	-195.87	-222.09	-227.4	-225.94	nd
2240	6565	-178.22	-194.4	-212.24	-224.05	-219.24
2245	6605	-191.99	-207.13	-212.27	-198.59	-213.81
2250	6645	-195.9	-235.1	-238.25	-246.83	-221.12
2255	6685	-213.71	-215.25	-236.56	-239.87	-220.25
2260	6792.7	-210.12	-233.88	-241.88	-249.7	-227.41
2265	6826.1	-222.74	-220.99	-235.55	-241.2	nd
2270	6859.5	-219.51	-234.54	-239.65	-232.38	-218.12
2275	6892.9	-208.19	-212.54	-219.25	-221.41	-223.65
2280	6926.3	-209.75	-224.76	-228.9	-240.26	-224.63
2285	6959.8	-211.32	-222.85	-232.11	-240.76	-209.38
2290	6993.2	-216.53	-217.72	-229.21	-246.4	-221.87
2295	7026.6	-211.05	-197.46	-215.54	-206.48	-210.41
2300	7060	-206.7	-204.47	-221.77	-233.07	-218.46
2305	7093.4	-221.1	-232.9	-237.53	-245.3	-224.68
2310	7126.8	-220.07	-223.53	-234.49	-247.21	-230.65
2315	7160.2	-206.56	-215.37	-226.37	-233.3	-214.46
2320	7193.6	-217.25	-232.06	-228.58	-242.9	-223.31
2325	7217	nd	nd	nd	nd	nd
2330	7233.8	-214.22	-222.18	-227.38	-228.44	-220.94
2335	7250.5	-219.17	-211.74	-232.04	-242.92	-226.59
2340	7267.2	-214.57	-210.28	-227.75	-234.5	-213.11
2345	7283.9	-226.27	-225.16	-233.23	-247.92	-219.4
2350	7300.7	-220.94	-240.25	-171.45	-257.13	-236.31
2355	7317.4	-223.96	-231.14	-237.69	-244.55	-227.23
2360	nd	-225.37	-239.06	-240.08	-243.54	-228.61
2650	8737.1	nd	nd	nd	nd	nd
2900	10372	nd	nd	nd	nd	nd

APPENDIX K. Acid leaf wax concentrations for Lake George, ND

Core	Drive	Depth in Drive (cm)	Depth below lake floor (cm)	C ₁₆ (ug/g sed)	C ₁₈ (ug/g sed)	C ₂₀ (ug/g sed)	C ₂₂ (ug/g sed)	C ₂₄ (ug/g sed)	C ₂₆ (ug/g sed)	C ₂₈ (ug/g sed)	C ₃₀ (ug/g sed)	C ₃₂ (ug/g sed)
A	1	0	20	57.9	58.6	42.0	152.9	535.3	363.2	357.2	337.7	154.6
A	1	10	30	55.1	35.1	37.4	94.3	330.2	311.8	298.6	277.9	144.2
A	1	20	40	7.1	14.2	22.3	24.9	87.2	60.2	43.8	31.1	13.7
A	1	30	50	34.1	2.2	12.8	5.8	20.4	17.7	18.7	7.0	0.0
A	1	40	60	10.8	19.9	20.1	43.1	150.9	165.1	166.7	96.2	39.4
A	1	50	70	10.6	14.6	17.4	43.4	151.7	158.9	141.2	73.8	30.7
A	1	60	80	12.3	11.2	79.4	86.0	102.2	117.3	130.4	73.0	32.3
A	1	70	90	7.9	13.5	18.3	254.0	111.0	125.7	147.6	95.1	45.7
A	1	80	100	11.8	21.3	27.9	807.7	241.6	252.5	244.2	141.9	58.5
A	1	90	110	46.4	22.2	19.0	47.6	176.1	151.6	106.4	59.4	23.8
A	2	0	120	24.5	21.3	20.3	50.9	178.0	214.9	239.4	172.9	88.1
A	2	8	128	23.5	30.7	25.7	50.2	175.7	158.8	142.5	97.0	45.2
A	2	16	136	33.1	29.6	21.3	52.4	183.5	172.9	148.1	98.3	45.5
A	2	24	144	27.7	34.0	28.4	71.1	241.7	229.5	214.3	148.3	66.1
A	2	32	152	6.9	11.2	11.9	4.7	16.4	76.2	70.7	44.3	21.6
A	2	40	160	23.5	27.6	23.1	67.9	237.5	201.1	197.0	142.7	75.2
A	2	48	168	16.4	14.7	13.9	36.4	134.6	92.4	68.6	43.0	19.4
A	2	56	176	10.4	20.3	16.9	36.3	104.8	92.5	93.5	67.1	34.4
A	2	64	184	12.7	36.6	33.6	61.3	238.7	247.1	280.0	206.0	102.3
A	2	72	192	20.7	36.5	34.2	62.8	232.2	200.7	207.3	144.1	71.1
A	2	80	200	15.9	34.9	32.4	83.4	380.8	329.0	319.5	216.4	102.4
A	2	88	208	5.5	18.1	15.9	28.9	105.9	88.2	79.6	48.3	22.1
A	2	96	216	7.4	39.5	22.7	39.4	119.5	103.4	109.7	83.5	44.5
A	3	0	220	46.6	58.0	32.0	53.0	159.9	114.8	114.3	81.2	38.0
A	3	8	228	102.7	129.1	67.1	121.0	283.8	168.3	156.6	105.2	54.9
A	3	16	236	32.3	40.6	23.4	39.2	99.8	66.0	63.0	40.1	21.6
A	3	24	244	81.8	116.6	70.4	117.6	268.0	171.6	158.1	110.1	60.9
A	3	32	252	56.4	67.6	41.6	82.5	167.6	120.5	114.1	78.6	41.6
A	3	40	260	32.5	34.6	22.1	39.6	81.4	60.4	64.3	48.7	27.2
A	3	48	268	18.0	21.0	15.2	28.3	48.6	30.0	26.0	17.9	10.1
A	3	56	276	13.3	10.6	6.4	9.3	14.0	9.5	12.3	11.3	7.4
A	3	64	284	34.3	29.5	21.7	45.0	99.1	82.9	106.9	85.5	46.7
A	3	72	292	20.3	20.7	18.1	37.8	69.0	52.4	53.5	35.9	18.3
A	3	80	300	150.8	145.6	18.4	36.3	72.1	45.5	50.9	40.1	22.5
A	3	88	308	33.2	40.5	38.3	67.4	18.7	95.3	86.1	56.6	26.9
A	4	0	320	18.7	28.7	21.9	43.9	95.4	60.1	54.3	38.3	19.6
A	4	8	328	30.7	29.6	29.8	63.2	110.2	77.7	82.3	58.2	29.5
A	4	16	336	52.8	30.1	28.8	63.7	145.2	93.6	91.1	71.9	38.0
A	4	24	344	25.9	22.8	19.1	37.8	59.9	41.8	46.9	33.3	19.1
A	4	32	352	46.8	44.7	52.1	95.0	182.8	114.1	102.1	73.4	36.4
A	4	40	360	40.4	39.5	38.3	66.3	136.8	100.6	98.6	75.6	43.3
A	4	48	368	33.2	25.8	22.1	38.6	85.1	50.6	40.0	28.7	16.8
A	4	56	376	137.6	79.3	96.5	147.9	339.6	287.8	267.3	165.3	18.5
A	4	64	384	66.9	47.7	67.2	119.2	251.7	158.0	123.3	74.6	33.5
A	4	72	392	31.5	29.1	49.7	83.6	59.5	53.0	34.7	16.3	13.3
A	4	80	400	33.2	34.5	34.9	65.7	205.9	179.9	170.0	120.1	60.3
A	4	88	408	31.3	25.3	27.9	65.2	196.2	165.6	16.2	105.5	48.6
A	4	96	416	10.7	20.5	24.4	54.5	119.1	67.3	57.8	40.3	19.9
A	4	104	424	48.7	49.3	60.3	104.0	206.9	129.0	112.0	78.0	37.4

APPENDIX K. (cont.)

Core	Drive	Depth in Drive (cm)	Depth below lake floor (cm)	C ₁₆ (ug/g sed)	C ₁₈ (ug/g sed)	C ₂₀ (ug/g sed)	C ₂₂ (ug/g sed)	C ₂₄ (ug/g sed)	C ₂₆ (ug/g sed)	C ₂₈ (ug/g sed)	C ₃₀ (ug/g sed)	C ₃₂ (ug/g sed)
A	5	0	440	36.1	46.0	38.9	71.9	181.3	120.9	114.3	78.7	39.4
A	5	8	448	52.7	43.9	48.5	85.9	172.1	110.6	100.8	73.2	37.8
A	5	16	456	11.4	21.4	29.4	47.7	82.8	51.3	43.2	16.2	11.6
A	5	24	464	46.4	50.9	59.4	106.9	326.3	294.7	289.6	188.8	84.8
A	5	32	472	22.4	1.7	16.6	30.9	58.6	37.9	45.1	26.4	12.5
A	5	40	480	64.6	68.5	63.9	132.1	313.6	208.6	196.9	133.8	70.5
A	5	48	488	49.5	43.7	62.6	106.7	293.3	225.5	225.3	179.0	92.8
A	5	56	496	45.7	45.4	50.2	93.7	214.0	136.6	135.7	73.2	32.7
A	5	64	504	48.3	61.7	69.7	116.9	255.1	172.2	140.9	94.5	52.4
A	5	72	512	98.5	84.4	118.4	223.4	423.3	232.1	181.5	127.7	63.9
A	5	80	520	48.9	50.9	64.2	218.9	160.7	107.9	86.4	58.5	29.9
A	5	88	528	25.9	37.7	38.2	63.0	115.6	97.2	108.6	87.2	48.4
A	6	0	540	67.9	65.3	31.5	54.9	146.4	115.2	101.9	68.6	30.9
A	6	8	548	20.3	40.2	36.5	55.3	70.5	52.2	57.1	43.3	23.5
A	6	16	556	57.3	65.6	100.3	153.5	320.9	249.3	215.3	148.8	69.3
A	6	24	564	19.3	23.7	26.6	50.2	85.9	59.8	56.8	43.4	23.2
A	6	32	572	44.7	38.7	50.8	62.0	111.1	285.2	241.9	130.6	39.2
A	6	40	580	12.0	31.5	55.9	77.5	185.3	112.6	83.9	48.5	22.7
A	6	48	588	64.1	46.6	53.2	93.0	186.9	108.0	74.0	44.3	22.0
A	6	56	596	34.9	24.5	23.8	54.2	118.7	82.0	62.8	34.2	15.0
A	6	64	604	27.6	21.8	25.3	41.5	6.7	41.5	42.8	32.3	18.6
A	6	72	612	36.3	33.6	34.5	55.3	144.2	92.1	55.8	31.8	14.7
A	6	80	620	15.4	12.4	9.6	20.3	45.5	33.0	25.2	15.1	10.6
A	6	88	628	38.6	25.4	22.2	52.0	132.4	94.5	72.7	48.2	20.8
A	7	0	640	42.0	47.2	27.6	44.1	88.9	54.9	46.9	31.6	16.3
A	7	8	648	20.4	24.0	16.1	25.2	40.3	28.7	27.8	21.9	12.8
A	7	16	656	21.2	22.5	25.9	62.4	138.5	74.0	58.9	37.9	20.0
A	7	24	664	46.4	40.0	45.6	108.8	239.6	123.1	109.1	67.8	33.1
A	7	32	672	19.2	42.8	75.2	96.0	202.3	151.0	117.9	74.4	38.0
A	7	40	680	20.3	23.2	21.7	54.8	128.6	81.4	47.7	22.3	9.4
A	7	48	688	42.2	28.7	23.9	58.9	127.3	84.8	61.0	37.1	18.7
A	7	56	696	26.0	26.8	28.0	64.3	119.0	68.4	49.7	32.4	16.1
A	7	64	704	23.9	46.5	59.2	120.5	216.6	132.6	115.2	87.4	46.7
A	7	72	712	10.7	21.6	24.9	52.6	90.4	53.7	40.5	26.6	14.9
A	7	80	720	5.1	8.6	9.4	19.9	29.3	18.9	15.0	9.8	5.6
A	7	88	728	9.4	13.6	12.1	27.5	42.3	26.0	20.7	14.4	7.8
A	8	0	740	40.6	52.2	26.1	48.0	98.3	63.6	57.7	41.3	23.0
A	8	8	748	27.2	30.4	23.8	41.5	95.2	62.1	56.3	39.0	20.7
A	8	16	756	63.8	44.8	56.3	90.8	151.4	95.6	68.1	40.3	18.9
A	8	24	764	10.3	8.7	16.6	39.1	59.6	34.0	36.4	17.1	10.5
A	8	32	772	47.1	46.7	57.9	98.7	159.2	97.7	89.8	69.2	37.1
A	8	40	780	136.3	80.6	89.3	119.7	188.6	124.0	125.8	100.0	57.2
A	8	48	788	75.3	47.1	35.4	93.7	167.8	91.6	74.2	53.4	29.6
A	8	56	796	27.4	30.4	17.2	32.0	70.9	55.6	49.2	38.3	24.2
A	8	64	804	56.7	31.6	23.0	73.4	184.2	123.8	102.9	69.7	37.3
A	8	72	812	66.3	30.1	27.0	99.2	281.9	188.6	165.9	113.8	56.5
A	8	80	820	19.5	21.4	18.5	46.6	92.9	56.8	44.8	31.5	16.7
A	8	88	828	9.4	12.6	10.4	28.8	58.9	44.7	32.1	21.0	11.1
A	9	0	840	34.1	43.0	33.3	68.2	167.9	131.5	129.4	96.9	49.5

APPENDIX K. (cont.)

Core	Drive	Depth in Drive (cm)	Depth below lake floor (cm)	C ₁₆ (ug/g sed)	C ₁₈ (ug/g sed)	C ₂₀ (ug/g sed)	C ₂₂ (ug/g sed)	C ₂₄ (ug/g sed)	C ₂₆ (ug/g sed)	C ₂₈ (ug/g sed)	C ₃₀ (ug/g sed)	C ₃₂ (ug/g sed)
A	9	8	848	40.0	42.9	38.9	72.8	183.8	147.2	147.9	109.2	59.2
A	9	16	856	6.6	9.1	8.5	15.0	34.8	28.0	25.5	19.2	10.6
A	9	24	864	5.8	9.4	11.4	24.7	64.4	50.7	49.4	45.6	27.4
A	9	32	872	nd	nd	nd	nd	nd	nd	nd	nd	nd
A	9	40	880	18.0	15.6	16.5	40.1	90.7	71.4	79.6	66.4	37.8
A	9	48	888	111.1	57.9	61.0	124.4	234.4	15.8	159.0	123.1	58.6
A	9	56	896	69.6	42.2	51.1	84.9	180.1	131.3	111.4	81.4	38.1
A	9	64	904	226.0	85.5	84.5	203.0	670.9	391.2	302.9	233.5	99.9
A	9	72	912	100.4	54.5	62.9	108.6	193.2	144.3	121.6	98.1	44.3
A	9	80	920	148.9	69.3	73.4	129.2	244.1	151.0	124.7	96.0	39.1
A	9	88	928	68.1	40.7	51.2	87.4	141.3	87.1	74.1	51.8	24.2
A	10	0	940	98.6	77.2	81.1	191.4	628.9	527.7	501.4	389.5	202.7
A	10	8	948	63.9	51.0	51.2	91.8	188.4	115.1	88.2	57.8	27.8
A	10	16	956	114.3	53.2	53.7	150.3	461.8	327.5	361.4	249.4	129.3
A	10	24	964	129.2	79.4	97.2	209.4	573.9	400.0	343.0	272.1	138.1
A	10	32	972	208.2	117.4	153.1	284.1	761.9	600.5	480.5	355.6	184.4
A	10	40	980	57.2	35.9	35.5	73.7	152.7	85.0	67.9	51.4	28.0
A	10	48	988	145.5	75.6	72.4	140.4	294.6	271.9	140.8	118.3	62.5
A	10	56	996	138.5	76.1	70.9	129.4	264.1	164.1	135.1	107.3	48.5
A	10	64	1004	110.4	52.8	106.4	135.3	384.2	293.1	258.0	184.2	88.9
A	10	72	1012	143.1	52.3	52.3	109.8	365.5	290.9	275.0	215.8	123.9
A	10	80	1020	7.2	4.0	3.3	6.7	8.2	5.9	7.0	5.4	3.4
A	10	88	1028	89.5	22.9	16.6	52.7	114.5	67.0	44.5	26.8	12.7
A	10	96	1036	54.1	28.1	29.7	89.6	249.2	177.6	182.4	167.1	98.3
A	11	0	1038	80.3	53.9	41.1	81.2	178.5	124.0	112.9	82.4	39.4
A	11	8	1046	91.5	30.5	26.9	89.9	336.5	277.5	249.4	176.3	106.9
A	11	16	1054	57.6	23.9	24.7	86.1	290.9	228.3	194.0	154.7	91.0
A	11	24	1062	27.6	64.2	57.3	131.5	420.4	350.7	328.3	265.2	118.5
A	11	32	1070	59.0	25.9	24.1	52.3	195.1	184.5	161.3	113.8	60.5
A	11	40	1078	30.7	17.7	15.7	42.9	140.1	126.1	122.0	110.1	63.8
A	11	48	1086	54.3	31.2	24.3	70.4	149.4	107.0	63.8	47.5	25.9
A	11	56	1094	51.2	28.5	31.0	88.9	245.0	191.3	217.3	202.9	127.5
A	11	64	1102	18.1	15.1	20.2	64.0	240.4	153.5	163.9	163.8	105.5
A	11	72	1110	165.3	81.4	77.6	209.3	609.6	506.9	605.8	541.2	332.1
A	11	80	1118	91.6	48.9	42.5	137.5	468.7	414.9	375.7	299.8	185.5
A	11	88	1126	36.4	22.6	25.3	79.0	215.6	153.7	167.8	158.6	104.1
A	12	0	1128	129.5	80.8	65.7	152.8	467.1	407.2	417.6	322.0	174.4
A	12	8	1136	44.4	22.7	21.5	71.7	224.6	226.4	214.4	198.2	121.1
A	12	16	1144	86.5	46.5	29.6	68.6	161.5	75.9	44.7	22.7	0.0
A	12	24	1152	12.3	9.2	7.1	27.8	61.6	38.7	30.4	21.8	10.8
A	12	32	1160	78.1	46.1	57.0	131.0	405.8	386.0	392.6	326.3	187.7
A	12	40	1168	60.1	39.0	27.0	49.5	92.7	55.3	44.1	32.4	16.9
A	12	48	1176	44.9	30.9	25.6	64.1	179.6	150.2	178.8	185.9	127.8
A	12	56	1184	58.2	38.2	33.1	96.4	274.2	218.6	260.7	234.1	143.8
A	12	64	1192	145.7	78.0	77.5	174.9	500.7	378.8	398.3	316.2	173.3
A	12	72	1200	106.8	54.4	52.8	102.9	234.6	162.0	154.1	154.1	86.8
A	12	80	1208	112.2	68.4	56.9	113.1	297.9	158.2	103.4	63.8	30.7
A	13	0	1213	101.6	74.7	40.6	91.9	218.5	130.1	87.5	59.8	30.3
A	13	8	1221	99.0	44.1	29.9	101.4	263.5	175.6	185.0	167.6	101.0
A	13	16	1229	127.9	69.8	53.2	180.3	510.6	309.1	271.1	231.8	140.0
A	13	24	1237	255.8	108.7	78.7	324.1	1797.1	581.9	470.7	384.4	194.0
A	13	32	1245	32.9	30.4	18.2	60.4	170.6	145.5	97.9	75.9	40.4
A	13	40	1253	54.2	36.8	22.4	68.2	146.1	74.9	47.4	33.2	15.8
A	13	48	1261	169.9	92.6	73.8	334.7	1157.2	831.6	80.8	424.8	158.5
A	13	56	1269	107.5	91.7	51.5	144.2	372.5	231.5	158.1	103.2	50.8
A	13	64	1277	72.1	62.2	25.0	87.2	238.1	143.0	93.1	61.6	34.0
A	13	72	1285	66.6	32.4	25.3	87.3	190.6	113.2	85.9	68.0	35.6
A	13	80	1293	35.2	20.6	19.9	54.1	128.4	86.5	78.9	64.7	33.9
A	13	88	1301	57.5	34.6	40.3	95.2	236.6	180.6	95.9	67.3	32.2

APPENDIX L. Hydrogen stable isotope data for Lake George, ND.

Drive	Depth in Drive (cm)	Depth below lake floor (cm)	Estage	C ₂₂	C ₂₄	C ₂₆	C ₂₈	C ₃₀
1	0	20	55.132	nd	-169.17	nd	nd	nd
1	2	22	63.18	nd	-172.94	nd	nd	nd
1	3	23	67.204	-198.58	-190.34	-209.98	-215.35	nd
1	5	25	75.252	-200.27	-197.64	-203.17	-208.83	nd
1	7	27	83.299	-156.56	-176.48	-181.80	-183.08	nd
1	10	30	95.368	nd	-198.67	nd	nd	nd
1	12	32	103.41	nd	-197.32	-201.90	-118.28	nd
1	17	37	123.52	nd	-199.4	-201.33	-204.04	nd
1	22	42	143.61	nd	-168.88	nd	nd	nd
1	25	45	155.66	nd	-202.19	nd	nd	nd
1	27	47	163.69	nd	-191.00	nd	nd	nd
1	32	52	183.75	nd	-197.72	-205.49	-206.21	nd
1	35	55	195.77	nd	-191.96	nd	nd	nd
1	37	57	203.78	nd	-196.17	nd	nd	nd
1	40	60	215.79	nd	-193.32	nd	nd	nd
1	42	62	223.79	nd	-195.60	nd	nd	nd
1	45	65	235.78	nd	-203.94	-210.62	-210.19	nd
1	47	67	243.77	nd	-196.57	nd	nd	nd
1	50	70	255.74	-154.21	-188.93	-184.44	-197.46	-182.16
1	52	72	263.71	nd	-200.77	nd	nd	nd
1	55	75	275.66	nd	-195.51	nd	nd	nd
1	57	77	283.62	nd	-177.14	nd	nd	nd
1	60	80	295.54	nd	-196.95	nd	nd	nd
1	62	82	303.49	nd	-189.25	nd	nd	nd
1	65	85	315.39	nd	-186.79	nd	nd	nd
1	67	87	323.32	nd	-184.82	nd	nd	nd
1	70	90	335.2	-156.44	-181.55	-193.74	-199.18	-192.52
1	72	92	343.11	nd	-190.47	nd	nd	nd
1	75	95	354.96	nd	-191.24	-207.73	-210.61	nd
1	77	97	362.85	nd	-186.23	-200.68	-207.24	nd
1	80	100	374.68	-153.62	-173.79	-184.15	-192.2	-194.25
1	82	102	382.56	nd	-177.12	nd	nd	nd
1	85	105	394.36	nd	-190.31	-209.33	-211.25	nd
1	87	107	402.22	nd	-192.03	-206.99	-213.08	nd
1	90	110	413.99	nd	-167.83	nd	nd	nd
1	92	112	421.83	nd	-177.05	nd	nd	nd
1	95	115	433.58	nd	-181.72	nd	nd	nd
2	0	120	453.11	nd	-185.30	nd	nd	nd
2	3	123	464.81	nd	-185.85	nd	nd	nd
2	5	125	472.6	nd	-190.27	nd	nd	nd
2	8	128	484.27	nd	-184.88	nd	nd	nd
2	11	131	495.91	nd	-183.71	nd	nd	nd
2	13	133	503.67	nd	-185.87	nd	nd	nd
2	16	136	515.28	nd	-189.16	nd	nd	nd
2	19	139	526.88	nd	-178.21	nd	nd	nd
2	21	141	534.59	nd	-190.64	nd	nd	nd
2	24	144	546.15	-154.08	-182.68	-182.1	-200.68	-188.96
2	27	147	557.69	nd	-181.99	nd	nd	nd
2	29	149	565.38	nd	-186.94	nd	nd	nd

APPENDIX L. (cont.).

Drive	Depth in Drive (cm)	Depth below lake floor (cm)	Estage	C ₂₂	C ₂₄	C ₂₆	C ₂₈	C ₃₀
2	32	152	576.88	-172.89	-185.87	-201.49	-186.32	nd
2	35	155	588.36	nd	-177.39	nd	nd	nd
2	37	157	596.01	nd	-184.63	nd	nd	nd
2	40	160	607.45	-155.03	-171.44	-185.23	-200.98	-199.69
2	43	163	618.87	nd	-175.57	nd	nd	nd
2	45	165	626.48	nd	-168.77	nd	nd	nd
2	48	168	637.86	-143.21	-157.17	-175.26	-194.91	-180.04
2	51	171	649.22	nd	-161.47	nd	nd	nd
2	53	173	656.78	nd	-155.47	nd	nd	nd
2	56	176	668.11	-148.47	-170.94	-175.66	-179.16	-185.1
2	59	179	679.4	nd	-194.40	nd	nd	nd
2	61	181	686.92	nd	-191.57	nd	nd	nd
2	64	184	698.17	-185.39	-189.95	-199.36	-206.71	-201.36
2	67	187	709.41	nd	-191.33	nd	nd	nd
2	69	189	716.88	nd	-194.92	nd	nd	nd
2	72	192	728.07	-175.85	-187.52	-190.34	-199.12	-196.76
2	75	195	739.24	nd	-191.79	nd	nd	nd
2	77	197	746.67	nd	-193.56	nd	nd	nd
2	80	200	757.8	-170.05	-186.88	-197.72	-202.25	-204.29
2	83	203	768.92	nd	-192.98	nd	nd	nd
2	85	205	776.32	nd	-185.75	nd	nd	nd
2	88	208	787.4	-159.53	-172.97	-184.99	-184.68	-183.25
2	91	211	798.47	nd	-191.96	nd	nd	nd
2	93	213	805.85	nd	-185.20	nd	nd	nd
2	96	216	816.9	-162.26	-182.80	-185.82	-199.99	-195.06
3	0	220	831.61	-166.73	-178.88	-185.73	-206.02	-205.95
3	3	223	842.64	nd	-185.40	nd	nd	nd
3	5	225	849.98	nd	-188.78	nd	nd	nd
3	8	228	861	-185.24	-182.86	-182.59	-197.11	-199
3	11	231	872	nd	-191.58	nd	nd	nd
3	13	233	879.34	nd	-192.78	nd	nd	nd
3	16	236	890.34	nd	-189.62	nd	nd	nd
3	19	239	901.34	nd	-192.11	nd	nd	nd
3	21	241	908.67	nd	-184.87	nd	nd	nd
3	24	244	919.67	-160.61	-177.18	-179.28	-198.26	-194.65
3	27	247	930.67	nd	-186.22	nd	nd	nd
3	29	249	938.01	nd	-182.00	nd	nd	nd
3	32	252	949.01	nd	-190.45	nd	nd	nd
3	35	255	960.02	nd	-184.86	nd	nd	nd
3	37	257	967.37	nd	-189.61	nd	nd	nd
3	38	258	971.04	-186.46	-180.65	-205.04	-208.61	nd
3	40	260	978.39	nd	-176.72	nd	nd	nd
3	43	263	989.43	nd	-180.13	nd	nd	nd
3	45	265	996.79	nd	-173.83	nd	nd	nd
3	48	268	1007.8	nd	-180.09	nd	nd	nd
3	51	271	1018.9	nd	-174.46	nd	nd	nd
3	53	273	1026.3	nd	-189.25	-205.05	-209.45	nd
3	59	279	1048.5	nd	-186.82	nd	nd	nd
3	63	283	1063.3	nd	-188.94	nd	nd	nd

APPENDIX L. (cont.).

Drive	Depth in Drive (cm)	Depth below lake floor (cm)	Estage	C ₂₂	C ₂₄	C ₂₆	C ₂₈	C ₃₀
3	64	284	1067	nd	-193.82	nd	nd	nd
3	65	285	1070.7	nd	-179.89	nd	nd	nd
3	66	286	1074.5	nd	-189.28	nd	nd	nd
3	67	287	1078.2	nd	-180.92	nd	nd	nd
3	68	288	1081.9	nd	-181.44	-205.53	-210.26	nd
3	69	289	1085.6	nd	-184.89	-208.31	-204.13	nd
3	70	290	1089.4	nd	-177.15	nd	nd	nd
3	71	291	1093.1	nd	-178.91	nd	nd	nd
3	72	292	1096.8	nd	-188.16	nd	nd	nd
3	73	293	1100.6	nd	-178.94	nd	nd	nd
3	75	295	1108	nd	-178.45	nd	nd	nd
3	76	296	1111.8	nd	-182.36	nd	nd	nd
3	77	297	1115.5	nd	-173.46	nd	nd	nd
3	78	298	1119.3	nd	-180.20	nd	nd	nd
3	79	299	1123	nd	-186.38	nd	nd	nd
3	80	300	1126.8	nd	-194.05	nd	nd	nd
3	81	301	1130.6	nd	-184.40	nd	nd	nd
3	82	302	1134.3	nd	-188.77	nd	nd	nd
3	83	303	1138.1	nd	-179.53	nd	nd	nd
3	85	305	1145.6	nd	-183.17	nd	nd	nd
3	88	308	1157	-187.9	-179.92	-183.57	-202.35	-199.9
3	91	311	1168.3	nd	-186.96	nd	nd	nd
4	0	320	1202.6	-178.78	-177.78	-188.55	-188.41	-196.69
4	3	323	1214.2	nd	-187.58	nd	nd	nd
4	5	325	1221.9	nd	-192.60	nd	nd	nd
4	8	328	1233.4	-185.75	-185.23	-188.37	-199.02	-200.14
4	11	331	1245	nd	-185.56	nd	nd	nd
4	13	333	1252.8	nd	-187.10	nd	nd	nd
4	16	336	1264.5	-178.37	-183.25	-181.9	-193.06	-197.33
4	16	336	1264.5	-178.37	-173.25	-181.9	-193.06	-197.33
4	19	339	1276.2	nd	-182.22	nd	nd	nd
4	21	341	1284.1	nd	-185.91	nd	nd	nd
4	24	344	1295.9	nd	-188.86	nd	nd	nd
4	27	347	1307.7	nd	-186.91	nd	nd	nd
4	29	349	1315.7	nd	-187.55	nd	nd	nd
4	32	352	1327.6	-192.97	-187.56	-189.58	-199.62	-196.78
4	35	355	1339.6	nd	-191.87	nd	nd	nd
4	37	357	1347.6	nd	-185.25	nd	nd	nd
4	40	360	1359.6	-182.66	-188.71	-192.92	-203.04	-202.83
4	43	363	1371.8	nd	-190.08	nd	nd	nd
4	45	365	1379.9	nd	-183.46	nd	nd	nd
4	48	368	1392	nd	-184.87	nd	nd	nd
4	51	371	1404.3	nd	-194.65	nd	nd	nd
4	53	373	1412.5	nd	-196.38	nd	nd	nd
4	56	376	1424.8	-184.6	-185.32	-194.16	-200.57	-198.35
4	59	379	1437.2	nd	-190.66	nd	nd	nd
4	61	381	1445.5	nd	-188.61	nd	nd	nd
3	62	382	1449.6	nd	-185.08	nd	nd	nd
4	64	384	1458	-192.2	-186.88	-197.8	-211.91	-203.66

APPENDIX L. (cont.).

Drive	Depth in Drive (cm)	Depth below lake floor (cm)	Estage	C ₂₂	C ₂₄	C ₂₆	C ₂₈	C ₃₀
4	67	387	1470.5	nd	-183.41	nd	nd	nd
4	69	389	1478.9	nd	-192.27	nd	nd	nd
4	72	392	1491.5	-187.33	-189.40	-197.8	-205.68	-201.46
4	75	395	1504.2	nd	-182.47	nd	nd	nd
4	77	397	1512.6	nd	-184.67	nd	nd	nd
4	80	400	1525.4	-195.68	-200.39	-206.87	-211.69	-197.41
4	83	403	1538.2	nd	-182.34	nd	nd	nd
4	85	405	1546.8	nd	-186.97	nd	nd	nd
4	88	408	1559.7	nd	-184.01	nd	nd	nd
4	91	411	1572.7	nd	-181.56	nd	nd	nd
4	93	413	1581.4	nd	-171.04	nd	nd	nd
4	96	416	1594.5	-190.77	-181.48	-189.56	-199.21	-208.53
4	99	419	1607.6	nd	-184.71	nd	nd	nd
4	101	421	1616.4	nd	-184.31	nd	nd	nd
4	104	424	1629.6	nd	-192.89	nd	nd	nd
5	0	440	1701.2	-192.44	-190.77	-198.89	-208.31	-208.79
5	3	443	1714.9	nd	-179.75	nd	nd	nd
5	5	445	1724	nd	-173.37	nd	nd	nd
5	8	448	1737.7	-191.33	-190.48	-198.14	-211.99	-201.92
5	11	451	1751.3	nd	-182.60	nd	nd	nd
5	16	456	1774.7	-192.55	-185.98	nd	nd	nd
5	13	457	1779.3	nd	-186.06	nd	nd	nd
5	19	459	1787.7	nd	-182.34	-200.35	-201.84	nd
5	24	464	1812.1	-180.6	-189.47	-194.57	-202.91	-210.23
5	27	467	1826.3	nd	-182.05	nd	nd	nd
5	29	469	1833.2	nd	-173.74	nd	nd	nd
5	32	472	1850	nd	-183.67	nd	nd	nd
5	35	475	1864.4	nd	-179.24	nd	nd	nd
5	37	477	1874	nd	-175.71	nd	nd	nd
5	40	480	1888.4	-191.52	-183.26	-197.75	-214.21	-199.65
5	48	488	1927.3	-193.96	-193.82	-200.24	-210.74	-212.63
5	56	496	1966.8	-184.99	-178.64	-189.6	-186.64	-197.09
5	72	512	2047.2	-206.42	-201.49	-207.41	-205.08	-195.55
5	80	520	2088.2	-173.68	-190.60	-190.36	-207.77	-196.51
5	88	528	2129.8	-193.44	-193.53	-200.54	-212.72	nd
6	0	540	2193.2	-187.85	-184.38	-192.32	-207.21	-203.03
6	4	544	2214.6	-183.68	-176.60	-197.49	-209.02	nd
6	12	552	2236.2	-178.97	-187.51	-205.72	-206.59	nd
6	16	556	2279.7	-194.76	-191.45	-191.87	-212.64	-198.7
6	24	564	2323.8	-188.19	-192.82	-199.13	-209.27	-205.05
6	32	572	2368.5	-194.6	-193.82	-197.58	-208.78	-198.46
6	40	580	2413.8	-191.26	-191.85	-199.91	-202.12	-197.07
6	48	588	2459.7	-193.9	-194.62	-201.39	-205.72	-200.29
6	72	612	2601	-196.36	-201.76	-199.54	-207.19	-205.8
6	80	620	2649.3	-199.62	-197.29	-200.44	-188.41	-193.07
6	88	628	2698.3	-186.87	-196.44	-204.89	-197.61	-177.56
7	16	656	2773	-188.41	-200.12	-199.79	-201.15	-197.35
7	24	664	2823.6	-187.22	-164.42	-166.72	-199.09	-199.8
7	32	672	2874.8	-198.84	-193.47	-206.01	-208.38	-202.29

APPENDIX L. (cont.).

Drive	Depth in Drive (cm)	Depth below lake floor (cm)	Estage	C ₂₂	C ₂₄	C ₂₆	C ₂₈	C ₃₀
7	40	680	2979.4	-197.98	-203.13	-201.04	-198.55	nd
7	48	688	3032.6	-196.1	-196.85	-202.65	-205.65	-193.19
7	56	696	3086.6	-192.55	-191.50	-203.09	-205.06	-202.77
7	60	700	3141.2	-190.89	-199.48	-209.65	-205.76	-164.95
7	64	704	3168.8	-203.22	-197.62	-205.39	-210.33	-211.02
7	68	708	3196.6	-187.54	-197.38	-172.41	-205.42	-203.06
7	72	712	3224.5	-184.25	-188.45	-187.41	-191.68	nd
7	76	716	3252.6	-199.94	-194.66	-207.77	-205.28	nd
7	80	720	3309.2	nd	-180.32	nd	nd	nd
7	88	728	3366.6	-190.92	-184.84	nd	nd	nd
7	94	734	3410	-178.76	-199.35	-189.66	-165.95	nd
8	0	740	3453.9	-194.78	-190.85	-188.93	-195.78	-194.05
8	4	744	3483.3	-198.97	-191.53	-201.96	-116.45	nd
8	8	748	3512.9	-187.49	-190.18	-193.85	-191.92	-210.69
8	12	752	3542.6	nd	-203.14	-220.88	10.088	nd
8	20	760	3572.5	nd	-195.35	-204.84	-187.34	nd
8	36	776	3632.8	-190.03	-187.54	-203.02	-206.69	nd
8	40	780	3693.8	-189.48	-189.14	-196.12	-207.56	nd
8	44	784	3755.4	-197.65	-194.79	-208.15	-199.56	nd
8	48	788	3817.7	-198.55	-190.39	-201.8	-210.41	-213.86
8	56	796	3880.6	-191.49	-202.27	-200.77	-199.6	nd
8	60	800	3912.3	-206.77	-209.06	nd	nd	nd
8	64	804	3944.2	-200.11	-192.63	-203.29	-211.16	-198.63
8	68	808	3976.2	-191.32	-198.61	-206.81	-132.97	nd
8	72	812	4008.4	-201.53	-203.09	-205.65	-204.84	-192.62
8	76	816	4040.7	-190.97	-187.74	-207.04	-209.46	nd
8	80	820	4073.3	-177.11	-189.29	-184.9	-189.31	-194.02
8	84	824	4105.9	nd	-180.38	-200.4	-211.73	nd
8	88	828	4138.8	nd	-192.28	-212.02	-209.77	nd
9	0	840	4238.2	-186.73	-186.64	-195.33	-205.61	-207.22
9	4	844	4271.7	-189.74	-190.60	-202.79	-205.89	nd
9	8	848	4339.2	-175.38	-187.34	-191.57	-203.76	-204.65
9	16	856	4373.2	nd	-178.39	nd	nd	nd
9	20	860	4407.3	nd	-187.03	-204.84	-202.98	nd
9	24	864	4441.7	-205.03	-200.50	-206.38	-213.05	nd
9	28	868	4476.1	nd	-199.24	-216.55	-202.27	nd
9	32	872	4510.8	nd	-197.74	-200.24	-187.17	nd
9	36	876	4545.6	nd	-206.31	-209.61	-201.96	nd
9	40	880	4580.6	-191.45	-189.99	-198.32	-203.52	nd
9	44	884	4615.7	-192.14	-193.88	-206.08	-207.76	nd
9	48	888	4651.1	-195.46	-188.86	-201.67	-210.2	-201.64
9	52	892	4686.6	-180.3	-169.93	-196.12	-200.15	nd
9	56	896	4722.2	-200.38	-195.37	-200.5	-201.48	-215.62
9	60	900	4758	-194.37	-194.33	-202.81	-211.35	nd
9	64	904	4794	-189.61	-181.16	-193.35	-204.38	-210.4
9	66	906	4812.1	-200.7	-194.14	-202.99	-201.08	nd
9	68	908	4830.2	-196.21	-190.93	-205.93	-212.5	nd
9	72	912	4866.5	-194.41	-179.28	-197.11	-205.82	-202.71
9	76	916	4903	-195.07	-180.35	-200.88	-211.05	nd

APPENDIX L. (cont.).

Drive	Depth in Drive (cm)	Depth below lake floor (cm)	Estage	C ₂₂	C ₂₄	C ₂₆	C ₂₈	C ₃₀
9	80	920	4939.7	-191.88	-184.73	-199.43	-203.4	nd
9	84	924	4976.5	-191.45	-176.68	-194.37	-201.18	nd
9	88	928	5013.5	-188.46	-180.95	-197.87	#DIV/0!	nd
9	92	932	5050.7	-197.52	-188.87	-196.9	-196.35	nd
10	0	940	5125.6	-188.35	-190.71	-197.88	-211.52	nd
10	4	944	5163.3	-196.77	-190.52	-204.04	-210.47	nd
10	8	948	5201.1	-194.89	-186.79	-196.96	-201.58	-208.53
10	12	952	5239.2	-199.67	-186.86	6.6945	-205.33	nd
10	16	956	5277.4	-186.83	-182.85	-202.24	-213.87	-212.86
10	20	960	5315.8	-185.77	-186.69	-191.5	-202.84	nd
10	24	964	5354.3	-196.24	-179.13	-197.3	-206.01	-206.56
10	28	968	5393.1	-203.11	-188.59	-176.05	-215.55	nd
10	36	976	5471.1	-197.68	-186.25	-205.66	-204.33	nd
10	40	980	5510.3	-189.11	-188.61	-196.03	-203.73	-205.61
10	44	984	5549.7	-209.39	-194.30	-207.69	-215.92	nd
10	48	988	5589.3	-186.99	-173.51	-162.42	-202.15	-203.46
10	52	992	5629.1	-200.6	-188.65	-202.19	-216.61	nd
10	56	996	5669.1	-191.27	-178.48	-191.67	-193.06	-198.17
10	64	1004	5749.6	-198.43	-198.43	-205.96	-211.83	-207.88
10	68	1008	5790.1	-196.69	-187.38	-199.63	-206.38	nd
10	76	1016	5871.6	nd	-195.56	-205.76	nd	nd
10	84	1024	5953.9	-195.95	-190.55	-205.76	-199.47	nd
10	88	1028	5995.3	-197.69	-197.55	-200.83	-187.94	-187.17
10	92	1032	6036.9	-189.69	-192.01	-201.63	-183.7	nd
10	96	1036	6078.7	-199.03	-205.21	-201	-215.75	-216.7
11	0	1038	6099.7	nd	-194.65	nd	nd	nd
11	4	1042	6141.8	-196.62	-187.37	-200.63	-201.74	nd
11	16	1054	6269.1	-194.75	-189.27	-200.72	-204.1	nd
11	20	1058	6312	-202.14	-200.19	-211.08	-213.86	nd
11	24	1062	6355	-190.04	-178.43	-204.25	-203.62	nd
11	28	1066	6398.2	-204.52	-198.08	-211.7	-205.1	nd
11	32	1070	6441.7	-196.94	-198.07	-204.51	-206.54	nd
11	36	1074	6485.3	-203.17	-199.73	-209.19	-196.29	nd
11	40	1078	6529.1	-202.17	-198.83	-204.43	-205.4	nd
11	44	1082	6573.1	-189.91	-191.19	-204.77	-209.09	nd
11	48	1086	6617.3	-205.51	-182.17	-168.75	-211.63	nd
11	52	1090	6661.7	-194.1	-202.88	-209.18	-208.43	nd
11	56	1094	6706.4	-202.48	-198.97	-208.93	-217.65	nd
11	60	1098	6751.2	-195.04	-191.99	-206.73	-204.95	-193.4
11	64	1102	6796.2	-202.31	-183.88	-201.34	-213.5	nd
11	72	1110	6886.8	-193.78	-198.56	-208.84	-210.4	nd
11	84	1122	7024.4	#DIV/0!	-180.08	nd	nd	nd
11	92	1128	7093.8	-194.73	-198.31	-181.24	-210.33	nd
12	4	1132	7140.4	-196.99	-196.94	-112.22	-209.31	nd
12	8	1136	7187.2	-203.87	-193.41	-182.01	-217.74	nd
12	16	1144	7234.2	-200.38	-201.93	-202.01	-200.17	nd
12	20	1148	7281.4	-198.01	-199.54	-204.39	-209.75	nd
12	24	1152	7376.4	-183.25	-186.69	-193.9	-197.59	nd
12	28	1156	7328.8	-202.66	-205.47	-208.37	-210.41	nd

APPENDIX L. (cont.).

Drive	Depth in Drive (cm)	Depth below lake floor (cm)	Estage	C ₂₂	C ₂₄	C ₂₆	C ₂₈	C ₃₀
12	32	1160	7472.3	-194.03	-193.24	-189.39	-210.64	nd
12	40	1168	7569.1	-199.41	-191.47	-202.72	-208.61	nd
12	44	1172	7617.8	-180.87	-197.14	-192.32	-219.21	nd
12	48	1176	7666.7	-200.11	-198.72	-205.17	-203.68	nd
12	52	1180	7715.8	-188.03	-189.91	-192.86	-206.9	nd
12	56	1184	7765.1	-206.83	-207.65	-209.73	-219.65	nd
12	60	1188	7814.6	-195.87	-193.46	-202.6	-107.81	nd
12	64	1192	7864.3	-193.88	-198.53	-204.27	-211.03	nd
12	68	1196	7914.1	-198.99	-187.49	-201.89	-208.21	nd
12	72	1200	7964.1	-203.54	-196.82	-206.46	nd	nd
12	76	1204	8014.3	-196.95	-184.65	-204.6	-208.42	nd
12	80	1208	8064.7	-194.52	-202.96	-195.1	nd	nd
13	0	1213	8127.8	-191.1	-181.95	-183.92	-203.28	nd
13	4	1217	8178.4	-197.58	-196.43	-206.61	-118.16	nd
13	8	1221	8229.2	-194.02	-189.21	-197.9	-216.34	nd
13	12	1225	8280.1	-195.27	-188.46	-207.69	-206.63	nd
13	16	1229	8331.2	-187.91	-187.78	-208.7	-213.51	nd
13	20	1233	8382.3	-195.72	-192.60	-205.3	-211.31	nd
13	22	1235	8407.9	nd	-174.68	nd	nd	nd
13	23	1236	8420.8	nd	-190.77	nd	nd	nd
13	24	1237	8433.6	-172.37	-161.06	-188.18	-194.96	nd
13	25	1238	8446.4	nd	-180.76	nd	nd	nd
13	26	1239	8459.3	nd	-179.00	nd	nd	nd
13	28	1241	8484.9	-188.45	-180.66	-199.62	-206.4	nd
13	32	1245	8536.4	-195.33	-184.42	-173.38	-209.94	nd
13	36	1249	8587.9	#DIV/0!	-180.13	nd	nd	nd
13	40	1253	8639.5	-191.84	-180.22	-196.01	nd	nd
13	44	1257	8691.2	-198.09	-190.76	-206.41	-208.31	nd
13	48	1261	8743	-182.33	-184.53	-201.77	-208.71	nd
13	56	1269	8846.7	-194.72	-184.22	-198.14	-203.13	nd
13	60	1273	8898.6	-207.46	-191.07	-194.9	-211.48	nd
13	64	1277	8950.5	-193.02	-188.08	-197.26	-203.8	nd
13	72	1285	9054.5	-192.65	-190.27	-209.18	-208.6	nd
13	76	1289	9106.6	-200.35	-195.49	-209.63	-215.26	nd
13	80	1293	9158.6	-204.55	-196.68	-207.33	-211.09	nd
13	84	1297	9210.7	-198.16	-190.98	-208.27	-214.14	nd
13	88	1301	9262.4	-190.75	-182.14	-182.54	-203.56	nd
13	92	1305	9314.5	nd	-197.23	-205.98	-198.1	nd

APPENDIX M. *n*-Alkane concentrations for Lake George, ND

Core	Drive	Depth in Drive (cm)	Depth below lake floor (cm)	C ₂₁ (ug/g sed)	C ₂₃ (ug/g sed)	C ₂₅ (ug/g sed)	C ₂₇ (ug/g sed)	C ₂₉ (ug/g sed)	C ₃₁ (ug/g sed)	C ₃₃ (ug/g sed)
A	1	0	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A	1	10	30	0.0	0.0	72.8	97.0	192.1	267.1	45.9
A	1	20	40	0.0	11.2	18.7	34.6	55.5	57.9	0.0
A	1	30	50	0.0	55.6	61.5	114.8	127.4	127.5	59.1
A	1	40	60	0.0	15.6	21.9	45.2	63.8	75.1	30.6
A	1	50	70	0.0	12.6	18.8	38.7	53.4	59.1	20.5
A	1	60	80	0.0	20.3	28.0	54.3	70.7	79.3	26.6
A	1	70	90	0.0	13.7	20.5	42.3	64.8	84.9	24.9
A	1	80	100	0.0	16.3	25.3	55.6	71.5	83.4	26.0
A	1	90	110	0.0	17.9	24.3	51.2	78.8	98.1	30.4
A	2	0	120	0.0	15.9	20.3	42.5	66.2	82.8	17.2
A	2	8	128	0.0	25.5	35.9	75.0	116.1	136.2	43.7
A	2	16	136	0.0	24.4	33.7	67.0	90.3	100.2	36.0
A	2	24	144	0.0	27.4	40.9	82.6	123.7	149.3	44.3
A	2	32	152	0.0	18.4	27.4	48.2	63.1	72.2	30.6
A	2	40	160	0.0	17.5	24.9	50.1	81.4	105.6	37.7
A	2	48	168	0.0	13.5	19.6	32.9	45.8	55.6	27.0
A	2	56	176	0.0	69.1	33.4	45.3	86.9	115.6	120.9
A	2	64	184	0.0	40.1	46.2	87.0	127.4	146.4	0.0
A	2	72	192	0.0	48.1	51.0	85.9	0.0	133.2	20.5
A	2	80	200	0.0	59.0	66.6	110.9	130.3	138.0	23.5
A	2	88	208	0.0	0.0	19.9	53.6	72.8	77.4	0.0
A	2	96	216	0.0	0.0	19.2	40.0	67.3	76.1	0.0
A	3	0	220	0.0	0.0	42.7	73.4	92.8	98.1	0.0
A	3	8	228	0.0	0.0	89.1	153.7	194.0	205.9	34.2
A	3	16	236	0.0	31.8	26.7	49.5	64.5	67.0	11.4
A	3	24	244	0.0	0.0	58.1	109.4	154.9	166.3	27.0
A	3	32	252	0.0	0.0	36.4	62.9	88.4	95.0	15.3
A	3	40	260	0.0	0.0	48.5	73.7	89.0	92.5	14.7
A	3	48	268	0.0	0.0	12.4	22.7	31.7	33.0	5.9
A	3	56	276	0.0	0.0	7.9	13.4	18.8	22.1	4.0
A	3	64	284	0.0	0.0	33.5	55.1	65.5	69.3	12.8
A	3	72	292	0.0	0.0	42.6	64.7	69.3	58.0	9.0
A	3	80	300	0.0	0.0	12.1	23.0	33.3	35.8	5.0
A	3	88	308	0.0	0.0	35.4	59.6	69.8	64.1	10.0
A	4	0	320	0.0	0.0	27.9	45.6	56.6	59.2	9.3
A	4	8	328	0.0	0.0	28.6	51.4	59.7	57.5	8.9
A	4	16	336	0.0	0.0	42.0	68.8	74.6	70.3	12.5
A	4	24	344	0.0	0.0	35.2	57.3	69.7	70.0	10.3
A	4	32	352	0.0	0.0	33.8	61.3	82.0	87.1	12.3
A	4	40	360	0.0	0.0	28.9	49.9	64.4	71.7	11.4
A	4	48	368	0.0	0.0	23.9	48.1	74.4	95.8	16.2
A	4	56	376	0.0	0.0	49.8	88.1	99.9	99.2	13.9
A	4	64	384	0.0	0.0	41.4	60.2	67.2	62.9	0.0
A	4	96	416	0.0	0.0	11.4	20.5	29.8	31.2	0.0
A	4	104	424	0.0	0.0	47.9	83.0	109.4	120.3	0.0
A	5	0	440	0.0	0.0	37.3	63.3	78.0	73.4	0.0
A	5	8	448	0.0	0.0	41.8	69.5	92.0	105.6	0.0
A	5	16	456	0.0	0.0	25.1	46.0	56.9	54.4	0.0

APPENDIX M. (cont.)

Core	Drive	Depth in Drive (cm)	Depth below lake floor (cm)	C ₂₁ (ug/g sed)	C ₂₃ (ug/g sed)	C ₂₅ (ug/g sed)	C ₂₇ (ug/g sed)	C ₂₉ (ug/g sed)	C ₃₁ (ug/g sed)	C ₃₃ (ug/g sed)
A	5	24	464	0.0	0.0	21.1	36.7	44.6	39.6	0.0
A	5	32	472	0.0	0.0	8.6	13.5	15.3	13.5	0.0
A	5	40	480	0.0	0.0	39.3	68.6	85.7	84.4	0.0
A	5	48	488	0.0	0.0	48.3	104.1	180.0	214.5	0.0
A	5	56	496	0.0	0.0	23.5	40.1	51349.9	52.2	0.0
A	5	64	504	0.0	0.0	43.5	79.1	97.0	96.7	0.0
A	5	72	512	0.0	0.0	48.1	84.5	108.3	119.1	0.0
A	5	80	520	0.0	0.0	28.0	49.2	59.6	54.8	0.0
A	5	88	528	0.0	0.0	20.4	38.2	48.8	48.0	0.0
A	6	0	540	0.0	0.0	34.6	64.2	88.5	92.9	0.0
A	6	8	548	0.0	0.0	19.0	35.7	50.3	58.5	0.0
A	6	16	556	0.0	0.0	51.1	96.1	127.9	149.9	0.0
A	6	24	564	0.0	0.0	25.1	44.9	57.0	63.0	0.0
A	6	32	572	0.0	0.0	51.5	93.8	104.8	95.3	0.0
A	6	40	580	0.0	0.0	35.1	58.3	57.0	46.7	0.0
A	6	48	588	0.0	0.0	31.9	58.5	66.1	58.6	0.0
A	6	56	596	0.0	0.0	36.0	56.6	47.9	36.5	0.0
A	6	64	604	0.0	0.0	20.0	37.5	53.9	62.3	0.0
A	6	72	612	0.0	0.0	28.1	47.8	54.5	51.9	0.0
A	6	80	620	0.0	0.0	20.5	30.7	27.0	20.6	0.0
A	6	88	628	0.0	0.0	23.6	36.2	34.9	29.7	0.0
A	7	0	640	0.0	0.0	27.7	50.6	62.2	59.3	0.0
A	7	8	648	0.0	0.0	21.9	38.7	49.5	47.0	0.0
A	7	16	656	0.0	0.0	16.4	26.4	32.5	31.7	0.0
A	7	24	664	0.0	0.0	19.4	28.0	30.8	25.2	0.0
A	7	40	680	0.0	0.0	24.1	33.7	25.2	16.5	0.0
A	7	48	688	0.0	0.0	16.6	24.6	23.7	18.6	0.0
A	7	56	696	0.0	0.0	16.0	24.3	23.7	19.4	0.0
A	7	64	704	0.0	0.0	25.9	42.8	49.1	46.6	0.0
A	7	72	712	0.0	0.0	14.4	23.5	27.1	22.7	0.0
A	7	80	720	0.0	0.0	4.5	7.0	7.2	5.7	0.0
A	7	88	728	0.0	0.0	7.7	12.5	15.2	14.5	0.0
A	8	0	740	0.0	0.0	18.4	30.2	34.6	29.8	0.0
A	8	8	748	0.0	0.0	18.6	31.6	36.8	32.1	0.0
A	8	16	756	0.0	0.0	21.9	33.8	34.3	30.7	0.0
A	8	24	764	0.0	0.0	10.7	16.1	17.8	16.5	0.0
A	8	32	772	0.0	0.0	18.1	29.2	31.9	25.3	0.0
A	8	40	780	0.0	0.0	25.9	40.9	45.9	46.4	0.0
A	8	48	788	0.0	0.0	21.3	35.4	43.5	45.5	0.0
A	8	56	796	0.0	0.0	13.0	18.9	19.8	16.4	0.0
A	9	24	864	0.0	0.0	11.7	18.7	28.4	38.1	11.8
A	9	32	872	0.0	19.4	23.8	40.4	63.1	54.3	15.1
A	9	40	880	0.0	0.0	10.5	18.5	32.0	48.1	13.9
A	9	48	888	0.0	22.3	44.2	75.4	113.8	129.5	28.6
A	9	56	896	0.0	23.9	29.4	48.0	60.1	65.3	16.0
A	9	64	904	0.0	27.5	51.8	92.6	140.9	167.4	41.8
A	9	72	912	0.0	32.0	57.2	108.6	174.0	197.7	37.1
A	9	80	920	0.0	35.6	61.9	107.1	146.9	146.6	29.7
A	9	88	928	0.0	0.0	34.4	71.9	108.8	132.3	27.4

APPENDIX M. (cont.)

Core	Drive	Depth in Drive (cm)	Depth below lake floor (cm)	C ₂₁ (ug/g sed)	C ₂₃ (ug/g sed)	C ₂₅ (ug/g sed)	C ₂₇ (ug/g sed)	C ₂₉ (ug/g sed)	C ₃₁ (ug/g sed)	C ₃₃ (ug/g sed)
A	10	0	940	0.0	33.9	55.3	103.4	172.1	228.8	55.7
A	10	8	948	0.0	18.3	29.7	57.4	92.2	116.2	28.8
A	10	16	956	0.0	156.0	36.0	59.2	95.1	133.9	28.5
A	10	24	964	0.0	28.4	42.0	84.3	132.8	180.1	41.8
A	10	32	972	0.0	20.9	34.5	69.7	111.3	136.5	31.5
A	10	40	980	0.0	14.7	25.4	50.9	82.2	122.9	27.2
A	10	48	988	0.0	38.8	63.0	132.3	208.2	256.3	67.5
A	10	56	996	0.0	34.5	53.6	104.1	153.9	177.4	39.3
A	10	64	1004	0.0	27.0	41.3	71.3	133.9	132.5	33.4
A	10	72	1012	0.0	16.5	25.9	49.8	86.9	103.7	23.6
A	10	80	1020	0.0	8.9	12.9	22.7	45.5	38.0	9.9
A	10	88	1028	0.0	16.3	23.7	34.5	61.1	46.9	13.1
A	10	96	1036	0.0	9.4	19.1	36.4	71.1	112.1	27.1
A	11	0	1038	0.0	19.2	32.4	53.0	81.2	97.2	23.8
A	11	8	1046	0.0	17.3	24.9	46.4	74.7	76.5	23.3
A	11	16	1054	0.0	12.2	19.7	30.1	50.1	56.5	15.9
A	11	24	1062	0.0	20.3	35.9	65.4	113.7	123.8	26.0
A	11	32	1070	0.0	13.5	20.8	39.5	59.0	64.7	15.2
A	11	40	1078	0.0	0.0	10.9	19.7	35.0	37.2	12.3
A	11	48	1086	0.0	11.8	21.5	42.0	73.5	107.1	28.9
A	11	56	1094	0.0	11.4	20.4	45.2	85.9	116.3	34.2
A	11	64	1102	0.0	0.0	14.6	29.7	53.8	72.5	21.6
A	11	72	1110	0.0	44.6	65.0	124.7	224.7	259.8	65.0
A	11	80	1118	0.0	20.5	29.2	57.0	83.5	92.5	27.2
A	11	88	1126	0.0	0.0	14.9	32.8	60.2	79.7	24.2
A	12	0	1128	0.0	28.7	43.7	80.4	127.0	169.1	42.8
A	12	8	1136	0.0	16.2	30.4	64.5	118.9	129.4	42.8
A	12	16	1144	0.0	19.6	25.6	49.6	73.0	75.4	17.8
A	12	24	1152	0.0	0.0	9.1	15.7	25.0	28.8	8.4
A	12	32	1160	0.0	22.3	35.0	72.7	131.1	151.1	41.5
A	12	40	1168	0.0	22.7	36.3	67.1	122.8	151.6	32.4
A	12	48	1176	0.0	0.0	17.5	38.1	80.0	114.8	34.3
A	12	56	1184	0.0	18.8	33.1	63.8	111.9	119.8	30.4
A	12	64	1192	0.0	35.8	49.0	92.6	151.9	145.7	36.4
A	12	80	1208	0.0	26.6	30.2	55.1	90.8	75.3	20.0
A	13	0	1213	0.0	24.6	33.7	59.7	93.5	111.6	28.5
A	13	8	1221	0.0	19.0	31.7	55.3	136.2	116.5	36.7
A	13	16	1229	0.0	38.7	60.3	107.2	198.9	189.1	47.6
A	13	24	1237	0.0	63.0	83.8	139.0	274.8	254.2	50.1
A	13	32	1245	0.0	14.5	22.3	44.6	86.5	71.6	23.8
A	13	40	1253	0.0	17.9	28.8	52.9	87.5	84.5	22.8
A	13	48	1261	0.0	266.5	231.9	356.3	361.4	273.5	65.4
A	13	56	1269	0.0	26.2	35.3	63.6	111.8	94.9	25.4
A	13	64	1277	0.0	19.3	29.2	51.6	108.3	77.5	23.6
A	13	72	1285	0.0	20.0	32.4	59.7	138.8	119.1	30.4
A	13	80	1293	0.0	10.2	20.1	39.4	127.0	88.7	22.6
A	13	88	1301	0.0	23.4	31.3	71.9	166.9	119.8	32.2

APPENDIX N. Long-chain alkenone data for Lake George, ND

Depth below lake floor (cm)	%37:4	UK37	LG Calib	C37:C38
23	36.263	-0.2657	23.09	
25	67.371	-0.6361	12.541	1.2502
27	38.291	-0.2926	22.325	0.3874
32	69.071	-0.6313	12.679	1.882
35	71.791	-0.6806	11.275	1.9171
37	0.8207	-0.7926	3.7102	3.2534
38	68.494	-0.5983	13.618	2.7798
42	0.7925	-0.7627	5.16	2.6547
45	0.7202	-0.6757	9.3806	2.2239
47	0.6997	-0.6509	10.582	1.9933
55	0.748	-0.7097	7.7294	
57	0.7098	-0.6708	9.6149	1.8769
62	0.6958	-0.6545	10.409	2.377
65	0.6699	-0.6266	11.759	2.3298
67	0.6806	-0.6402	11.102	2.4035
72	0.6348	-0.5882	13.623	2.5585
75	0.8006	-0.7522	5.6699	
77	0.6467	-0.6007	13.015	2.3498
85	0.6915	-0.6533	10.467	2.2859
87	0.7129	-0.6778	9.275	2.7139
92	0.6796	-0.61	12.564	2.7474
95	0.6362	-0.5668	14.662	2.1435
97	0.6699	-0.619	12.127	2.0571
102	0.5599	-0.4966	18.064	1.947
105	0.6558	-0.596	13.242	1.9191
107	0.7023	-0.6406	11.081	2.1437
112	0.6349	-0.5665	14.676	2.0702
115	0.6699	-0.6191	12.126	2.7221
123	0.5712	-0.5076	17.531	1.5922
125	0.7308	-0.6868	8.8411	2.6481
131	0.7493	-0.7184	7.3073	2.89
133	0.7759	-0.7416	6.1853	2.6969
139	0.6617	-0.6115	12.493	3.0168
141	0.6771	-0.6265	11.766	2.2308
147	0.6676	-0.6161	12.268	2.1399
149	0.7006	-0.65	10.625	2.4072
155	0.64	-0.5877	13.649	2.1608
157	0.6557	-0.6055	12.784	1.9549
163	0.5174	-0.4403	20.796	
165	0.505	-0.4266	21.459	1.4776
171	0.5539	-0.4701	19.349	1.5903
173	0.6005	-0.5301	16.442	1.6292
179	0.6438	-0.5892	13.573	1.2849
181	0.5969	-0.5369	16.11	1.5189
187	0.4201	-0.315	26.87	0.7258
211	0.7978	-0.6501	10.622	2.7542
213	0.7784	-0.6196	12.098	2.418
223	0.4125	-0.2966	27.767	3.1036
225	0.7898	-0.6369	11.261	2.4132

APPENDIX N. (cont.)

Depth below lake floor (cm)	%37:4	UK37	LG Calib	C37:C38
231	0.7603	-0.5937	13.358	1.9586
233	0.7285	-0.5518	15.39	1.7183
239	0.8038	-0.6591	10.184	2.3769
241		-0.6407	11.078	2.9171
247	0.7764	-0.6202	12.073	2.2669
249	0.7921	-0.641	11.063	2.6795
255	0.7636	-0.5989	13.102	1.8489
257	0.7394	-0.5629	14.851	1.9172
263	0.778	-0.6192	12.121	2.2769
265	0.7894	-0.6368	11.267	2.6091
271	0.319	-0.2135	31.797	2.9669
273	0.4673	-0.3861	23.427	4.4083
279	0.8023	-0.656	10.333	2.1669
288	66.696	-0.6272	12.795	1.4528
305	0.7132	-0.5274	16.57	2.0083
311	0.8061	-0.6601	10.135	2.6756
323	0.7774	-0.6174	12.206	1.9711
325	0.7817	-0.6241	11.882	2.3643
333	0.7099	-0.6583	10.223	2.1201
339	0.5974	-0.5265	16.613	2.3644
341	0.6575	-0.6221	11.976	2.9091
347	0.732	-0.6897	8.7001	2.762
349	0.6581	-0.6102	12.557	2.4163
355	0.5875	-0.544	15.768	2.2071
357	0.6421	-0.6022	12.941	3.2442
363	0.6867	-0.6353	11.341	2.6016
365	0.6757	-0.6241	11.88	2.4456
371	0.6939	-0.6385	11.182	2.4798
373	0.6669	-0.5595	15.014	3.1691
397	0.8098	-0.6752	9.4017	2.4857
403	0.6894	-0.6272	11.731	0.0099
405	0.6729	-0.6068	12.72	2.1909
411	0.7019	-0.6525	10.506	3.2946
544	57.628	-0.5504	14.982	2.8253
552	37.267	-0.2728	22.889	0.9757
700	54.959	-0.4895	16.717	1.4914
708	63.399	-0.5865	13.955	1.7186
724	62.208	-0.4749	17.132	
744	62.93	-0.5113	16.095	2.3397
752	57.688	-0.5253	15.697	2.2553
784	37.573	-0.3337	21.153	1.1791
792	69.834	-0.6517	12.097	2.2021
824	64.793	-0.6	13.569	1.2384
844	59.537	-0.5445	15.15	2.038
852	64.107	-0.5901	13.85	2.5259
860	68.094	-0.6201	12.996	2.4759
868	71.688	-0.675	11.433	2.3643
876	68.818	-0.6282	12.765	1.6828
884	75.225	-0.7081	10.49	2.1098

APPENDIX N. (cont.)

Depth below lake floor (cm)	%37:4	UK37	LG Calib	C37:C38
892	67.18	-0.6203	12.99	1.4335
900	69.619	-0.6481	12.2	1.4171
908	76.486	-0.7148	10.299	1.8922
916	40.259	-0.2035	24.863	0.6187
924	58.174	-0.5206	15.83	0.8024
932	67.521	-0.6343	12.593	2.3599
944	54.78	-0.4962	16.525	
952	65.044	-0.6033	13.474	1.8983
960	71.783	-0.6839	11.179	2.2482
968	55.648	-0.4663	17.378	0.9977
976	72.973	-0.6964	10.824	2.1803
984	75.687	-0.7312	9.834	2.3754
992	68.644	-0.6502	12.139	2.5178
1000	74.384	-0.6977	10.787	2.6783
1008	58.317	-0.5446	15.148	0.7588
1016	53.794	-0.4987	16.455	0.5863
1024	74.062	-0.7005	10.706	1.935
1032	62.964	-0.5857	13.977	1.455
1042	64.115	-0.5917	13.806	1.3992
1050	68.474	-0.6427	12.353	2.1133
1058	63.566	-0.5981	13.623	2.6913
1066	67.958	-0.6404	12.419	2.0122
1074	69.779	-0.6469	12.235	2.095
1082	75.644	-0.7238	10.043	2.0711
1090	65.422	-0.5689	14.456	2.7231
1098	72.747	-0.6844	11.166	2.9659
1106	64.272	-0.5618	14.657	1.6708
1114	66.012	-0.621	12.973	1.9782
1122	71.974	-0.6864	11.108	2.099
1130	75.367	-0.7129	10.354	1.6586
1132	67.022	-0.6109	13.258	1.4344
1140	73.03	-0.6981	10.775	2.485
1148	76.906	-0.7449	9.4434	2.2376
1172	75.571	-0.7188	10.185	2.5655
1180	75	-0.7067	10.532	2.4107
1188	70.92	-0.669	11.603	1.6485
1196	70.815	-0.659	11.89	1.8578
1204	73.251	-0.6943	10.884	2.7034
1212	79.764	-0.7686	8.7669	3.4259
1217	77.426	-0.7448	9.4444	3.0506
1225	76.004	-0.7326	9.7915	5.6889
1233	62.943	-0.6077	13.351	9.7573
1241	71.798	-0.617	13.086	5.106
1249	64.292	-0.5056	16.257	2.4449
1257	69.976	-0.6469	12.235	2.1002
1265	75.112	-0.7081	10.49	2.8126
1273	70.402	-0.6555	11.989	2.7041
1289	76.37	-0.723	10.065	2.1326
1297	79.3	-0.7602	9.007	2.4821
1305	76.937	-0.7357	9.7035	2.1269

APPENDIX O. Long-chain alkenone data for Lake George, ND freeze core.

ID	Depth in Freeze core (cm)	%37:4	C ₃₇ (ug / g sed)	C ₃₇ :C ₃₈	UK'37	UK37	UK38	Inferred Temp (°C)
1	0.37	74.517	65	3	0.144	-0.7086	-0.139	7.7849
2	0.73	73.857	78	3	0.145	-0.7007	-0.1302	8.1675
3	1.10	75.927	244	2	0.035	-0.7509	-0.1408	5.734
4	1.47	73.825	256	3	0.160	-0.6964	-0.1487	8.3764
5	1.84	72.068	277	2	0.154	-0.6775	-0.1426	9.2901
6	2.20	73.744	365	3	0.151	-0.6978	-0.1585	8.3097
7	2.57	69.171	328	2	0.149	-0.6457	-0.0932	10.836
8	2.94	67.064	226	2	0.149	-0.6216	-0.0602	12.003
9	3.30	63.755	209	2	0.149	-0.5835	-0.0291	13.85
10	3.67	65.356	85	2	0.143	-0.6039	-0.0362	12.861
11	4.04	67.641	155	2	0.155	-0.6263	-0.0433	11.773
12	4.40	64.959	120	2	0.136	-0.6021	-0.0482	12.95
13	4.77	64.82	233	2	0.158	-0.5926	-0.0257	13.408
14	5.14	63.721	416	2	0.165	-0.5774	-0.0159	14.148
15	5.51	64.163	299	1	0.162	-0.5837	-0.0147	13.839
16	5.82	68.439	119	0	0.139	-0.6406	-0.142	11.08
17	6.14	73.333	1901	2	0.153	-0.6924	-0.1216	8.5685
18	6.46	73.146	778	2	0.150	-0.6911	-0.042	8.6327
19	6.78	65.411	496	1	0.147	-0.6033	-0.0431	12.89
20	7.10	67.192	369	2	0.150	-0.6228	-0.0437	11.943
21	7.41	66.955	886	2	0.157	-0.6177	-0.032	12.194
22	7.73	65.278	4631	1	0.152	-0.5999	-0.0395	13.056
23	8.05	66.521	794	2	0.154	-0.6138	-0.025	12.382
24	8.37	65.767	643	3	0.153	-0.6052	-0.0135	12.797
25	8.69	65.405	491	3	0.151	-0.6019	-0.0071	12.96
26	9.00	65.405	491	1	0.151	-0.6019	-0.0133	12.96
27	9.32	65.257	508	1	0.162	-0.5961	-0.0072	13.238
28	9.64	66.104	593	3	0.173	-0.6025	0.0023	12.927
29	9.96	64.584	644	2	0.152	-0.5919	0.0003	13.444
30	10.28	63.153	303	1	0.145	-0.5781	0.0131	14.11
31	10.59	60.331	758	2	0.160	-0.54	-0.0035	15.962
32	10.91	62.273	706	1	0.153	-0.5651	-0.0038	14.743
33	11.23	63.308	465	1	0.164	-0.5728	0.0133	14.369
34	11.55	59.127	906	1	0.144	-0.5325	-0.0258	16.324
35	11.87	65.905	1015	2	0.152	-0.6071	-0.0599	12.705
36	12.18	69.283	677	1	0.156	-0.6448	-0.0827	10.877
37	12.50	71.485	803	2	0.152	-0.6714	-0.1219	9.5863
38	12.82	73.375	567	3	0.149	-0.694	-0.1742	8.492
39	13.14	75.068	883	3	0.157	-0.7115	-0.2021	7.6418
40	13.46	77.45	990	3	0.162	-0.738	-0.2032	6.3564
41	13.77	78.403	338	2	0.161	-0.7492	-0.1924	5.8123
42	14.09	76.741	365	nd	0.148	-0.733	nd	6.601
43	14.41	76.821	1300	2	0.163	-0.7303	-0.1583	6.73
44	14.73	74.313	995	2	0.150	-0.7046	-0.1333	7.9763
45	15.05	73.417	1149	2	0.146	-0.6954	-0.1061	8.4224
46	15.36	73.497	279	2	0.152	-0.6946	-0.1291	8.4647
47	15.68	70.419	nd	2	0.153	-0.6588	-0.0673	10.197
48	16.00	69.64	362	9	0.155	-0.6494	-0.0411	10.654
49	16.32	71.151	105	1	0.156	-0.6666	-0.0667	9.8225
50	16.67	68.608	nd	1	0.152	-0.6383	-0.06	11.192
51	17.03	69.65	nd	2	0.146	-0.6521	-0.0949	10.523
52	17.39	72.967	nd	2	0.152	-0.6887	-0.1124	8.7484
53	17.75	73.923	nd	2	0.164	-0.6966	-0.103	8.3669
54	18.10	71.765	453	2	0.142	-0.6775	-0.0997	9.2903
55	18.46	70.713	99	2	0.144	-0.6648	-0.0718	9.9062

APPENDIX O. (cont.)

ID	Depth in Freeze core (cm)	%37:4	C ₃₇ (ug / g sed)	C ₃₇ :C ₃₈	UK'37	UK37	UK38	Inferred Temp (°C)
56	18.82	71.483	nd	19	0.140	-0.6748	-0.0598	9.4232
57	19.17	64.076	nd	3	0.142	-0.5896	-0.0678	13.556
58	19.53	63.999	nd	1	0.136	-0.5911	-0.0149	13.481
59	19.89	69.608	nd	0	0.130	-0.6566	-0.0078	10.304
60	20.24	74.738	nd	2	0.157	-0.7077	-0.0807	7.8291
61	20.60	76.602	nd	2	0.156	-0.7294	-0.1129	6.7732
62	20.96	72.798	nd	2	0.134	-0.6916	-0.1001	8.607
63	21.32	74.678	nd	2	0.151	-0.7085	-0.0944	7.7889
64	21.67	76.106	nd	2	0.163	-0.7222	-0.1138	7.1247
65	22.03	72.071	nd	2	0.140	-0.6817	-0.089	9.0906
66	22.39	67.623	nd	1	0.160	-0.6244	-0.0412	11.867
67	22.74	63.706	nd	1	0.132	-0.5893	-0.0322	13.57
68	23.10	60.356	nd	1	0.141	-0.5478	-0.0125	15.583
69	23.46	59.032	nd	4	0.118	-0.5418	-0.029	15.873
70	23.81	54.629	nd	nd	0.114	-0.4944	-0.0592	18.172
71	24.17	58.844	nd	1	0.151	-0.5264	-0.053	16.621
72	24.53	58.25	nd	1	0.130	-0.5284	-0.07	16.525
73	24.89	59.392	nd	1	0.124	-0.5436	-0.0846	15.788
74	25.24	55.392	nd	1	0.127	-0.4972	-0.0378	18.037
75	25.60	49.396	nd	1	0.105	-0.441	-0.0306	20.76
76	25.96	47.444	nd	1	0.108	-0.4179	0.1728	21.882
77	26.31	52.16	nd	1	0.138	-0.4558	-0.0493	20.043
78	26.67	50.655	nd	1	0.112	-0.4513	-0.0729	20.26
79	27.03	52.499	nd	1	0.122	-0.4672	-0.0551	19.49
80	27.39	45.731	nd	1	0.117	-0.3939	-0.0313	23.045
81	27.75	45.929	nd	1	0.112	-0.3986	-0.0427	22.816
82	28.11	42.458	nd	1	0.106	-0.3634	-0.0672	24.523
83	28.48	30.195	nd	1	0.111	-0.2242	-0.0317	31.277
84	28.84	70.419	nd	nd	0.153	-0.6588	nd	10.197
85	29.20	21.014	nd	1	0.135	-0.1036	0.0554	37.123
86	29.56	29.143	nd	1	0.146	-0.1877	-0.0136	33.047
87	29.92	29.379	nd	1	0.129	-0.2024	-0.0006	32.331
88	30.28	33.939	nd	1	0.129	-0.2542	-0.0156	29.819
89	30.64	32.728	nd	1	0.139	-0.2336	0.0095	30.82
90	31.00	nd	nd	nd	nd	nd	nd	nd
91	31.36	nd	nd	nd	nd	nd	nd	nd
92	31.72	nd	nd	nd	nd	nd	nd	nd
93	32.09	nd	nd	nd	nd	nd	nd	nd
94	32.45	62.384	nd	1	0.145	-0.5693	-0.1041	nd
95	32.81	54.233	nd	1	0.151	-0.4731	-0.0705	19.206
96	33.17	55.418	nd	1	0.158	-0.4836	-0.0762	18.697
97	33.53	nd	nd	nd	nd	nd	nd	nd
98	33.95	52.824	nd	1	0.132	-0.4659	0.0032	19.552
99	34.37	52.693	nd	1	0.122	-0.4692	-0.0251	19.394
100	34.79	53.923	nd	1	0.124	-0.4821	-0.0465	18.77
101	35.21	58.867	nd	1	0.133	-0.534	-0.0492	16.253
102	35.63	56.642	nd	1	0.126	-0.512	-0.0273	17.32
103	36.05	61.824	nd	1	0.135	-0.5669	-0.0477	14.657
104	36.47	53.414	nd	1	0.389	-0.3532	-0.0343	25.022
105	36.89	69.756	nd	1	0.138	-0.6557	-0.0454	10.347
106	37.31	79.823	nd	3	0.143	-0.7693	-0.2782	4.8381
107	37.73	72.924	nd	2	0.136	-0.6923	-0.3344	8.5749
108	38.15	75.579	nd	2	0.134	-0.723	-0.3347	7.0865
109	38.57	73.622	nd	2	0.157	-0.6948	-0.436	8.4532
110	38.99	76.687	nd	2	0.151	-0.7316	-0.3729	6.6683

APPENDIX O. (cont.)

ID	Depth in Freeze core (cm)	%37:4	C ₃₇ (ug / g sed)	C ₃₇ :C ₃₈	UK'37	UK37	UK38	Inferred Temp (°C)
111	39.41	77.16	nd	2	0.146	-0.7383	-0.3331	6.3442
112	39.83	73.157	nd	2	0.151	-0.6911	-0.3347	8.6321
113	40.25	75.567	nd	2	0.132	-0.7234	-0.3353	7.0666
114	40.67	78.431	nd	3	0.152	-0.7514	-0.3354	5.7065
115	41.09	78.828	nd	3	0.137	-0.7592	-0.3802	5.3288
116	41.51	78.579	nd	3	0.135	-0.7569	-0.4069	5.439
117	41.93	77.813	nd	3	0.543	-0.6576	-0.3835	10.255
118	42.33	81.066	nd	4	0.146	-0.783	-0.4199	4.1744
119	42.73	78.224	nd	3	0.132	-0.7535	-0.3609	5.6048
120	43.13	74.304	nd	3	0.145	-0.7057	-0.3052	7.9239
121	43.53	77.038	nd	3	0.157	-0.7344	-0.2912	6.5302
122	43.93	68.037	nd	2	0.164	-0.6281	-0.2948	11.69
123	44.33	69.371	nd	2	0.131	-0.6536	-0.2924	10.452
124	44.73	67.269	nd	2	0.168	-0.6178	-0.0725	12.186
125	45.13	64.25	nd	3	0.281	-0.5421	-0.1952	15.857
126	45.53	70.104	nd	2	0.140	-0.6592	-0.1361	10.177
127	45.93	67.815	nd	2	0.141	-0.6329	-0.3218	11.456
128	46.33	71.146	nd	3	0.153	-0.6674	-0.3056	9.781
129	46.73	71.239	nd	2	0.165	-0.6648	-0.1306	9.9055
130	47.13	69.837	nd	2	0.160	-0.65	-0.169	10.624
131	47.53	64.46	nd	1	0.144	-0.5935	-0.1202	13.365
132	47.93	63.63	nd	2	0.144	-0.5838	-0.1203	13.836
133	48.33	67.511	nd	2	0.153	-0.6255	-0.1498	11.812
134	48.73	71.21	nd	2	0.160	-0.6661	-0.2514	9.844
135	49.13	nd	nd	nd	nd	nd	nd	nd
136	49.53	nd	nd	nd	nd	nd	nd	nd
154	49.93	nd	nd	nd	nd	nd	nd	nd

APPENDIX P. Hydrogen stable isotopes of acids for Lake George, ND freeze core.

Depth in Freeze core (cm)		C ₁₆	C ₁₈	C ₂₀	C ₂₂	C ₂₄	C ₂₆	C ₂₈	C ₃₀
4.5	1.28	nd	nd	nd	nd	nd	nd	nd	nd
9	3.30	-199.95	-202.54	nd	-167.38	-175.99	-189.83	-172.13	-198.24
11.5	4.22	nd	nd	nd	nd	-189.03	nd	nd	nd
13.5	4.95	nd	nd	nd	nd	-182.69	nd	nd	nd
15.5	5.66	nd	nd	nd	nd	-174.80	nd	nd	nd
18	6.46	-213.70	-217.52	nd	-177.21	-174.53	-195.51	-164.93	-205.27
19	6.78	-210.77	-204.43	-158.30	-180.87	-190.97	-194.52	-167.72	-208.81
20	7.10	nd	nd	nd	nd	-188.40	-195.90	-209.68	nd
21	7.41	nd	nd	nd	nd	-191.64	-196.06	-209.24	-206.40
22	7.73	-205.46	-211.74	-174.14	-178.95	-190.47	-195.34	-173.99	-207.63
23	8.05	-212.37	-208.28	nd	-172.46	-191.44	-183.69	-149.13	-205.12
24	8.37	-214.61	-230.92	-193.37	-180.16	-197.64	-196.67	-172.63	-187.85
25	8.69	-207.00	-214.39	-191.63	-182.41	-184.61	-193.82	-179.41	-206.86
26	9.00	-207.29	-213.64	nd	-168.79	-195.34	-188.47	-208.67	nd
27	9.32	-218.57	-217.99	-195.10	-180.23	-191.95	-193.76	-168.22	-203.23
28	9.64	-202.53	-214.85	-196.45	-177.16	-192.36	-196.27	-167.59	-211.35
29	9.96	-207.27	-208.21	nd	nd	-192.79	-190.89	-202.17	5.71
30	10.28	-210.27	-216.95	nd	-171.41	-191.35	-195.85	-207.67	-185.31
31	10.59	-202.78	-212.07	nd	-178.30	-180.72	-188.83	-109.92	-216.67
32	10.91	-207.18	-214.33	nd	-182.81	-184.88	-194.58	-205.80	-201.63
33	11.23	-194.74	-215.86	nd	nd	-188.93	-191.59	-200.15	-196.56
34	11.55	-207.71	-219.20	nd	nd	-186.76	-195.92	-205.67	-212.81
35	11.87	-207.35	-218.06	-164.18	-178.64	-185.89	-193.87	-171.22	-211.29
36	12.18	-211.41	-231.36	-182.16	-184.71	-187.69	-197.37	-176.83	-208.98
37	12.50	-186.63	-226.80	nd	-176.26	-187.76	-194.71	-172.26	-204.38
38	12.82	-200.76	-225.71	nd	-172.64	-192.65	-191.09	-166.36	-206.15
39	13.14	-217.16	-243.29	-199.10	-186.31	-187.46	-195.03	-176.63	-216.15
40	13.46	-208.16	-244.79	nd	-180.94	-190.79	-199.92	-175.09	-210.77
41	13.77	-208.06	-238.69	-186.31	-182.77	-190.20	-194.00	-171.25	-207.73
42	14.09	-106.99	-237.69	nd	-181.88	-190.63	-191.88	nd	-205.29
43	14.41	-244.92	nd	nd	-181.51	-183.98	-146.44	nd	nd
44	14.73	-197.12	-224.10	nd	-172.05	-205.58	-191.14	-163.43	-204.37
45	15.05	-205.12	-226.07	-159.10	-179.74	-181.65	-195.70	-165.88	-207.27
46	15.36	-197.70	-218.58	nd	-176.81	-184.33	-191.63	nd	-201.83
47	15.68	-196.85	-214.16	nd	-157.42	-189.09	-189.48	-160.77	nd
48	16.00	-189.94	-211.80	-176.85	-175.83	-182.75	-190.56	-161.34	-199.42
49	16.32	-193.82	-210.61	-161.01	-167.12	-184.25	-186.54	-160.62	-180.94
50	16.67	-201.09	-201.52	-155.97	-172.28	-178.45	-187.62	-164.97	-193.92
51	17.03	-199.00	-202.58	nd	-177.78	-186.35	-194.53	-163.92	-204.54
52	17.39	-196.21	-196.98	nd	-165.52	-185.79	-183.94	-170.41	-198.73
52	17.75	-200.56	-198.64	-176.77	-179.01	-184.08	-197.45	-168.63	-199.13
54	18.10	-193.82	-207.01	-173.14	-170.35	-187.35	-191.64	-158.96	-184.84
55	18.46	-199.08	-196.88	nd	-151.78	-181.75	-188.54	-170.70	nd
56	18.82	-192.64	-193.18	-182.52	-172.15	-182.65	-192.17	-169.26	-194.36
57	19.17	-177.06	-167.21	-151.67	-163.97	-183.61	-195.31	-182.12	nd
58	19.53	-199.52	-197.68	-167.74	-172.77	-178.39	-187.38	-169.83	-189.25
59	19.89	-184.54	-182.89	nd	-156.23	-185.45	-183.48	-156.88	nd
60	20.24	-189.54	-185.31	-185.60	-180.85	-183.74	-190.06	-151.75	-186.56
61	20.60	-190.43	-195.87	-161.77	-183.63	-190.67	-191.85	-146.05	-187.70
62	20.96	-184.48	-195.82	nd	-159.15	-190.27	-186.77	-150.16	-174.19
63	21.32	-181.54	-190.40	-153.95	-172.40	-190.61	-151.39	-156.06	-195.01
64	21.67	-186.78	-189.96	-179.27	-180.15	-203.87	-191.45	-150.32	-187.55
65	22.03	-185.62	-197.89	-194.64	-166.84	-193.62	-184.24	-147.84	-193.62
66	22.39	nd	nd	nd	nd	-185.64	nd	nd	nd
67	22.74	-197.74	-185.64	-193.04	-194.73	-181.83	-193.00	-147.78	-191.77
68	23.10	nd	nd	nd	nd	-190.12	nd	nd	nd
69	23.46	-149.41	nd	nd	nd	-175.67	nd	nd	nd
70	23.81	-184.77	-177.79	nd	nd	-170.03	-176.81	-144.66	nd
71	24.17	-180.14	-186.20	nd	-153.16	-174.33	-147.57	-149.05	nd
72	24.53	-146.28	-172.75	nd	-107.74	-162.24	-159.43	-140.38	-227.87
73	24.89	-207.38	-179.65	nd	-180.50	-158.65	-178.89	-152.62	-209.98
74	25.24	nd	nd	nd	nd	-181.05	nd	nd	nd
75	25.60	-210.99	-195.92	nd	-164.94	-172.90	-193.26	-147.26	-199.75

APPENDIX P. (cont.)

	Depth in Freeze core								
ID	(cm)	C ₁₆	C ₁₈	C ₂₀	C ₂₂	C ₂₄	C ₂₆	C ₂₈	C ₃₀
76	25.96	-194.47	-207.00	-190.39	-166.08	-188.50	-190.00	-150.88	-185.21
77	26.31	-204.76	-228.43	nd	-169.67	-191.61	-176.68	-181.50	-192.67
78	26.67	-225.77	-191.64	-196.07	-162.85	-182.60	-171.87	-156.94	-186.18
79	27.03	-215.55	-197.84	-163.23	-184.55	-191.08	-188.21	-151.92	-161.58
80	27.39	-187.60	-199.86	-166.64	-171.28	-184.13	-184.37	nd	nd
81	27.75	-193.64	-204.48	nd	-177.56	-186.96	-183.47	-149.43	-199.61
82	28.11	-185.03	-199.09	nd	nd	-183.30	-170.54	nd	nd
83	28.48	nd	nd	nd	nd	-184.37	nd	nd	nd
84	28.84	-199.93	-221.32	nd	-177.21	-189.66	-189.18	nd	nd
85	29.20	nd	nd	nd	nd	-187.89	nd	nd	nd
86	29.56	nd	nd	nd	nd	-191.86	nd	nd	nd
87	29.92	nd	nd	nd	nd	-181.28	-175.66	nd	nd
88	30.28	nd	nd	nd	-180.60	-186.61	-192.37	-200.43	-205.05
89	30.64	-164.80	-211.21	nd	nd	-199.62	-183.22	nd	nd
90	31.00	-201.72	-203.39	nd	-206.65	-196.64	-177.40	-149.50	-196.68
91	31.36	-174.78	-198.88	nd	-180.98	-184.21	-177.55	nd	-178.81
92	31.72	-197.99	nd	nd	-194.80	-193.30	-194.40	-199.28	-194.02
94	32.09	-181.94	-209.61	nd	-179.04	-200.70	-188.89	-150.02	-183.64
95	32.45	-193.19	nd	nd	nd	-201.39	nd	nd	nd
96	32.81	-197.09	-198.81	-207.88	-187.52	-177.45	-192.77	-141.39	-196.98
97	33.17	-192.80	-203.33	-197.78	-179.38	-179.71	-191.88	-148.35	-181.99
100	33.53	-172.69	nd	nd	-176.89	-186.73	-188.17	-145.45	-191.17
101	33.95	-180.70	-191.08	nd	-177.36	-192.46	-185.82	nd	-187.41
102	34.37	-179.83	nd	nd	nd	-201.17	-186.59	nd	nd
103	34.79	-149.14	nd	nd	nd	-193.27	-189.51	nd	nd
105	35.21	-179.16	nd	nd	-182.05	-196.00	-191.68	-146.08	-183.83
107	35.63	-206.06	-181.04	-168.28	-167.11	-188.52	-200.22	-166.02	-202.37
108	36.05	-194.12	-180.76	-178.30	-194.13	-211.99	-193.18	-149.32	-195.01
110	36.47	-198.23	-187.13	-184.90	-191.45	-191.46	-192.79	-154.91	-190.23
111	36.89	-196.30	-179.66	-179.16	-179.80	-193.50	-190.66	-155.49	-186.84
112	37.31	-191.11	-180.78	-170.30	-176.76	-188.56	-190.44	-149.25	-204.34
113	37.73	nd	nd	nd	nd	-185.41	-170.43	-193.20	-180.04
114	38.15	-190.25	-183.62	-164.13	-182.10	-195.05	-191.95	-154.65	-193.84
115	38.57	-201.74	-183.62	-191.18	-182.10	-173.91	-191.95	-154.65	-207.63
116	38.99	-197.57	-166.05	-164.13	-172.71	-192.51	-144.67	-194.52	-146.64
117	39.41	-180.70	-187.80	-169.59	-170.66	-182.31	-191.98	-151.03	-155.59
118	39.83	-171.95	-166.71	-173.41	-165.90	-182.01	-161.16	-150.78	-184.11
119	40.25	-191.15	-174.19	-176.46	-168.33	-170.89	-128.69	-192.52	-194.92
120	40.67	-186.85	nd	nd	nd	-176.71	-114.94	nd	nd
121	41.09	-177.15	nd	nd	nd	-173.42	-126.71	nd	nd
122	41.51	-152.43	-102.36	nd	nd	-168.68	-148.04	nd	nd
123	41.93	-126.75	-85.40	nd	-129.00	-177.59	-153.56	-189.31	nd
124	42.33	-171.58	-173.29	nd	-122.57	-180.21	-172.83	-145.20	nd
125	42.73	-129.40	-84.56	-123.61	-142.84	-189.24	-178.49	-163.04	-191.90
127	43.13	-139.75	-116.09	-151.82	-156.96	-199.79	-196.32	-154.50	-202.41
128	43.53	-167.92	-114.37	nd	-153.12	-209.52	-192.10	-204.31	nd
130	43.93	-140.02	-113.13	nd	-146.58	-205.63	-179.16	-146.21	nd
135	44.33	-184.60	-186.95	-153.92	-141.31	-200.57	-184.98	-155.00	-189.28
140	44.73	-181.32	-171.25	-161.40	-159.24	-186.64	-127.54	-143.56	-172.05
141	45.13	-176.25	-107.36	-146.17	-152.37	-165.61	-199.22	nd	-173.49
142	45.53	-192.50	-153.08	-162.89	-163.84	-208.58	-199.81	-163.05	-207.53
143	45.93	-196.71	-172.15	-172.69	-167.43	-210.85	-200.09	-162.99	-204.34
144	46.33	-179.95	-149.78	-164.04	-161.31	-207.95	-201.84	nd	-201.50
146	46.73	-189.77	-168.91	-176.41	-170.72	-212.74	-200.64	-156.66	-208.27
147	47.13	-186.82	-163.88	-175.50	-161.91	-213.24	-198.89	-153.80	-210.51
148	47.53	-184.32	-150.12	-155.06	-160.86	-209.01	-195.05	nd	-186.33
151	47.93	-186.78	-167.07	-177.11	-166.54	-196.06	-201.93	-147.15	-206.19
152	48.33	-196.10	-171.30	-160.39	-174.91	-212.73	-197.36	-167.49	-207.07
153	48.73	-186.49	-167.13	-162.39	-156.24	-207.14	-201.76	-159.65	-198.61
154	49.13	-167.70	-152.73	-129.45	-154.92	-211.11	-199.72	-144.47	-196.56
155	49.53	-193.47	-173.57	-171.96	-154.33	-212.58	-193.32	-172.33	-204.91
156	49.93	-198.02	-169.48	-174.01	-173.79	-190.48	-200.81	-161.34	-203.28
159	50.33	-191.32	-171.03	-181.01	-174.35	-211.18	-201.68	-167.57	-207.78
161	50.73	-198.25	-171.20	-181.84	-170.26	-211.08	-196.31	-205.11	-196.98
162	51.04	-191.94	-171.52	-172.17	-168.27	-210.07	-199.56	-159.38	-203.74
164	51.35	-181.33	-114.89	-145.75	-156.22	-208.37	-197.04	-200.65	-195.07

APPENDIX Q. Hydrogent stable isotopes alkanes of Lake George, ND freeze core.

ID	Depth in Freeze core		C ₂₃	C ₂₅	C ₂₇	C ₂₉
	(cm)					
64	21.67	nd	-189	-203	-202	
65	22.03	nd	-198	nd	nd	
66	22.39	nd	-203	-209	-211	
67	22.74	nd	nd	nd	nd	
68	23.10	nd	-193	nd	nd	
69	23.46	nd	-193	nd	nd	
70	23.81	nd	-202	-205	-207	
71	24.17	nd	-197	-204	-209	
72	24.53	nd	-199	-203	-209	
73	24.89	nd	-197	-203	nd	
74	25.24	nd	-195	-204	nd	
75	25.60	nd	-196	-196	-199	
76	25.96	nd	-199	-195	-198	
77	26.31	nd	-203	-198	-201	
78	26.67	nd	-207	-207	-211	
79	27.03	nd	-198	nd	nd	
80	27.39	-199	-205	-206	-212	
82	28.11	-203	-208	-208	-213	
84	28.84	-206	-211	-209	-216	
88	30.28	-208	-215	-210	-219	
89	30.64	-219	-217	-213	-220	
90	31.00	-209	-216	-210	-215	
91	31.36	-217	-220	-212	-216	
92	31.72	-116	-220	-213	-218	
94	32.09	-216	-223	-215	-219	
96	32.81	-215	-216	-211	-217	
97	33.17	-216	-217	-211	-216	

Appendix R. Northern Great Plains lakes hydrogen stable isotopes for surface sediment acids

ID	Lat	Long	C22	C24	C26	C28	C30	C32
CUBCREEK	40.24	-97.05	-85.2	-96.8	-74.7	-77.1	nd	nd
DEWEYR	41.40	-100.39	nd	-99.4	-111.4	-116.4	-109.3	nd
HACKBERRY	41.44	-102.26	-72.1	-61.5	-64.3	-58.8	-63.1	-83.5
ROUNDUP	41.75	-102.41	-66.8	-57.5	-51.2	-65.6	nd	nd
SWAN	42.14	-100.46	-54.6	-79.6	-84.0	-59.4	-72.0	-51.5
W.LONG	42.32	-100.43	-96.5	-99.6	-105.6	-91.1	-100.0	-83.9
CARTER PJ	42.68	-103.52	-127.1	-129.0	-124.6	-132.3	-127.3	-88.9
MENG	42.96	-103.59	-90.6	-107.1	-112.2	-99.0	-92.6	nd
ALBERT	44.53	-97.15	-66.6	-62.2	-67.4	-58.4	-88.5	-47.2
MEDICINE	44.82	-97.35	-91.9	-81.2	-106.8	-115.3	nd	nd
WAUBAY	45.40	-97.43	-90.8	-98.8	-110.1	-99.4	-122.6	-119.7
ENEMY	45.40	-97.29	-69.6	-98.9	-95.3	-86.7	-83.5	-81.7
COLDWATER	46.02	-99.07	-86.5	-94.1	-96.9	nd	nd	nd
ALKALI	46.03	-97.38	-130.2	-119.5	-125.4	-80.5	nd	nd
ALKALINE	46.67	-99.55	-92.9	-97.7	-98.3	-96.4	nd	nd
GEORGE	46.75	-99.50	-107.9	-105.4	-107.9	-130.1	nd	nd
MOON	46.85	-98.60	-113.3	-110.9	-117.3	-122.1	-122.6	-119.0
SPIRITWOOD	47.08	-98.58	-113.6	-116.1	-117.2	-110.9	nd	nd
CARLSON	47.92	-101.47	-109.8	-90.7	-99.6	-90.3	-102.7	nd
SKOAL	47.92	-101.47	-123.4	-120.8	-125.9	-119.7	nd	nd
RICE	48.00	-101.60	-120.0	-124.4	-117.4	-89.8	nd	nd
KETTLE	48.60	-103.62	-109.3	-98.1	-110.1	-110.9	-123.6	nd
BRUSH	48.60	-104.12	-128.6	-127.1	-134.9	-114.6	nd	nd

Appendix S. Northern Great Plains physical lake data for July 2007

Depth (m)	T (oC)	Cond (mS/cm)	TDS	Salinity (g/L)	DO (%)	pH
South Dakota						
<i>Albert</i>						
0	31.1	1995	1.162	0.89	286	8.46
0.5	31.05	1991	1.161	0.89	301	8.5
1	28.56	1913	1.157	0.89	305	8.52
2	24.6	1779	1.166	0.91	228.5	8.46
<i>Medicine</i>						
0	25.37	14.65	9.523	8.5	219	8.37
1	25.36	14.65	9.524	8.5	223.7	8.39
2	25.32	14.65	9.524	8.5	231.3	8.4
3	24.53	14.63	9.503	8.48	206.8	8.41
4	23.96	14.59	9.486	8.47	142.4	8.38
5	23.34	14.59	9.48	8.47	57.4	8.36
6	22.97	14.58	9.479	8.47	9.9	8.36
7	17.96	16.39	10.57	9.56	5	8.17
8	6.92	15.54	10.11	8.96	4.1	7.71
<i>Bitter</i>						
0	24.3	3209	2.112	1.7	160	8.83
1	24.25	3200	2.11	1.7	160.6	8.81
2	24.2	3196	2.109	1.7	159.5	8.8
3	24.15	3192	2.109	1.7	158.2	8.79
4	24.13	3189	2.108	1.69	157.1	8.78
4.5	23.24	3152	2.113	1.7	102.4	8.75
<i>Waubay</i>						
0	24.91	2216	1.44	1.13	159.2	8.86
1	24.91	2210	1.439	1.13	163	8.84
2	24.91	2208	1.438	1.13	163.7	8.83
3	24.91	2208	1.438	1.13	163.6	8.83
4	24.91	2207	1.437	1.13	163	8.84
5	24.91	2208	1.437	1.13	163.1	8.84
6	24.91	2208	1.438	1.13	163	8.84
7	24.56	2200	1.439	1.13	132.6	8.83
<i>Enemy Swim</i>						
0	24.82	407	0.266	0.2	150.1	8.95
1	24.68	406	0.265	0.2	150.3	8.91
2	24.69	406	0.265	0.19	149.3	8.83
3	24.55	406	0.266	0.2	147.9	8.79
4	23.96	403	0.267	0.2	140.9	8.76
5	23.84	402	0.267	0.2	139.7	8.76

Appendix S. (cont.)

Depth (m)	T (oC)	Cond (mS/cm)	TDS	Salinity (g/L)	DO (%)	pH
North Dakota						
<i>Coldwater</i>						
0	26.2	2240	1.424	1.12	141.4	8.89
1	25.24	2196	1.421	1.12	137.9	8.88
2	25.13	2188	1.419	1.11	134.8	8.87
3	25.04	2184	1.418	1.11	131.7	8.86
4	24.22	2155	1.422	1.12	92	8.81
5	23.95	2142	1.421	1.12	59.7	8.78
6	23.8	2137	1.421	1.12	43	8.76
<i>Isabel</i>						
0	26.42	1733	1.097	0.85	115.1	8.84
1	26.41	1731	1.096	0.85	112.7	8.83
1.5	26.41	1731	1.096	0.85	110.7	8.83
<i>Alkaline</i>						
0	24.06	4263	2.821	2.31	133.6	8.93
1	24.08	4268	2.825	2.31	133.5	8.94
2	23.89	4253	2.825	2.31	129.3	8.97
<i>Lake George</i>						
0	24.98	16584	10.78	9.72	150.9	8.98
1	24.9	16559	10.79	9.73	154.3	9
2	24.64	16472	10.78	9.73	153	9.02
3	24.34	16381	10.78	9.73	151.8	9.03
4	23.66	16121	10.77	9.72	145.8	9.04
5	22.33	15659	10.72	9.7	125.7	9.05
6	21.64	15405	10.7	9.67	108.3	9.05
7	21.28	15286	10.7	9.67	99.2	9.06
8	20.81	15123	10.69	9.66	86.3	9.05
9	20.3	14962	10.68	9.67	68.2	9.06
10	19.1	14585	10.69	9.68	44.5	9.06
11	15.41	13551	10.76	9.76	11.7	9.07
12	11.81	12532	10.93	9.9	3.2	9.05
13	6.53	11367	11.42	10.28	2.2	9.02
14	3.55	10588	11.66	10.42	1.7	9.01
15	2.3	10179	11.68	10.38	1.6	8.98
16	1.64	10001	11.73	10.4	1.3	8.97
17	1.38	9906	11.74	10.39	1.1	8.94
18	1.23	9859	11.74	10.38	1	8.94