

2010

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Toney, Jaime L.; Huang, Yongsong; Fritz, Sherilyn C.; Baker, Paul A.; Grimm, Eric; and Nyren, Paul, "Climatic and Environmental Controls on the Occurrence and Distributions of Long Chain Alkenones in Lakes of the Interior United States" (2010). *Papers in the Earth and Atmospheric Sciences*. 124.

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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 ~11 °C versus 17 °C in our warmest sites), brackish to mesosaline (salinity = 8.5–9.7 g/L), and alkaline (pH = 8.4–9.0) 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 C_{37:4} alkenone; however, we discovered an unprecedented lacustrine alkenone distribution in a cluster of lakes, with a total absence of 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 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.

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 cos-

mopolitan haptophyte algae (*Emiliani huxleyii* 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 et al., 2005).

LCAs also have been reported in lakes (Cranwell, 1985; Zink et al., 2001; Chu et al., 2005; D'Andrea and Huang, 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 paleotemperature 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 TEX_{86} 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

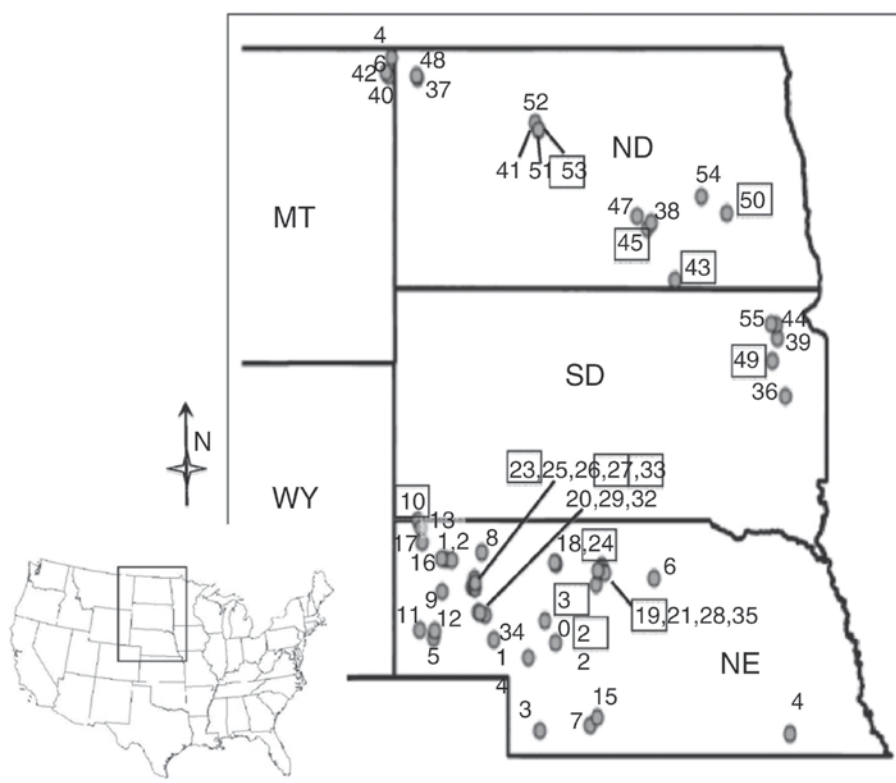


Figure 1. Map showing the location of investigated lakes sites. Numbers correspond to lakes listed in Table 1. Numbers enclosed by a square represent sites with alkenone presence in the modern lake sediments.

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

ID#	LCA	Lake	State	Lat.	Long.	Max. depth (m)	Conductivity (µS/cm)	pH	DO (%) 0.5m	July Lake T	Salinity (g/L)	Alkalinity (mg/L CaCO ₃)	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	181	X	X
2		Chadron	NE	42.45	103.00	4.32	301	8.96	8.18	20.96	0.11	192	X	X
3		Champion	NE	40.28	101.45	1.30	392	8.66	6.80	23.66	0.19	194	X	X
4		Cub Creek S	NE	40.24	97.05	2.87	166	8.08	7.10	22.44	0.07	76	X	X
5		Cochran	NE	41.46	103.32	X	1444	9.27	6.48	20.62	0.77	293	X	X
6		Cozad	NE	42.22	99.44	X	348	9.06	6.84	19.33	0.17	253	X	X
7		Hayes Center	NE	40.35	100.56	1.86	472	8.77	8.23	23.60	0.24	310	X	X
8		Isham Dam	NE	42.55	102.47	4.46	767	9.32	7.40	20.74	0.39	367	X	X
9		Kilpatrick	NE	42.05	103.17	1.90	475	9.41	7.20	20.60	0.24	313	X	X
10	*	Meng	NE	42.96	103.59	1.07	9847	9.65	7.63	18.27	5.57	317	X	X
11		Merritt	NE	41.56	103.56	20.03	180	8.66	9.98	18.63	X	77	X	X
12		Minatare	NE	41.55	103.30	5.87	722	8.65	7.62	18.22	0.35	243	X	X
13		Norman	NE	42.86	103.54	0.77	477	9.19	8.28	16.37	0.27	257	X	X
14		Ogallala	NE	41.21	101.65	10.56	752	8.53	8.87	16.79	0.46	181	X	X
15		Wellfleet	NE	40.45	100.44	2.50	363	8.70	7.83	23.24	0.18	305	X	X
16		Whitney	NE	42.47	103.18	2.42	390	8.77	6.76	18.64	0.19	251	X	X
17		CP Johnson	NE	42.68	103.52	3.87	304	8.58	8.46	18.79	0.11	179	X	X
18		Two Mile	NE	42.40	101.16	X	235	10.34	9.37	18.34	0.11	154	CO ₃	Na(Ca)
19	*	Big Alkali	NE	42.38	100.36	2.11	544	8.76	9.08	19.84	0.26	312	CO ₃	Na
20		Border Lake	NE	41.79	102.54	0.90	31,621	10.02	6.77	17.44	0.20	38,924	CO ₃ (SO ₄)	Na
21		Dewey Lake	NE	42.33	100.39	1.65	339	8.59	8.97	18.64	0.15	191	CO ₃	Ca
22	*	Dry Lake	NE	41.40	101.17	0.96	3475	9.25	8.12	19.93	2.22	1856	CO ₃	Na
23	*	Freshwater Lake	NE	42.22	102.60	1.07	41,285	9.39	7.41	21.77	49.45	16,040	SO ₄	X
24	*	Goose Lake	NE	42.42	101.19	0.91	4008	8.92	9.16	17.27	4.06	2826	CO ₃	Na
25		Jesse Lake	NE	42.11	102.64	0.53	61,412	9.85	6.04	18.33	64.91	59,280	CO ₃ (SO ₄)	X
26		Jesse Road	NE	42.10	102.60	0.68	28,978	9.51	6.74	19.93	25.16	27,660	CO ₃ (SO ₄)	X
27	*	Palmer	NE	42.09	102.59	0.85	10,469	9.36	8.37	20.28	8.63	6915	CO ₃ (SO ₄)	X
28		Pony Lake	NE	42.29	100.31	1.18	283	9.07	9.53	19.46	0.14	147	CO ₃	Na
29		Roundup Lake	NE	41.75	102.41	1.01	1145	8.70	8.87	18.39	0.75	661	CO ₃	Na
30	*	Sand Beach	NE	41.68	101.36	2.13	2653	9.17	8.72	20.52	1.08	1604	CO ₃	Na
31		Swan Lake	NE	42.14	100.46	0.74	180	9.38	9.02	20.62	0.08	100	CO ₃	Ca
32		Three Claim	NE	41.78	102.50	X	3001	8.82	8.36	15.53	3.39	1978	CO ₃	X
33	*	Wickson Lake	NE	42.16	102.59	0.84	19,169	9.15	7.53	20.31	5.50	3184	CO ₃ (SO ₄)	K(Na)
34		Hackberry	NE	41.44	102.26	X	578	X	X	X	0.29	386	CO ₃	Na(Mg)(K)
35		West Long	NE	42.32	100.43	1.23	321	8.94	9.30	19.25	0.17	171	CO ₃	X
<i>Northern Great Plains Lakes</i>														
36		Albert	SD	44.53	97.13	2.3	1995	8.46	0.89	31.05	0.89	40	SO ₄	Mg(Ca)
37		Alkali	ND	48.58	103.60	<0.5	28,608	X	18.07	23.97	18.07	1680	X	X
38		Alkaline	ND	46.66	99.56	2.9	4263	8.93	2.31	24.08	2.31	300	X	Mg(Na)
39		Bitter	SD	45.27	97.27	5.2	3209	8.83	1.70	24.25	1.70	200	SO ₄ (CO ₃)	Mg(Na)

continued

Table 1. (continued)

ID#	LCA	Lake	State	Lat.	Long.	Max. depth (m)	Conductivity ($\mu\text{S}/\text{cm}$)	pH	DO (%) 0.5m	July Lake T	Salinity (g/L)	Alkalinity (mg/L CaCO ₃)	Dominant anion	Dominant cation
N40		Brush	MT	48.60	104.11	18	5547	9.00	3.23	21.56	3.23	240	SO ₄	Na
41		Carlson	ND	47.92	101.47	4.5	1181	9.15	0.64	21.07	0.64	160	X	X
42		Clear	MT	48.65	104.15	1.7	7608	9.19	4.58	21.09	4.58	600	CO ₃	Na
43	*	Coldwater	ND	46.01	99.07	6.8	2240	8.89	1.12	25.24	1.12	160	SO ₄ (CO ₃)	Mg(Na)
44		Enemy Swim	SD	45.44	97.29	5.7	407	8.95	0.20	24.68	0.20	80	X	X
45	*	George	ND	46.74	99.49	60	16,584	8.98	9.72	24.90	9.72	520	SO ₄	Na
46		Goose	MT	48.84	104.06	<0.5	13,1807	X	92.20	X	92.20	10,200	X	X
47		Isabel	ND	46.82	99.74	2.2	1733	8.84	0.85	26.41	0.85	240	CO ₃ (SO ₄)	Na(Mg)
48		Kettle	ND	48.61	103.62	9.7	1624	8.79	0.87	21.86	0.87	200	X	X
49	*	Medicine	SD	44.98	97.36	8.3	14,754	8.37	8.50	25.36	8.50	160	SO ₄ (CO ₃)	Na
50	*	Moon	ND	46.86	98.16	8.2	5355	8.98	2.74	27.35	2.74	260	SO ₄ (CO ₃)	Na(Mg)
51		Nelson	ND	47.92	101.47	4	1650	9.20	0.91	20.84	0.91	160	SO ₄	X
52		Rice	ND	48.01	101.53	5.8	1751	8.95	0.93	22.73	0.93	160	SO ₄	X
53	*	Skoal	ND	47.92	101.47	1.8	12,018	8.90	7.68	20.07	7.68	300	SO ₄	X
54		Spiritwood	ND	47.07	98.61	14	2050	9.27	0.99	25.66	0.99	100	SO ₄ (CO ₃)	Na(Mg)
55		Waubay	SD	45.45	97.38	7.3	2216	8.86	1.13	24.91	1.13	180	SO ₄	Mg

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) (Figure 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 0–1 cm of a gravity surface corer (Figure 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 (60 m × 320 µm × 0.10 µm) 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 >4 µg 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 h. 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 Figure 2a-d. The C₃₆ 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 C₃₆ compound, as demonstrated by hydrogenation of C₃₆ (Figure 2b). Amazingly, the distributions of C₃₆ and C_{36:1} closely mimic the alkenone distributions (Figure 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 (Figure 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 (Figure 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 H₂/PtO₂.

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	10,713	27,783	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	12,210	35.25
Medicine	245	28,680	117.06
Moon	73	2541	34.67
Skool	277	8955	32.34
Coldwater	125.1	1898	15.17
Brush	281.8	2304	8.18

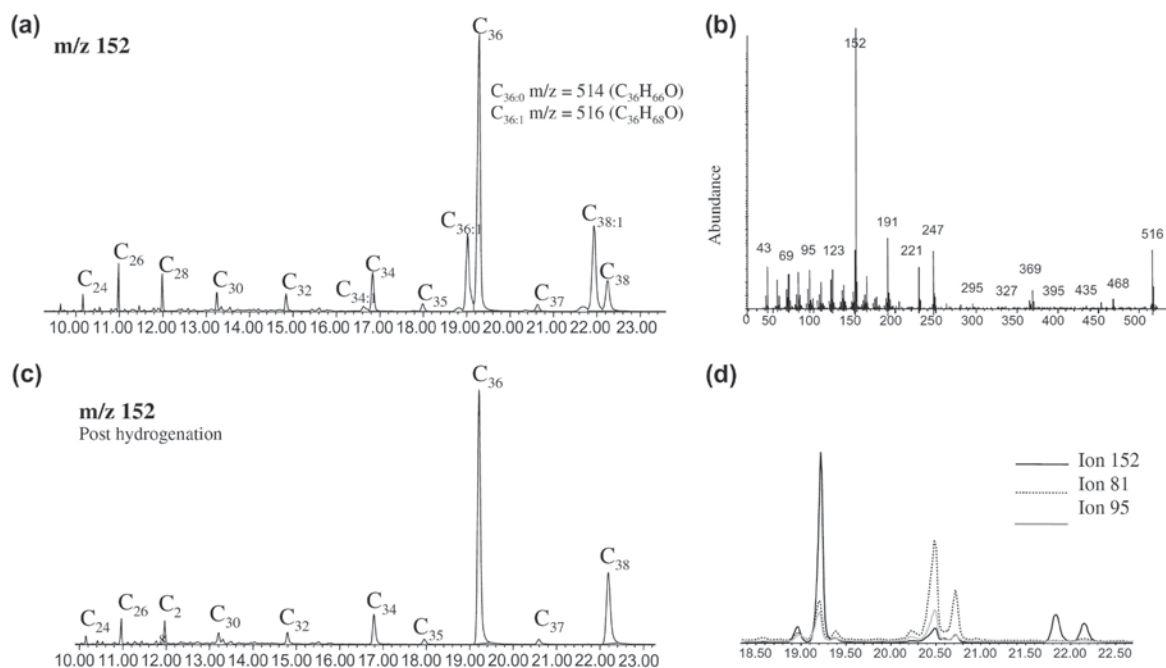


Figure 2. GC-MS data for a novel compound series found in a number of study sites: (a) Ion chromatogram of the compound series (m/z 152), (b) same as (a) but after hydrogenation, (c) mass spectrum of the $C_{36:0}$ homologue of the compound series, (d) Ion chromatogram showing elution of the unknown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

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 Figure 3a-k. The alkenones from 8 sites (Figure 3d-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 8.4–9.4. 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 (Figure 4a and b). All filters contain $C_{37:4'}$, $C_{37:3'}$, $C_{37:2'}$, $C_{38:4\text{et}'}$, $C_{38:3\text{et,me}'}$, $C_{38:2\text{et,me}'}$, $C_{39:4'}$ 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 $\mu\text{g/L}$ water, and the C_{37} LCAs are not resolvable. Water samples collected through the ice in February 2009 from 0, 5, 10, and 13 m (max water depth at site 13.5 m) were sampled for LCAs, but none were detected.

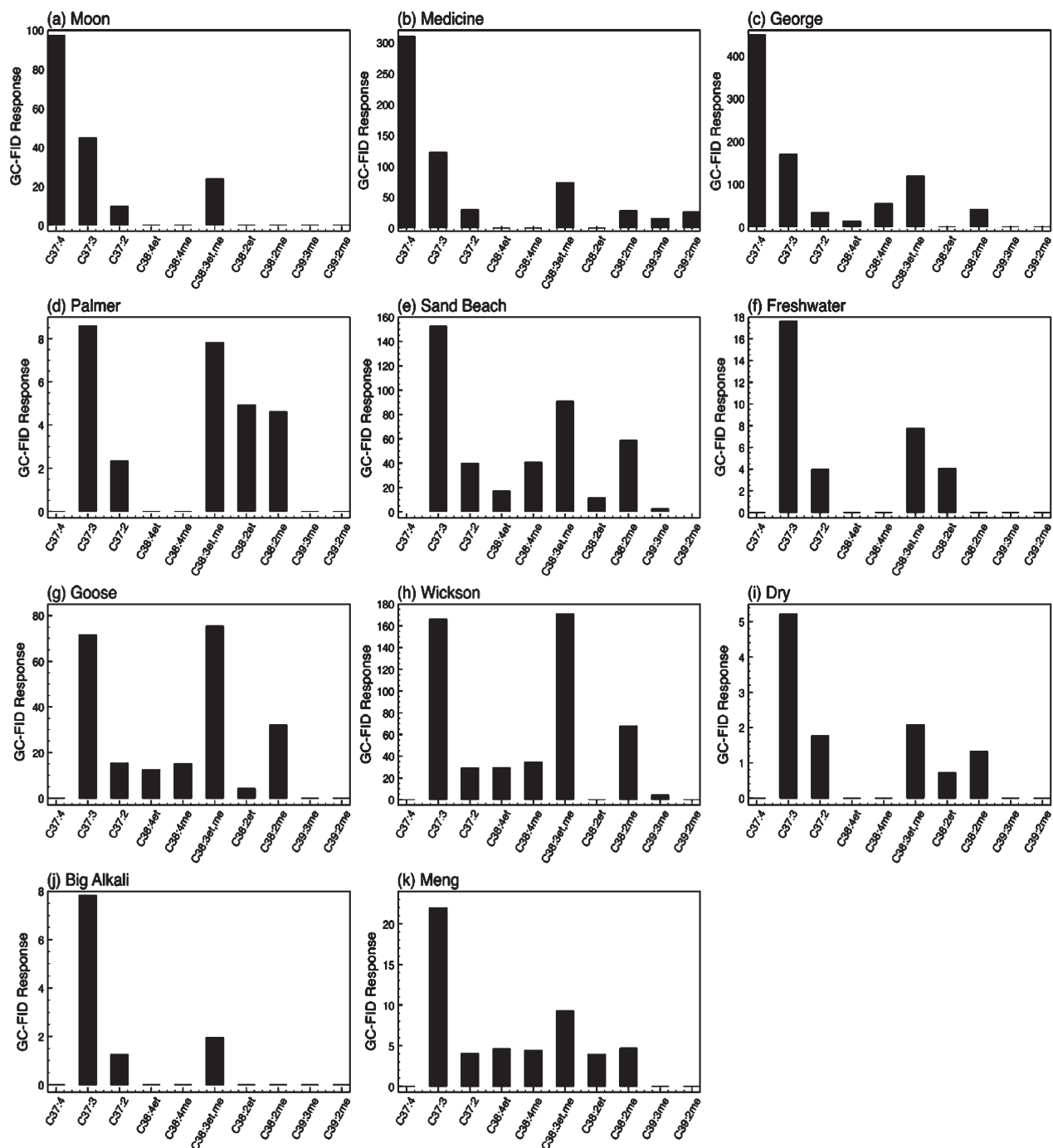


Figure 3. Histograms showing the relative abundance of long chain methyl and ethyl ketones for lake sites with quantifiable alkenone peaks. Lakes (a)–(c) are in the NGP and contain the $C_{37:4}$ alkenone, which is absent from the LCA profiles from the NE lakes shown in (d)–(k). (NGP, Northern Great 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.)

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 over-

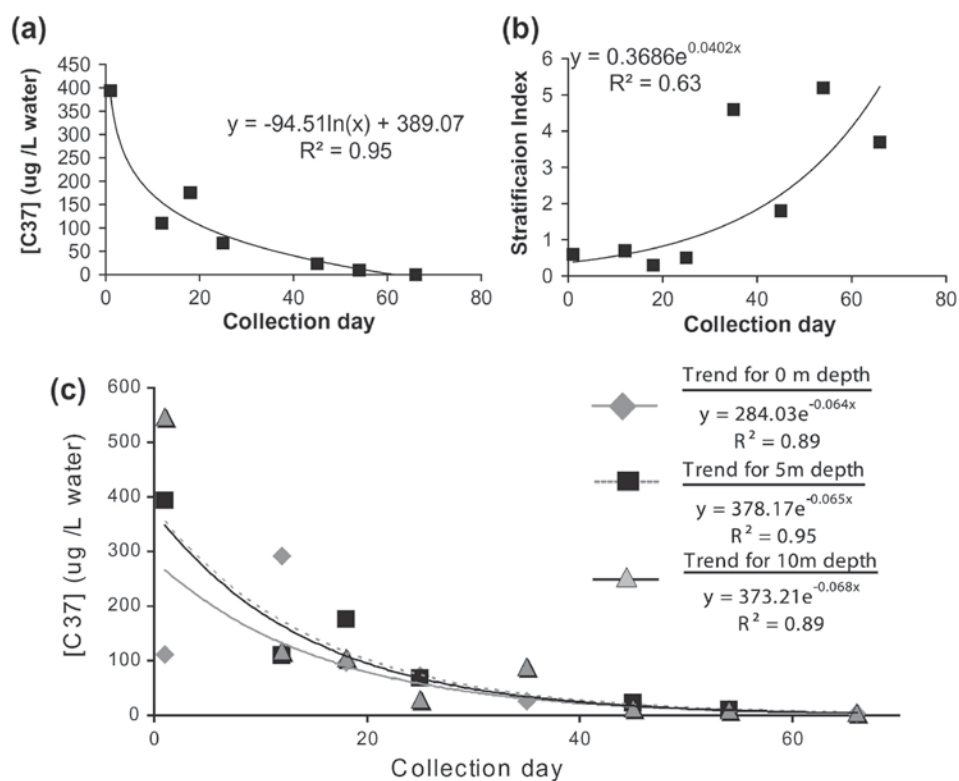


Figure 4. (a) Alkenone concentration data from water column sampling at 5 m depth from Lake George, ND. Day 1 = May 16, 2008. (b) The stratification index (surface water temperature subtract the temperature at 10 m water depth) for Lake George, ND. (c) Alkenone concentration data from water column sampling 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).

lying 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 ($SCR = 0.01$ – 2.59) that lack $C_{37:4}$ versus the alkenone-containing NGP sites ($SCR = 15.17$ – 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 hap-

tophytes 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 (Saras 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

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 Conc. (µg g ⁻¹ sed)	C ₃₈ Conc. (µg g ⁻¹ sed)	C _{38:3} Conc. (µg g ⁻¹ sed)	UK37 ^a	UK37 ^b	UK3738 ^c	UK38 ^d	UK38 ^e	C ₃₇ /C ₃₈	%C _{37:4}	MAAT ^f (°C)
<i>Core Top Sediment Samples</i>												
Moon Lake	ND	1.93*	0.17	0.07	0.18	-0.58	-0.14	0.00	0.00	6.40	62	11
Medicine Lake	SD	669.99	6.40	2.93	0.19	-0.61	-0.12	0.28	0.28	4.54	67	12
Lake George	ND	313.95	2.86	1.27	0.6	-0.64	-0.12	-0.07	0.25	2.88	69	11
Sand Beach Lake	NE	16.57	0.48	0.19	0.21	0.21	0.06	0.09	0.39	0.87	0	17
Freshwater Lake	NE	3.84*	0.55	0.20	0.18	0.18	0.18	0.00	0.00	1.83	0	15
Goose Lake	NE	4.49*	0.24	0.11	0.18	0.18	0.06	0.14	0.30	0.62	0	16
Wickson Lake	NE	37.35	1.30	0.64	0.15	0.15	0.01	0.12	0.28	0.64	0	16
Dry Lake	NE	1.24*	0.34	0.11	0.25	0.25	-0.30	0.39	0.39	2.71	0	16
Palmer Lake	NE	2.83	0.49	0.17	0.21	0.21	0.14	0.37	0.37	1.04	0	16
Big Alkali Lake	NE	1.17*	0.19	0.06	0.14	0.14	0.29	0.00	0.00	9.51	0	17
Meng Lake	NE	4.74*	0.29	0.10	0.16	0.16	-0.02	0.01	0.33	1.98	0	16
Coldwater Lake	ND	1.98*	X	X	X	X	X	X	X	X	X	12
Skoal Lake	ND	X	X	X	X	X	X	X	X	X	X	12
<i>In-situ Water Column Filter Samples</i>												
LG 5/16 0 cm	ND	111.72	29.63	15.89	0.15	-0.70	0.19	-0.12	0.24	3.77	74	8.6
LG 5/16 5 cm	ND	393.59	101.19	53.23	0.16	-0.71	0.19	-0.14	0.24	3.89	75	8.3
LG 5/16 10 cm	ND	547.19	140.08	73.95	0.16	-0.72	0.20	-0.10	0.26	3.91	75	8.0
LG 5/27 0 cm	ND	291.70	73.73	39.64	0.15	-0.67	0.20	-0.05	0.28	3.96	71	11.7
LG 5/27 5 cm	ND	109.61	29.50	15.15	0.15	-0.69	0.20	-0.10	0.28	3.72	73	11.3
LG 5/27 10 cm	ND	118.36	32.18	18.71	0.15	-0.69	0.20	-0.07	0.26	3.68	73	11.0
LG 6/03 0 cm	ND	96.44	27.48	16.05	0.15	-0.62	0.21	-0.02	0.31	3.51	67	13.5
LG 6/03 5 cm	ND	175.16	51.16	30.40	0.14	-0.63	0.20	-0.04	0.29	3.42	68	13.6
LG 6/03 10 cm	ND	104.74	30.64	18.42	0.14	-0.65	0.21	-0.01	0.30	3.42	69	13.2
LG 6/10 0 cm	ND	72.54	23.72	14.37	0.15	-0.55	0.22	0.00	0.33	3.06	60	14.4
LG 6/10 5 cm	ND	67.20	18.49	11.47	0.16	-0.52	0.21	0.02	0.31	3.63	59	13.8
LG 6/10 10 cm	ND	29.28	10.11	6.26	0.15	-0.53	0.23	0.06	0.34	2.90	59	13.9
LG 6/20 0 cm	ND	26.42	0.00	0.00	0.20	-0.35	0.20	X	X	X	46	19.0
LG 6/20 5 cm	ND	20.12	5.55	4.05	0.20	-0.38	0.22	0.27	0.27	3.62	48	15.2
LG 6/20 10 cm	ND	88.17	22.34	15.58	0.22	-0.45	0.24	0.30	0.30	3.95	55	14.4
LG 6/30 0 cm	ND	20.95	8.71	6.25	0.20	-0.50	0.22	0.28	0.28	2.40	57	19.7
LG 6/30 5 cm	ND	23.07	9.19	6.83	0.16	-0.49	0.22	0.26	0.26	2.51	57	18.6
LG 6/30 10 cm	ND	13.12	5.58	4.07	0.07	-0.57	0.17	0.27	0.27	2.35	60	17.9
LG 7/09 0 cm	ND	9.51	3.97	2.94	0.15	-0.50	0.20	0.26	0.26	2.39	57	21.9
LG 7/09 5 cm	ND	9.78	4.09	2.88	0.16	-0.44	0.22	0.30	0.30	2.39	52	21.2
LG 7/09 10 cm	ND	10.16	5.33	3.25	0.16	-0.50	0.26	0.09	0.36	1.91	57	16.7
LG 7/21 0 cm	ND	2.95	4.55	3.43	0.00	-0.63	0.20	0.25	0.25	0.65	63	23.5

Continued.

Table 3. (Continued).

Lake	State	C ₃₇ Conc. (µg g ⁻¹ sed)	C ₃₈ Conc. (µg g ⁻¹ sed)	C _{38:3} Conc. (µg g ⁻¹ sed)	UK37 ^a	UK37 ^b	UK3738 ^c	UK38 ^d	UK38 ^e	C ₃₇ /C ₃₈	% C _{37:4}	MAAT ^f (°C)
LG 7/21 5 cm	ND	X	X	X	X	-0.53	0.00	X	X	X	53	21.2
LG 7/21 10 cm	ND	4.08	X	X	X	-0.56	0.00	X	X	X	56	19.8
LG 6/9/09 6 m	ND	2.62	22.29	2.03	0.00	-0.69	0.00	0.00	0.00	0.43	69	5.7
LG 6/9/09 7 m	ND	13.34	21.29	3.68	0.00	-0.77	0.32	0.59	0.59	0.63	77	3.3
LG 6/9/09 8 m	ND	16.04	X	X	X	-0.81	0.00	0.00	0.00	X	81	2.4
LG 6/9/09 9 m	ND	16.36	X	X	X	-0.75	0.00	0.00	0.00	X	75	3.3
LG 6/9/09 15 m	ND	4.21	X	X	X	-0.77	0.00	0.00	0.00	X	77	1.6
LG 5/28/09 5 m	ND	17.81	X	X	0.10	-0.63	0.10	0.10	0.26	X	67	11.2

X signifies that sample concentrations were too low to quantify.

^a $U^{K'} = 37:2 / (37:2 + 37:3)$ (Sikes and Volkman, 1993).

^b $U^{K'} = (37:2 - 37:4) / (37:2 + 37:3 + 37:4)$ (Rosell-Melé et al., 1995).

^c $U^{K'} = (38:2 + 37:2) / (38:2 + 38:3 + 37:2 + 37:3)$ (Pearson et al., 2008).

^d $U^{K'} = (38:2 - 38:4) / (38:2 + 38:3 + 38:4)$ (Pearson et al., 2008).

^e $U^{K'} = 37:2 / (37:2 + 37:3)$ (Pearson et al., 2008).

^f LG, Lake George Filter.

^g Mean annual air temperature.

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

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) and Clear Lake aquifer (SO₄ < 200 mg/L). 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 µS/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 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 (60 mM) 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 (0.1–49.5 g L⁻¹) (Table 1), all but one site with alkenones in the sediments have salinity >1 g L⁻¹. Thus, we suggest that the 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 C_{37} alkenone distribution among the study lakes is the presence or absence of the tetra-unsaturated ketone (Figure 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 C_{37} alkenones may be a function of salinity, with higher percentage of $C_{37:4}$ in lower salinity lakes (Chu et al., 2005; Liu et al., 2006). 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 ($0.1\text{--}49.5\text{ g L}^{-1}$) (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 , $U_{37}^{K'}$, $U_{37:38}^{K'}$, 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 (Zink et al., 2001; D'Andrea and Huang, 2005); 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 versus 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 taxonomy

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 $C_{37:4}$ ($C_{37}:C_{38} = 2.4 \pm 2.9$) (Figure 5). In these two groups of lakes, NE Sandhills versus 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 *Chrysothila 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., 2007; 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; Marlowe et al., 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.

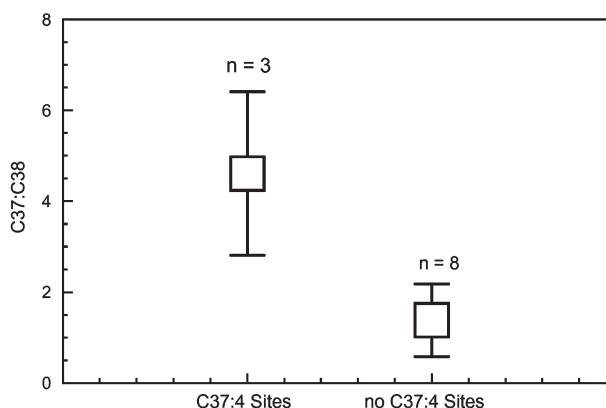


Figure 5. The $C_{37}:C_{38}$ ratio distribution for lakes that contain the $C_{37:4}$ LCA versus lakes that do not contain $C_{37:4}$.

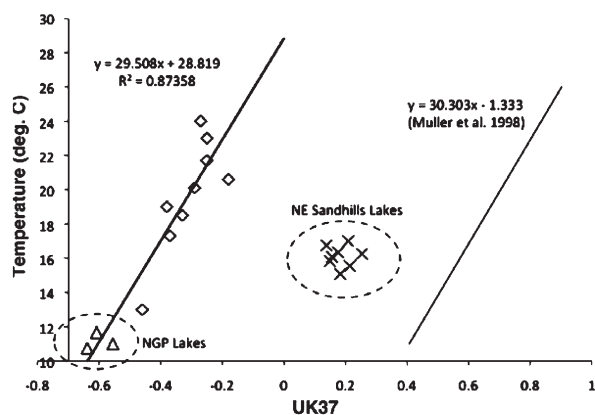


Figure 6. The previously published Zink et al. (2001) lacustrine LCA U_{37}^K calibration (diamonds) with the NGP core top U_{37}^K (triangles), the NE Sand Hill sites U_{37}^K (X) and the accepted marine linear calibration from Müller et al. (1998). (NGP, Northern Great Plains; MAAT, mean annual air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

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 SO_4).

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) (Figure 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 (Figure 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 (Figure 4a). Alkenone concentrations in lake water at 5 m depth decreased logarithmically, while

stratification index increased exponentially (Figure 4b and 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, 5, 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 ($r^2 = 0.75$) (Figure 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% and 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 ($r^2 = 0.73$) (Figure 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 (Figure 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

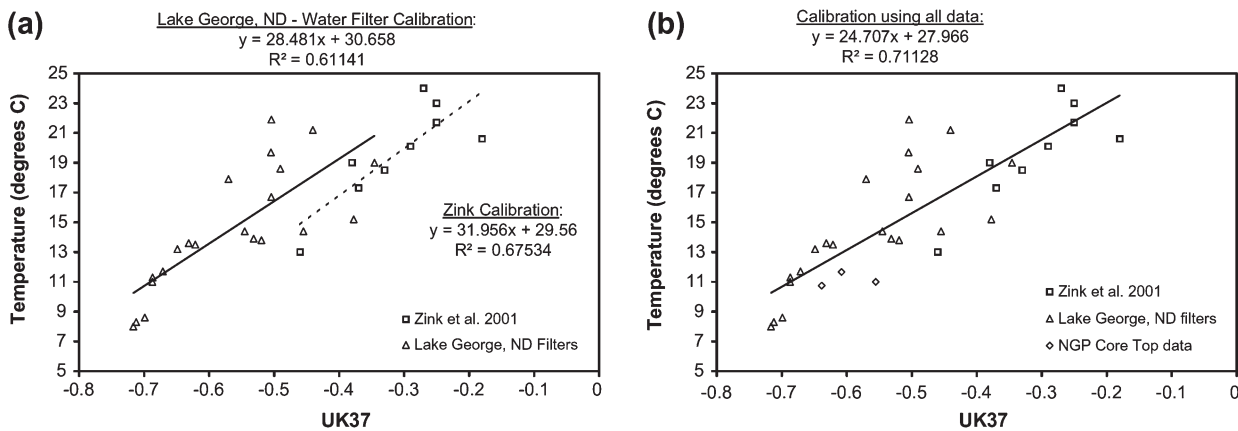


Figure 7. (a) The lacustrine LCA U_{37}^K calibration from the 2008 Lake George, ND for water column filters with *in-situ* lake water temperature (linear regression $r^2 = 0.74$, $p < 1.4 \times 10^{-8}$), (b) The proportion of $C_{37:4}$ from Lake George, ND water column filters plotted versus *in-situ*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

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 8.4–9.0; (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 an approach is used to derive LCA temperature calibration; (5) We demonstrate that the $U_{37}^{K'}$ in our study sites 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.

Acknowledgments

This work was supported by grants from the National Science Foundation to Y. Huang (EAR06-02325), Sheryllyn Fritz and Eric Grimm (EAR06-02154) and Paul Baker (EAR06-01779). We appreciate the constructive suggestions of Klaus Zink and two anonymous reviewers, whose feedback greatly strengthened the manuscript. We would like to thank Marcelo Alexandre for assistance during laboratory analysis; John and Aris Holz for water chemistry analysis of the NE sites; Danuta Bennett, John Holz, and Tad Barrow for assistance in collection of NE Sand Hill samples; and M. Isler for access at Lake George. Many thanks to Rick Bohn for collecting water samples without which this work would not be possible.

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