Assessing environmental controls on the distribution of long-chain alkenones in the

Canadian Prairies

Julien Plancq[a*], Bianca Cavazzin[a], Steve Juggins[b], Heather A. Haig[c], Peter R. Leavitt[c],

Jaime L. Toney[a]

[a] School of Geographical and Earth Sciences, University of Glasgow, Glasgow G12 8QQ,

Scotland, UK

[b] School of Geography, Politics and Sociology, Newcastle University, Newcastle-upon-Tyne,

NE1 7RU, UK

[c] Limnology Laboratory, Department of Biology, University of Regina, Regina, SK, Canada

S4S 0A2

[*] Corresponding author. E-mail address: julien.plancq@glaswow.ac.uk; tel.: +44 (0) 141 330

5449.

Abstract

Long-chain alkenones (LCAs) have been used for decades to reconstruct quantitative sea-surface temperature records, but they also have a great potential for paleotemperature reconstructions in lacustrine settings. Here, we investigated how the presence and abundance of LCAs in surface sediments from 106 lakes varied with environmental conditions in lakes of the Northern Great Plains (Canadian Prairies) in southern Saskatchewan, Canada. Consistent with preliminary research, we found LCAs in 55% of surveyed lakes, with mean concentrations of 143 μg/g dry sediment, but very high concentrations (up to 2.3 mg/g dry

26   sediment) in 7% of lakes. Statistical analyses indicate that salinity and stratification play key

27   roles in determining LCA presence and abundance supporting previous research in Spain and

28   the Northern Great Plains, US. Overall, the alkenone unsaturation index (U_{37}^K) was not

29   correlated significantly with average summer water temperature, probably because the timing

30   of maximum LCA production occurs during spring season. We conclude that improved

31   seasonal sampling is required within the study lakes to better identify the timing and habitat

32   of haptophyte production, and allow development of environmental temperature

33   reconstruction tools.

34

35   Keywords: alkenones, lakes, haptophyte, salinity, stratification, U_{37}^K.

36

37   1. Introduction

38   Long-chain alkenones (LCAs) are C_{35}-C_{42} di-, tri- and tetra-unsaturated methyl and ethyl

39   ketones whose biosynthesis in modern oceans is restricted to a few unicellular haptophyte

40   algae of the Isochrysidale Order, including the calcifying species (coccolithophores)

41   *Emiliania huxleyi* and *Gephyrocapsa oceanica* (Marlowe et al., 1984; Volkman et al., 1980,

42   1995), and the coastal non-calcifying species *Ruttnera lamellosa* (formerly *Chrysotila*

43   *lamellosa*) and *Isochrysis galbana* (Marlowe et al., 1984, 1990). The degree of unsaturation

44   of C_{37} alkenones has been shown to vary as a function of the environmental temperature

45   experienced during haptophyte growth (Brassell et al., 1986; Prahl and Wakeham, 1987), and

46   the so-called alkenone unsaturation indices (U_{37}^K, $U_{37}^{K'}$) have been developed in both

47   laboratory cultures and marine surface sediments as proxies for sea surface temperature

48   (Prahl et al., 1988; Müller et al., 1998; Conte et al., 2006). During the last three decades, the

49    $U_{37}^{K'}$ proxy has been successfully applied to reconstruct past marine temperature records at

50 various sites and timescales (e.g., Rosell-Melé et al., 1995; Müller et al., 1998; Pahnke and

51 Sachs, 2006).

52 More recently, LCAs have been reported worldwide in continental lakes (e.g., Cranwell,

53 1985; Zink et al., 2001; Chu et al., 2005; Longo et al., 2016). Unlike the open oceans, where

54 the LCA production is believed to be limited to *E. huxleyi* and *G. oceanica*, genomic analyses

55 from lacustrine sediments and particulate organic matter in lake water have shown the

56 existence of multiple LCA-producing haptophyte species within and among lakes (e.g.,

57 Coolen et al., 2004; D'Andrea et al., 2006; Theroux et al., 2010; Toney et al., 2012). Theroux

58 et al. (2010) identified three distinct phylogenetic groups within the Isochrysidale Order.

59 Group I (aka "Greenland phylotype", D'Andrea et al., 2006) seems to include haptophyte

60 species restricted to freshwater and oligohaline lakes, whereas Group II includes *R. lamellosa*

61 and *I. galbana*, as well as, related species found in brackish waters and saline lakes. Group III

62 includes the marine producers *E. huxleyi* and *G. oceanica*. In principle, the presence of

63 multiple species complicates development of biogeochemical metrics of past lake

64 temperatures as the relationship between temperature and the alkenone unsaturation index

65 could differ between haptophyte species (Theroux et al., 2010; Toney et al., 2010; Randlett et

66 al., 2014).

67 Moreover, the environmental parameters controlling alkenone distribution in inland waters

68 remain unclear as LCAs have been found in freshwater (e.g., Zink et al., 2001; D'Andrea and

69 Huang, 2005; Longo et al., 2013, 2016; D'Andrea et al., 2016) and saline lakes (e.g., Pearson

70 et al., 2008; Toney et al., 2010; Liu et al., 2008, 2011; Zhao et al., 2014; Song et al., 2016),

71 with ionic composition varying from sulfate- (e.g., Pearson et al., 2008; Toney et al., 2010) to

72 carbonate-rich solutions (e.g., Chu et al., 2005; D'Andrea and Huang, 2005). Salinity has

73 been suggested as an important factor on the occurrence and concentration of LCAs

74 (D'Andrea and Huang, 2005; Lopez et al., 2005; Pearson et al., 2008; Toney et al., 2010; Liu

75 et al., 2011; Chivall et al., 2014), with thresholds of salinity (<3 g/L and >100 g/L)

76 considered unsuitable for LCA producers to prosper (Liu et al., 2011; Song et al., 2016). In

77 addition, seasonality of environmental variables (e.g., temperature, salinity, nutrient),

78 especially in high latitude regions where lakes are ice covered during winter, may have a

79 strong effect on phytoplankton succession (e.g., Lampert and Sommer, 1997; Wetzel, 2001;

80 Korkonen et al., 2017). In fact, lacustrine haptophytes experience sudden increase in

81 abundance (bloom events), likely linked to seasonal changes in nutrient loading and

82 irradiance levels (D'Andrea et al., 2011; Toney et al., 2010; Huguet et al., 2011; Theroux,

83 2012).

84 Despite these potential complications, LCAs have been demonstrated to vary with lake

85 temperature in some sites, and a number of calibrations of the U^K_{37}, $U^{K'}_{37}$, and $U^{K''}_{37}$ indices have

86 been established in cultures (e.g., Sun et al., 2007; Toney et al., 2012; Theroux et al., 2013;

87 Zheng et al., 2016) and *in-situ* in freshwater (e.g., Zink et al., 2001; D'Andrea et al., 2011,

88 2016; Theroux et al., 2013; Longo et al., 2016) and saline lakes (e.g., Chu et al., 2005; Toney

89 et al., 2010; Zheng et al., 2016). Together, these findings suggest that quantitative

90 reconstruction of environmental temperature is possible.

91 The development of LCAs as metrics for past lacustrine temperatures may be important

92 for the Great Plains of North America. This region is sensitive to temperature and moisture

93 changes and often experiences prolonged periods of drought and floods that have major

94 impacts on agriculture production and ecosystem function. Although lacustrine proxies for

95 past droughts and pluvial intervals have been developed (e.g., diatom- and ostracod-inferred

96 salinity; e.g., Cywinska and Delorme, 1995; Michels et al., 2007), there are few unambiguous

97 proxies of past temperature in inland waters before the instrumental period. Quantitative

98 paleotemperature reconstructions are thus crucial to help decipher the climate mechanisms

99 responsible for drought occurrences. Studies from Northern Great Plains, USA, (Theroux et

100 al., 2010; Toney et al., 2010, 2012) suggest that LCA and haptophyte distributions may be

101 suitable for quantitative temperature reconstruction, particularly in well-studied sites. Toney

102 et al. (2011) reported the presence of LCAs in 13 lakes in the Canadian Prairies, southern

103 Saskatchewan, where the development of an alkenone paleothermometer is particularly

104 promising, because they appear to have only one haptophyte species based on the LCA

105 distribution. However, the low sample size (n=19) was insufficient to conclusively determine

106 which environmental parameters control the presence of LCA-producing haptophytes, or to

107 derive a temperature calibration based on lacustrine surface sediments.

108   Here, we assess LCA presence and distributions in surface sediments collected from a

109 broader survey of 87 additional lakes in the Canadian Prairies. Water chemistry data

110 measured at the time of sampling for each lake allows us to better statistically constrain the

111 environmental parameters favouring LCA presence and concentration in these 106 lakes. We

112 also investigated the relationship between LCA and environmental temperature to further

113 develop potential applications of an alkenone unsaturation index, such as U_{37}^K.

114

115 **2. Methods**

116

117 2.1. Sites and sampling

118   Between July 29th and August 30th 2013, 106 lakes from the Canadian Prairies (southern

119 Saskatchewan, Canada) (Fig. 1) were sampled during a field campaign for studies on modern

120 drought risks and ecosystem sustainability. These lakes occur in the sub-humid (north east)

121 to the semi-arid (south-west) areas of the Canadian Prairies, a region where evaporation

122 exceeds precipitation by 20-60 cm/year and lakes are sustained by the influx of snowmelt and

123 groundwater (Pham et al., 2008, 2009). Climate data for the study region, obtained from the

124 WorldClim database, indicate mean (+ SD) air temperatures and precipitation of; 2.5 ± 1.0°C

125   and 27.9 ± 2.5 mm in spring, 16.9 ± 0.7°C and 56.9 ± 6.0 mm in summer, 3.6 ± 0.8°C and

126   23.6 ± 4.1 mm in autumn and -14.2 ± 2.0°C and 16.9 ± 1.9 mm in winter. Survey lakes span a

127   wide spatial extent of 4° latitudinal gradient (Fig. 1) and a large range of salinity from fresh

128   (0.1 g/L) to hyper-saline (102 g/L), with diverse ionic composition.

129   *In-situ* lake parameters, including salinity, conductivity, pH, depth and temperature, were

130   measured at 0.5-m depth intervals (lakes <10 m depth) or 1-m depth intervals (lakes >10 m

131   depth) for the entire water column using a YSI Pro Plus meter (YSI Inc., Yellow Springs,

132   Ohio, USA) following Pham et al. (2008). Major ions including sodium (Na^+), chloride (Cl^-),

133   calcium (Ca^{2+}), magnesium (Mg^{2+}), sulfate (SO_4^{2-}), bicarbonate (HCO_3^-), and carbonate

134   (CO_3^{2-}) were determined for each lake and analysed at the University of Alberta Water

135   Chemistry Laboratory using standard procedures (Stainton et al., 1977). Water chemistry,

136   especially surface salinity, of saline lakes may undergo significant changes over the seasonal

137   cycles, and our *in-situ*, one-time measurements of salinity may not capture the seasonal

138   variation of ionic content in these lakes (Pham et al., 2009). However, while extremely dry

139   years lead to spatially coherent increases in lake salinity from spring to later summer in the

140   Canadian prairie lakes (Pham et al. 2009), there is little evidence of progressive increases in

141   salinity in years with elevated precipitation despite a net precipitation deficit (Pham et al.

142   2009), such as those which preceded our 2013 survey. These patterns are described in detail

143   as part of the Energy-*mass* flux framework, outlined in Leavitt et al. (2009). Thus, it is likely

144   that salinity does not significantly change from spring to summer in most of our survey

145   lakes.

146   Surface sediments were collected from each lake using an Ekman grab sampler.

147   Subsamples of sediments (top centimetre of the collected sediments) were freeze-dried at

148   0.01 Pa for 36 hours before use for analyses of organic constituents.

149

150   2.2 LCA analyses

151   Freeze-dried sediments were homogenized and extracted with dichloromethane

152   (DCM):methanol (MeOH) (9:1, v:v) using a Dionex model ASE350 accelerated solvent

153   extractor. Following evaporation of the solvent, the total lipid extracts were separated into

154   neutral and acid fractions by elution through a LC-NH$_2$ SPE column using DCM:isopropyl

155   alcohol (1:1, v/v) followed by ether with 4% acetic acid (v/v) as eluents, respectively. The

156   neutral fractions were further separated into four fractions of increasing polarity by

157   chromatography over a silica gel column packed with 35-70 μm particles using hexane,

158   DCM, ethyl acetate:hexane (1:3, v/v) and MeOH as eluents.

159   LCAs are contained in the second fraction (DCM) and were detected and quantified using

160   gas chromatography with a flame-ionization detector (GC-FID). To remove compounds that

161   can co-elute with LCAs such as alkenoates, LCA fractions were saponified by heating at

162   60°C overnight in 1N KOH in MeOH:H$_2$O (95:5, by volume). After heating, the reaction

163   mixture was cooled to room temperature, quenched with NaCl (5% by volume), extracted

164   using hexane, and rerun on the GC-FID.

165   GC-FID analyses were performed on an Agilent 7890B Series GC system configured with

166   an Agilent VF-200 ms capillary column (60 m length, 0.25 mm internal diameter, 0.10 µm

167   film thickness) (Longo et al., 2013). Hydrogen was used as the carrier gas at a 36 cm/s

168   column flow rate. The GC method used splitless injection (320°C), and the oven temperature

169   was programmed from 50°C (hold for 1 min) to 255°C at 20°C/min, then to 300°C at

170   3°C/min, followed to 10°C/min increase to 320°C and hold for 10 min. Samples were also

171   run with the same temperature program on an Agilent 7890B Series GC coupled with a

172   5977A GC-EI Mass Spectrometer (GC-MS) to confirm the identity the LCAs using the

173   known ion chromatograms and by comparison of mass spectral data and GC retention times

174   with published data (de Leeuw et al., 1980; Marlowe et al., 1984). LCAs were quantified

175   using hexatriacontane (n-C_{36} alkane) as an internal standard added to the sample before

176   injection. The reproducibility achieved for duplicate LCA quantifications was estimated to be

177   better than ± 10% (coefficient of variation). The alkenone unsaturation index U^K_{37} and other

178   indices (for example $U^{K'}_{37}$, $U^{K''}_{37}$, U^K_{38}) were calculated for all lakes following Brassell et al.

179   (1986), Pearson et al. (2008), and Zheng et al. (2016). LCA distribution parameters such as

180   C_{37}/C_{38} (Volkman et al., 1995), $\%C_{37:4}$ (Rosell-Melé, 1998), and RIK_{37} (Longo et al., 2016)

181   were also computed.

182

183   2.3 Statistical analyses

184   Approximately 45% of the lakes sampled had LCAs below detection limit and where

185   present, LCAs showed a strong right-skewed distribution. Consequently, the relationships

186   between LCAs and environmental parameters were modelled using a two-stage hurdle

187   process (Zuur et al., 2012) that models the presence-absence of LCAs and LCA concentration

188   as two distinct processes. For the first part, or "hurdle", we used a binomial generalised

189   linear model based on the full dataset to model the presence and absence of LCAs

190   above/below the detection limit. For observations with LCAs above the detection limit (i.e.,

191   those that crossed the hurdle), we modelled LCA concentration as a function of selected

192   environmental variables using a lognormal model. The hurdle model considers

193   presence/absence and concentration of LCAs separately, which is appropriate as

194   environmental factors controlling LCA presence may be different from those controlling

195   LCA concentration once observed (Taranu et al., 2017). Limnological datasets such as ours

196   contain large numbers of potential predictors which are often highly intercorrelated. Variable

197   selection is often used to find the "minimum adequate model" that contains a small subset of

198   variables that explains, in a statistical sense, the data as well as the full set. However,

199   variable selection with highly correlation predictors is problematic (Whittingham et al.,

2006), and it is increasingly recognised that there is no "best" model, but often a series of models that explain the data equally well.

Predictive models were developed to account for the observation that many limnological variables are potentially correlated. For both parts of the hurdle model, we tested the significance of each variable (water chemistry, limnological, climatic, landscape) separately using a chi-squared or F test for the presence-absence and concentration models, respectively. We then fitted a large set of models using all combinations of the subset of significant variables (Calcagno and Mazancourt, 2010). Using this set of models we then used Akaike's Information Criterion (AIC) to identify (a) the best model, and (b) all other models within 2 AIC units of the best model, as these are effectively indistinguishable from the best model and are equally plausible. We then fitted a final model using the list of predictor variables that appear in any of the selected models.

Variance partitioning of either the variance explained for the lognormal model or pseudo-R-squared (binomial model; Nagelkerke, 1991) was used to quantify the importance or explanatory power of each predictor in the final model and to decompose the explained variance into independent and shared components (Borcard et al., 1992). To provide an additional visualisation of the relationship between LCAs and environmental factors, we used classification and regression trees (CARTs) to model LCA presence and absence, and concentration, respectively. CARTs are binary recursive tree-based models that identify a set of decision rules that best predicts either a binary outcome (classification trees) or quantitative variable (regression tree). At each stage of fitting a CART tree, the algorithm identifies a variable and threshold value that best predicts the response variable. Since our interest here is in explanation rather than prediction, we used AIC to identify the best tree rather than cross-validation (Hothorn and Zeileis, 2015). CARTs have the disadvantage that they can only display a single model, when there may be several best models, but have the

225 advantage that they can reveal interactions between variables not easily visualised using other

226 methods (Hastie et al., 2009).

227 The relationships between various alkenone-based indices (%$C_{37:4}$, C_{37}/C_{38}; U^{K}_{37}, $U^{K'}_{37}$,

228 $U^{K''}_{37}$), alkenone isomer composition, and environmental factors were further explored using

229 linear regressions and constrained ordinations. For the latter, we assessed the significance

230 and importance of each predictor with a Monte Carlo permutation test and variance

231 partitioning respectively (Borcard et al., 2011).

232 The distribution of LCA concentration and many of the explanatory variables showed a

233 right-skewed distribution: these were either $\log_{10}$ (all water chemistry, lake area, LCA

234 concentration) or square-root- (sample depth) transformed prior to analysis to normalize their

235 distributions. We also included average air temperature, obtained from the WorldClim

236 database with a spatial resolution of approximately 1 km^2.

237 All statistical analyses were performed using R software for statistical computing and

238 graphics (R Development Core Team, 2017) with the following packages: *glmulti* (Calcagno

239 and Mazancourt, 2010), *vegan* (Oksanen et al., 2017), and *partykit* (Hothorn and Zeileis,

240 2015).

241

242 **3. Results**

243

244 3.1. Environmental data

245 Survey lakes exhibited large gradients for most environmental parameters (Table 1 and

246 supplementary Table S1) including depth (0.1-28.5 m), salinity (0.1-102.2 g/L) and mean

247 water temperature (8.6-23.7°C). In general, individual lakes had higher concentrations of

248 sulfate (SO_4^{2-}) (31.5-120,223 mg/L) than bicarbonate (HCO_3^-) (156-5625 mg/L), carbonate

249 (CO_3^{2-}) (<0.01- 5528 mg/L) or chloride (Cl^-) (3.1-45,022 mg/L). In addition, lakes exhibited

different degrees of thermal and chemical stratification, ranging from polymixis to permanent

stratification (meromixis). Chemistry and hydrology of about two-thirds of these lakes has

been discussed in Pham et al. (2009). Elevated sulfate concentration in the Saskatchewan

region is mostly derived from oxidation of the underlying bedrock (Last and Ginn, 2005;

Hayashi and van der Kamp, 2009).

3.2. LCA occurrence and abundances

Of the 106 lake surface sediments examined in this study, 58 (55%) contained detectable

concentrations of LCAs (Fig. 1; Table 1). Total concentrations of LCAs range from 2.5 to

27,625 μg/g TOC (0.02-2312 μg/g dry sediment), with very high concentrations (> 1000 μg/g

TOC or 100 μg/g dry sediment) recorded in 9 of the LCA-containing lakes (15% of basins)

(Table 1). The highest concentrations of LCAs generally occurred in the northern section of

the sampling area (> 51°N; Fig. 1).

The majority of LCA-containing surface sediments (53%) featured high $\%C_{37:4}$, with

values > 60%. Five lakes featured a profile mainly composed of the $C_{37:3}$ alkenone ($\%C_{37:4}<$

40%) (Fig. 2). In addition, one lake displayed a predominance of C_{38} alkenones over C_{37}

alkenones, with particularly elevated concentrations of the $C_{38:3}$ alkenone (Fig. 2). Among the

lakes, four of them showed the presence of the $C_{37:3b}$ alkenone isomer, including one

freshwater (< 0.5 g/L) and three oligohaline (0.5-5 g/L) lakes (Table 1 and Fig. 2). RIK_{37}

(ratio between $C_{37:3a}$ and $C_{37:3b}$ isomers; Longo et al., 2016) values ranged 0.47-0.57 (Table

1). C_{37}/C_{38} ratios varied between 0.4 and 7.7, with a mean value of 2.7 ± 1.7 (Table 1). No

significant correlation was found between the U_{37}^{K} unsaturation index and depth-integrated

water-column temperature or surface water temperature at the time of sampling (August

2013), or with air (spring or summer) temperature. No significant correlation was found

274 using other indices (such as the $U_{37}^{K\prime}$, $U_{37}^{K\prime\prime}$, or the U_{38}^K), or through regression analysis of

275 individual LCAs with air (spring or summer) and water temperature.

276

277 3.3. Factors influencing LCA presence and concentration

278   Figure 3 shows the significant predictor variables explaining the presence/absence of

279 LCAs, the explanatory power (variance) of each variable, and the explained variance

280 decomposed into independent and shared components. Salinity, water pH, depth, water

281 temperature and stratified (stratification of the lake) were the most significant predictor

282 variables explaining the occurrence of LCAs in the Canadian prairie lakes (supplementary

283 Table S2). Salinity was the most important variable (24% of variance explained), and most

284 of the variation explained by salinity (20%) was independent of other variables. Water pH

285 explained a small part (3.5%), but its explanatory power was mostly independent of other

286 parameters. Stratified, depth and water temperature explained moderate amounts of

287 variability (5%, 11% and 14%, respectively) but their explanatory powers were largely

288 confounded with each other.

289   To provide an additional visualisation of the relationship between LCAs and

290 environmental factors, we used a classification tree (Fig. 4). A split in salinity at 2.4 g/L was

291 observed, representing a threshold for the presence of LCAs. At low salinity (<2.4 g/L),

292 stratification was important for the presence, but LCAs were rare in lakes with high pH (>

293 8.5). At higher salinity (>2.4 g/L), LCAs were rare in shallow lakes (<0.6 m). LCAs occurred

294 mainly in deeper lakes (>0.6 m), but they were slightly less common in very saline lakes

295 (>44.4 g/L).

296   Salinity, depth, water temperature and presence of stratification were the most significant

297 predictor variables of modelled LCA concentrations (Fig. 5 and supplementary Table S2).

298 Salinity was the most important variable (31% of variance explained), and most of the

299   variation explained by salinity was independent of other variables. Depth explained a much

300   smaller proportion of variance (7%) and its explanatory power was entirely confounded with

301   other variables. Stratification and water temperature explained moderate amounts (16% and

302   24%, respectively), although their explanatory powers were largely confounded with each

303   other. The regression tree (Fig. 6) showed a salinity threshold of 4.9 g/L. A further distinction

304   was noted at low salinities (<4.9 g/L), with slightly higher LCA concentrations between 0.8

305   and 4.9 g/L than in less saline waters. At higher salinities (>4.9 g/L), LCAs showed higher

306   concentrations in stratified lakes than in unstratified basins.

307   Relative (%) importance of carbonate and sulfate and metrics of total salinity were

308   identified as the main predictors of alkenone isomer compositions, but explained variances

309   were generally low (8.2%, 5% and 2.5%, respectively). Most variation explained by

310   carbonate (4.6%) was independent of other variables, whereas the explanatory power of

311   sulfate (4.8%) and salinity (1.4%) was largely confounded with other variables.

312   Statistical analyses between various LCA-based indices and environmental factors showed

313   a significant but weak relationship between % carbonate (among anions) and $\%C_{37:4,}$ U^K_{37},

314    $U^{K''}_{37}$, or C_{37}/C_{38} (supplementary Table S2). No significant relationship was found between

315   salinity and $\%C_{37:4}$, or between salinity and U^K_{37}, $U^{K''}_{37}$, or C_{37}/C_{38} (supplementary Table S2).

316   For the $U^{K'}_{37}$ index, no significant correlation was observed with any environmental variables.

317

318   **4. Discussion**

319

320   4.1. LCA distribution and haptophyte taxonomy

321   The majority of LCA-containing lakes showed high proportion of $C_{37:4}$ alkenone (Fig. 2),

322   which is a common feature of lacustrine systems (e.g., Sun et al., 2004; D'Andrea et al.,

323   2011; Longo et al., 2016). A large variation in C_{37}/C_{38} ratio values (0.5-7.7; mean value =

324 3.3) was observed in these lakes, but values were in the same range as those recorded for

325 *Isochrysis galbana* and *Ruttnera lamellosa* (Marlowe et al., 1984; Rontani et al., 2004) and

326 lakes within the US Great Plain sites (Toney et al., 2010). In contrast, five Canadian lakes

327 were characterized by elevated $C_{37:3}$ alkenone content (Fig. 2), a profile typical of offshore

328 marine environments (e.g., Prahl et al., 1988; Sikes and Volkman, 1993) and rarely reported

329 in lacustrine sediments (e.g., Li et al., 1996; Theissen et al., 2005; Toney et al., 2010; Hou et

330 al., 2016). These lakes present lower C_{37}/C_{38} ratio variations and values (1.4-2.4; mean value

331 = 1.8) similar to those recorded for *Emiliania huxleyi* (Marlowe et al., 1984), from Chinese

332 lakes (Sun et al., 2004; Chu et al., 2005), and in US sites with $C_{37:3}$-dominant profiles (Toney

333 et al., 2010). Finally, a single lake displayed a predominance of C_{38} over C_{37} LCAs, with

334 especially elevated levels of tri-unsaturated components. A few lacustrine studies have

335 reported such a profile in alkaline, evaporitic or saline settings (Thiel et al., 1997; Sun et al.,

336 2004; Chu et al., 2005; Pearson et al., 2008). C_{37}/C_{38} ratio for this particular lake was low

337 (0.4) and very similar to that of the Spanish lakes (mean value = 0.1) studied by Pearson et al.

338 (2008). Pearson et al. (2008) suggested that salinity may play a role in LCA composition,

339 with a salinity threshold of 40 g/L for C_{37} LCAs and C_{38}-dominated LCA profiles being

340 characteristic of hypersaline lakes. In contrast, the sole basin with C_{38} LCAs reported here

341 was not hypersaline (10 g/L) and we report that C_{37} LCAs were also present in one lake with

342 salinity >40 g/L (Table 1), suggesting that salinity, per se, is not the sole factor influencing

343 the dominance of C_{38} alkenones.

344   Differences in LCA profiles among basins may reflect variation in the community

345 composition of LCA-producing haptophyte species in the studied Canadian lakes. Studies in

346 the US Great Plains (Toney et al., 2010; Theroux et al., 2010; Toney et al., 2012) showed the

347 presence of two haptophyte species from Group II among and within saline lakes, with OTU7

348 haptophyte or Hap B producing mainly $C_{37:3}$ alkenones, while OTU8 haptophyte or Hap A

349   produces predominantly $C_{37:4}$ alkenones. Part of the reason for this difference in distribution

350   can be attributed to the offset in the timing of their bloom, where Hap A blooms in the early

351   spring during low temperatures (2-12°C) and Hap B blooms nearly a month later with a

352   temperature range of (12-18°C) (Theroux, 2012). In addition, Toney et al. (2012) showed that

353   Hap A species dominates LCA production in sulfate-rich lakes (sulfate:carbonate ratio SCR

354   >1), while production is dominated by Hap B in carbonate-dominated lakes with SCR<1.

355   Here, the majority of the study lakes were sulfate-rich with SCR values much greater than

356   one, except for Reflex Lake (Table 1 and supplementary Table S1). All together, these

357   observations would suggest that Hap A and Hap B are mainly responsible for the sedimentary

358   LCA profile in the Canadian lakes, with few exceptions.

359   The Group I phylotype also thrived in some of the Canadian freshwater (< 0.5 g/L) and

360   oligohaline (0.5-5 g/L) lakes, as suggested by Longo et al. (2016) based on the presence of

361   the alkenone isomer $C_{37:3b}$. The RIK_{37} index (ratio between $C_{37:3a}$ and $C_{37:3b}$ isomers) has also

362   been proposed as a taxonomic indicator for multi-species effect, with RIK_{37} values of 0.51-

363   0.60 indicating lakes hosting only Group I haptophytes and RIK_{37} values between 0.60 and

364   1.0 for lakes containing both Group I and Group II haptophytes (Longo et al., 2016). In this

365   study, we found the $C_{37:3b}$ isomer in four lakes (Fig. 2), including one freshwater and three

366   oligohaline lakes, and RIK_{37} values ranging 0.47-0.57 (Table 1), indicating the exclusive

367   presence of Group I haptophytes in these lakes. In all other sites, RIK_{37} values of 1 ($C_{37:3b}$

368   isomer absent) suggest the exclusive presence of Group II haptophytes. Given these patterns,

369   we will conduct genomic analyses in the future to confirm the presence of Hap A, Hap B, and

370   Group I haptophytes in these Canadian lakes.

371

372   4.2. Relationship between LCAs and environmental parameters

373   Overall, our statistical analyses of the relationship between LCA distribution and major

374   environmental gradients showed that LCA occurrence and abundance was influenced by a

375   combination of salinity, water temperature, basin morphometry (depth), and water-column

376   stratification (Figs. 3-6). These results are consistent with previous studies that have shown

377   that LCA abundance is generally higher in lakes with elevated sulfate content and salinity

378   (e.g., Pearson et al., 2008; Toney et al., 2010; Liu et al., 2011; Zhao et al., 2014). Sulfate

379   content does not appear as a main variable for LCA distribution in our statistical analyses,

380   likely because most of our study lakes are actually sulfate-rich. In contrast, salinity appears as

381   a main factor on LCA presence and abundance. Salinity thresholds highlighted in our

382   statistical analyses (2.4-44.4 g/L) support the observation that higher LCA concentrations

383   occur in brackish/saline lakes rather than in fresh and hypersaline lakes (e.g., Liu et al, 2011;

384   Song et al., 2016).

385   While salinity is was the main variable regulating LCAs in this study, it was more difficult

386   to separate the unique effects of water temperature (more LCAs with lower temperature),

387   depth and stratification, likely because the thermal regime varied with the degree of lake

388   stratification. In fact, highest LCA concentrations are generally recorded in lakes exhibiting

389   permanent stratification and deep-water anoxia (Table 1). Stratification and deep-water

390   anoxia have been suggested to play a role in LCA abundance in relation with the life cycle of

391   lacustrine haptophytes (Toney et al., 2010). In the US Great Plains, the movement of LCAs

392   within the water column is affected mainly by the degree of mixing and stratification

393   experienced during the haptophyte bloom (Toney et al., 2010). Indeed, it appears that

394   lacustrine haptophytes include a benthic vegetative stage within their life cycle (Toney et al.,

395   2010), like recently evidenced for the marine haptophyte *Isochrysis galbana* (Ellegaard et al.,

396   2016). In addition, some lacustrine phytoplankton cells survive during winter season as

397   resting cells in the bottom sediment (Salmi and Salonen, 2016). Thus, we conclude that

398 bottom waters could represent a physical "refuge" for haptophyte cells during times of the

399 year when conditions are unsuitable for population growth, such as winter.

400   In the Canadian Prairies, elevated sulfate content, derived from oxidation of pyrite in the

401 underlying bedrock, is common in lakes that have significant groundwater contribution (Last

402 and Ginn, 2005; Hayashi and van der Kamp, 2009). This environmental sulfur (as sulfate) is

403 an essential element for all photosynthetic algae (e.g., Takahashi et al., 2011; Giordano and

404 Prioretti, 2015); however, the influx of sulfate-rich groundwater could also favor chemical

405 stratification (meromixis) and deep-water anoxia (Pham et al., 2008, 2009). Thus, at present,

406 it is unclear how biotic demand and abiotic factors may interact to favour haptophytes and

407 LCA production or preservation. In particular, further studies will be thus needed to further

408 assess the influence of sulfate on LCAs and determine whether high LCA concentration in

409 these Canadian lakes is the result of increased production or merely improved post-

410 depositional preservation in the anoxic, aphotic bottom waters (Leavitt, 1993).

411   While the vast majority of our Canadian study lakes are saline, our survey also included

412 25 freshwater lakes (Table 1). Only 6 of these freshwater lakes contained detectable LCAs

413 and our statistical analyses showed that LCAs occurred mainly in stratified alkaline

414 freshwater lakes with pH up to 8.5 (Fig. 4). These observations are consistent with those in

415 Alaskan freshwater lakes, where high LCA concentration was found in deep lakes with high

416 pH, alkalinity and conductivity (Longo et al., 2016). Interestingly, these patterns suggest that

417 the main environmental parameters influencing LCA occurrence and abundance may differ

418 between freshwater and saline lakes, although it is still unclear how salinity, morphology, and

419 stratification may interact with the basic biology of LCA-producing haptophytes.

420

421 4.3. Relationship between LCA-based indices and environmental parameters

422   The %$C_{37:4}$ has been suggested as a salinity proxy as the relative proportion of the $C_{37:4}$

423   alkenone has been reported to vary inversely with salinity in marine (e.g., Rosell-Melé, 1998;

424   Schulz et al., 2000; Kaiser et al., 2017) and lacustrine environments (e.g., Liu et al., 2008;

425   2011; Song et al., 2016). As with some previous works (e.g., Sikes and Sicre, 2002; Toney et

426   al., 2010), we found no significant relationship of %$C_{37:4}$ with salinity (supplementary Table

427   S2), possibly because the production of $C_{37:4}$ alkenone varies among haptophyte species and

428   strains (Theroux et al., 2010). The U_{37}^K and $U_{37}^{K''}$ indices, which include the $C_{37:4}$ alkenone in

429   their definition, are also not influenced by salinity variation (supplementary Table S2),

430   conversely to what has been observed in Chinese lakes (Liu et al., 2011; Song et al., 2016).

431   On the other hand, a significant but weak correlation was observed between the relative

432   abundance of $C_{37:4}$ alkenone and relative importance of carbonate among anions

433   (supplementary Table S2). Similarly, a significant but weak correlation was found between

434   U_{37}^K, $U_{37}^{K''}$ and the % carbonate (supplementary Table S2). The effect of carbonate on the

435   proportion of $C_{37:4}$ alkenone, if any, remains unknown.

436

437   4.4. Relationship between LCA unsaturation indices and temperature

438   Although temperature was a substantial (24%) predictor of variation in LCA

439   concentrations in the statistical analyses of lake survey data, there was no significant

440   relationship between mean water-column temperature at the time of sampling (August 2013)

441   and common indices of environmental temperature (U_{37}^K, $U_{37}^{K'}$, $U_{37}^{K''}$, or U_{38}^K). This lack of

442   correlation may reflect the fact that temperature data was estimated on only a single date in

443   August, while 1-cm deep sediment samples integrate historical haptophyte populations at the

444   scale from seasons to years. Furthermore, late summer does not necessarily correspond to the

445   time of maximum haptophyte production. For example, Toney et al. (2011) obtained a weak

446   but positive and statistically significant correlation ($R^2 = 0.30$, $p = 0.067$), with average

447 surface temperature recorded in spring in some of these lakes, suggesting a predominantly

448 cool-season life cycle of haptophytes. The slope of the relationship was also similar to that

449 derived from site-specific calibration of the U_{37}^K index in Lake George, North Dakota,

450 suggesting that the change in LCA unsaturation as a function of temperature is similar

451 between Great Plains lakes in the US and Canada. In fact, when we apply the temperature

452 calibration of Lake George (Toney et al., 2010) at one site for which we have measured water

453 temperature data for 20-years (Diefenbaker Lake), the LCA-inferred temperature is 11.9°C,

454 which is very close to the average water temperature in June (11.2°C) and the average air

455 temperature in the preceding month (May) recorded at a nearby weather station (10.9°C;

456 Elbow Cs weather station).

457   As the LCA profile at Lake Diefenbaker suggests the presence of Group I haptophyte, we

458 also applied the three *in-situ* temperature calibrations currently developed for lakes where the

459 presence of Group I phylotype has been confirmed (D'Andrea et al., 2011, 2016; Longo et

460 al., 2016). Here, the LCA-inferred temperature varied between 6.2°C and 12.1°C, values

461 which were close to the average water temperature in May (6.8°C) or June (11.2°C).

462 Together, these data support the idea that haptophyte bloom occurs in spring after ice-out

463 (Toney et al., 2010). Unfortunately, year-round estimates of water temperature were not

464 available for study lakes and we did not find any correlation with air temperature data for the

465 spring season from weather stations close to the studied lakes.

466   Weak correlation between the LCA unsaturation indices and environmental temperature

467 may also reflect the presence of multiple haptophyte species with different thermal optima

468 (e.g., Hap A and Hap B), in a single lake. For example, Hap A produces mainly $C_{37:4}$

469 alkenones and blooms during spring, whereas Hap B produces mainly $C_{37:3}$ alkenones and

470 blooms during summer (Theroux, 2012). However, we did not find any significant

471 correlations with spring air temperature for lakes with high %$C_{37:4}$ and with summer air

472 temperature for lakes with low %$C_{37:4}$. This is likely because the temperature calibration

473 throughout the bloom events, which combines Hap A and Hap B temperature calibrations, is

474 actually very similar to the *in-situ* lake calibration of Toney et al. (2010) (Theroux, 2012).

475 Instead, we suggest that establishment of a valid calibration of environmental conditions and

476 the U_{37}^K index may require more intensive field sampling during the haptophyte bloom in

477 select candidate lakes to quantify the seasonality of LCA production and the appropriate

478 environmental temperature for comparison (Toney et al., 2010).

479

480 **4. Conclusion**

481

482 We conclude that long-chain alkenones are common in the Canadian lakes of the Northern

483 Great Plains, and that their presence and abundance is influenced by a combination of

484 salinity, water temperature, depth, and degree of water-column stratification. Based on this

485 study, sulfate may play a role in LCA distribution, even though it is unclear if its effect is

486 related to biological requirements or to change in the aquatic environment (groundwater,

487 stratification, etc.). While these environmental parameters clearly play a role in regulating the

488 life cycle of the LCA-producing haptophytes, further studies will be essential to define how

489 haptophyte ecology varies with seasonal and interannual changes in lake conditions. LCA

490 profiles suggested that most lakes contained haptophyte species from the Group II phylotype,

491 likely closely related to OTU8 haptophyte (Hap A) and OTU7 haptophyte (Hap B) found in

492 the US Great Plains. However while the presence of other species from Group I phylotype

493 was also confirmed, we note that they were not particularly common. Unexpectedly, we were

494 not able to calibrate the LCA unsaturation ratios (U_{37}^K, $U_{37}^{K'}$, $U_{37}^{K''}$ or U_{38}^K) with environmental

495 conditions at this time. Instead, further studies with a more intensive sampling at different

496 depths and seasons will be needed to calibrate the U_{37}^K index *in-situ* with the appropriate

environmental temperature. Genomic analyses will also be important to precisely determine the haptophyte species present in these Canadian lakes.

Acknowledgements

References

Borcard, D., Legendre, P., Drapeau, P., 1992. Partialling out the spatial component of ecological variation. Ecology 73, 1045–1055.

Borcard, D., Gillet, F., Legendre, P., 2011. Numerical Ecology with R. Springer, Dordrecht.

Brassell, S.C., Brereton, R.G., Eglinton, G., Grimalt, J., Liebezeit, G., Marlowe, I.T., Pflaumann, U., Sarnthein, M., 1986. Palaeoclimatic signals recognized by chemometric treatment of molecular stratigraphic data. Organic Geochemistry 10, 649–660.

Calcagno, V. Mazancourt, C.D., 2010. glmulti: An R Package for Easy Automated Model Selection with (Generalized) Linear Models. Journal of Statistical Software 34.

Chivall, D., M'Boule, D., Sinke-Schoen, D., Sinninghe Damsté, J.S., Schouten, S., van der Meer, M.T.J., 2014. Impact of salinity and growth phase on alkenone distributions in coastal haptophytes. Organic Geochemistry 67, 31–34.

521   Chu, G., Sun, Q., Li, S., Zheng, M., Jia, X., Lu, C., Liu, J., Liu, T., 2005. Long-chain

522   alkenone distributions and temperature dependence in lacustrine surface sediments from

523   China. Geochimica et Cosmochimica Acta 69, 4985–5003.

524   Conte, M.H., Sicre, M.-A., Rühlemann, C., Weber, J.C., Schulte, S., Schulz-Bull, D., Blanz,

525   T., 2006. Global temperature calibration of the alkenone unsaturation index ($U^{K'}_{37}$) in

526   surface waters and comparison with surface sediments. Geochemistry, Geophysics,

527   Geosystems 7. http://dx.doi.org/10.1029/2005GC001054.

528   Coolen, M.J.L., Muyzer, G., Rijpstra, W.I.C., Schouten, S., Volkman, J.K., Sinninghe

529   Damsté, J.S., 2004. Combined DNA and lipid analyses of sediments reveal changes in

530   Holocene haptophyte and diatom populations in an Antarctic lake. Earth and Planetary

531   Science Letters 223, 225–239.

532   Cranwell, P.A., 1985. Long-chain unsaturated ketones in recent lacustrine sediments.

533   Geochimica et Cosmochimica Acta 49, 1545–1551.

534   Cywinska, A., Delorme, L.D., 1995. Paleoenvironmental interpretations using fossil

535   freshwater ostracodes from Clearwater Lake core site, Saskatchewan, and Max Lake,

536   Manitoba. National Water Research Institute, Burlington, Ontario, NWRI Contribution 95-

537   1-7, 43 pp.

538   D'Andrea, W.J., Huang, Y., 2005. Long-chain alkenones in Greenland lake sediments: Low

539    $\delta^{13}C$ values and exceptional abundance. Organic Geochemistry 36, 1234–1241.

540   D'Andrea, W.J., Lage, M., Martiny, J.B.H., Laatsch, A.D., Amaral-Zettler, L.A., Sogin, M.L.,

541   Huang, Y., 2006. Alkenone producers inferred from well-preserved 18S rDNA in

542   Greenland lake sediments. Journal of Geophysical Research 111, G03013.

543   D'Andrea, W.J., Huang, Y., Fritz, S.C., Anderson, N.J., 2011. Abrupt Holocene climate

544   change as an important factor for human migration in West Greenland. Proceedings of the

545   National Academic of Sciences of the United States of America 108, 9765–9769.

546   D'Andrea, W.J., Theroux, S., Bradley, R.S., Huang, X., 2016. Does phylogeny control U^K_{37}-

547   temperature sensitivity? Implications for lacustrine alkenone paleothermometry.

548   Geochimica et Cosmochimica Acta 175, 168–180.

549   de Leeuw, J.W., van der Meer, F.W., Rijpstra, W.I.C., 1980. On the occurrence and structural

550   identification of long chain unsaturated ketones and hydrocarbons in sediments, In:

551   Douglas, A.D., Maxwell, J.R. (Eds.), Advances in Organic Geochemistry 1979. Pergamon,

552   pp. 2311–2317.

553   Ellegaard, M., Moestrup, Ø., Andersen, T.J., Lundholm, N., 2016. Long-term survival of

554   haptophyte and prasinophyte resting stage in marine sediment. European Journal of

555   Phycology 51, 328–337.

556   Giordano, M., Prioretti, L. 2015. Sulphur and algae: metabolism, ecology and evolution, In:

557   Borowitzka, M.A., Beardall, J., Raven, J.A. (Eds.), The Physiology of Microalgae.

558   Springer Dordrecht, Dordrecht, the Netherlands, pp. 185–209.

559   Hastie, T., Tibshirani, R., Friedman, J., 2009. The Elements of Statistical Learning. Data

560   Mining, Inference and Prediction, 2nd ed. Springer.

561   Hayashi, M., van der Kamp, G., 2009. Groundwater-wetland ecosystem interaction in the

562   semiarid glaciated plains of North America. Hydrogeology Journal 17(1), 203–214,

563   doi:10.1007/s10040-008-0367-1.

564   Hothorn, T., Zeileis, A., 2015. partykit: A Modular Toolkit for Recursive Partytioning in R.

565   Journal of Machine Learning Research 16, 3905–3909.

566   Hou, J., Huang, Y., Zhao, J., Liu, Z., Colman, S., An, Z., 2016. Large Holocene summer

567   temperature oscillations and impact on the peopling of the northeastern Tibetan Plateau.

568   Geophysical Research Letters 43, 1323–1330, doi:10.1002/2015GL067317.

569   Huguet, C., Fietz, S., Stockhecke, M., Sturm, M., Anselmetti, F.S., Rosell-Melé, A., 2011.

570   Biomarker seasonality study in Lake Van, Turkey. Organic Geochemistry 42, 1289–1298.

571   Kaiser, J., van der Meer, M.T.J., Arz, H.W., 2017. Long-chain alkenones in Baltic Sea

572   surface sediments: New insights. Organic Geochemistry 112, 93–104.

573   Korkonen, S.T., Ojala, A.E.K., Kosonen, E., Weckström, J., 2017. Seasonality of

574   chrysophyte cyst and diatom assemblages in varved Lake Nautajärvi – implications for

575   palaeolimnological studies. Journal of Limnology 76(2), 366–379.

576   Lampert, W., Sommer, U., 1997. Limnoecology: The ecology of lakes and streams. Oxford

577   University Press, Oxford: 382 pp.

578   Last, W.M., Ginn, F.M., 2005. Saline systems of the Great Plains of western Canada: an

579   overview of the limnogeology and paleolimnology. Saline Systems 1(10), 1–38.

580   Leavitt, P.R., 1993. A review of factors that regulate carotenoid and chlorophyll deposition

581   and fossil pigment abundance. Journal of Paleolimnology 9, 109–127.

582   Leavitt, P.R., Fritz, S.C., Anderson, N.J., Baker, P.A., Blenckner, T., Bunting, L., Catalan, J.,

583   Conley, D.J., Hobbs, W.O., Jeppesen, E., Korhola, A., McGowan, S., Rühland, K., Rusak,

584   J.A., Simpson, G.L., Solovieva, N., Werne, J., 2009. Paleolimnological evidence of the

585   effects on lakes of energy and mass transfer from climate and humans. Limnology and

586   Oceanography 54(6), 2330–2348.

587   Li, J., Philp, R.P., Pu, F., Allen, J., 1996. Long-chain alkenones in Qinghai Lake sediments.

588   Geochimica et Cosmochimica Acta 60, 235–241.

589   Liu, W.G., Liu, Z.H., Fu, M.Y., An, Z.H., 2008. Distribution of the C_{37} tetra-unsaturated

590   alkenone in Lake Qinghai, China: a potential lake salinity indicator. Geochimica et

591   Cosmochimica Acta 72, 988–997.

592   Liu, W.G., Liu, Z.H., Wang, H.Y., He, Y.X., Wang, Z., Xu, L.M., 2011. Salinity control on

593   long-chain alkenone distributions in lake surface waters and sediments of the northern

594   Qinghai-Tibetan Plateau, China. Geochimica et Cosmochimica Acta 75, 1693–1703.

595   Longo, W.M., Dillon, J.T., Tarozo, R., Salacup, J.M., Huang, Y., 2013. Unprecedented

596   separation of long-chain alkenones from gas chromatography with a

597   poly(trifluoropropylmethylsiloxane) stationary phase. Organic Geochemistry 65, 94–102.

598   Longo, W.M., Theroux, S., Giblin, A.E., Zheng, Y., Dillon, J.T., Huang, Y., 2016.

599   Temperature calibration and phylogenetically distinct distributions for freshwater

600   alkenones: Evidence from northern Alaskan lakes. Geochimica et Cosmochimica. Acta

601   180, 177–196.

602   Marlowe, I.T., Brassell, S.C., Eglinton, G., Green, J.C., 1984. Long-chain unsaturated

603   ketones and esters in living algae and marine sediments. Organic Geochemistry 6, 135–

604   141.

605   Marlowe, I.T., Brassell, S.C., Eglinton, G., Green, J.C., 1990. Long-chain alkenones and

606   alkyl alkenoates and the fossil coccolith record of marine sediments. Chemical Geology

607   88, 349–375.

608   Michels, A., Laird, K.R., Wilson, S.E., Thomson, D., Leavitt, P.R., Oglesby, R.J., Cumming,

609   B.F., 2007. Multidecadal to millennial-scale shifts in drought conditions on the Canadian

610   prairies over the past six millennia: implications for future drought assessment. Global

611   Change Biology 13(7), 1295–1307.

612   Müller, P.J., Kirst, G., Ruhland, G., von Storch, I., Rosell-Melé, A., 1998. Calibration of the

613   alkenone paleotemperature index $U^{K'}_{37}$ based on core-tops from the eastern South Atlantic

614   and the global ocean (60°N-60°S). Geochimica et Cosmochimica Acta 62, 1757–1772.

615   Nagelkerke, N.J.D., 1991. A note on a general definition of the coefficient of determination.

616   Biometrika 78, 691–692.

617   Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin,

618   P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.M., Szoecs, E., Wagner,

H., 2017. vegan: Community Ecology Package. R package version 2.4-3. https://CRAN.R-project.org/package=vegan

Pahnke, K., Sachs, J.P., 2006. Sea surface temperatures of southern midlatitudes 0–160 kyr B.P. Paleoceanography 21, PA2003.

Pearson, E.J., Juggins, S., Farrimond, P., 2008. Distribution and significance of long-chain alkenones as salinity and temperature indicators in Spanish saline lake sediments. Geochimica et Cosmochimica Acta 72, 4035–4046.

Pham, S.V., Leavitt, P.R., McGowan, S., Peres-Neto, P., 2008. Spatial variability of climate and land-use effects on lakes of the northern Great Plains. Limnology and Oceanography 53, 728–742.

Pham, S.V., Leavitt, P.R., McGowan, S., Wissel, B., 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, 101–118.

Prahl, F.G., Wakeham, S.G., 1987. Calibration of unsaturation patterns in long-chain ketone compositions for palaeotemperature assessment. Nature 330, 367–369.

Prahl, F.G., Muehlhausen, L.A., Zahnle, D.L., 1988. Further evaluation of long-chain alkenones as indicators of paleoceanographic conditions. Geochimica et Cosmochimica Acta 52, 2303–2310.

R Development Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Randlett, M.-E., Coolen, M.J.L., Stockhecke, M., Pickarski, N., Litt, T., Balkema, C., Kwiecien, O., Tomonaga, Y., Wehrli, B., Schubert, C.J., 2014. Alkenone distribution in Lake Van sediment over the last 270 ka: influence of temperature and haptophyte species composition. Quaternary Science Reviews 104, 53–62.

643 Rontani, J.-F., Beker, B., Volkman, J.K., 2004. Regiospecific oxygenation of alkenones in the

644   benthic haptophyte *Chrysotila lamellosa* Anand HAP 17. Phytochemistry 65, 3269–3278.

645 Rosell-Melé, A., Eglinton, G., Pflaumann, U., Sarnthein, M., 1995. Atlantic core-top

646   calibration of the U^{K}_{37} index as a sea-surface palaeotemperature indicator. Geochimica et

647   Cosmochimica Acta 59, 3099–3107.

648 Rosell-Melé, A., 1998. Interhemispheric appraisal of the value of alkenone indices as

649   temperature and salinity proxies in high-latitude locations. Paleoceanography 13, 694–

650   703.

651 Salmi, P., Salonen, K., 2016. Regular build-up of the spring phytoplankton maximum before

652   ice-break in a boreal lake. Limnology and Oceanography 61, 240–253.

653 Schulz, H.M., Schoner, A., Emeis, K.C., 2000. Long-chain alkenone patterns in the Baltic

654   Sea—an ocean–freshwater transition. Geochimica et Cosmochimica Acta 64, 469–477.

655 Sikes, E.L., Volkman, J.K., 1993. Calibration of alkenone unsaturation ratios ($U^{K'}_{37}$) for

656   paleotemperature estimation in cold polar waters. Geochimica et Cosmochimica Acta 57,

657   1883–1889.

658 Sikes, E.L., Sicre, M.A., 2002. Relationship of the tetra-unsaturated C_{37} alkenone to salinity

659   and temperature: implications for paleoproxy applications. Geochemistry, Geophysics,

660   Geosystems 3, 1–11.

661 Song, M., Zhou, A., He, Y., Zhao, C., Wu, J., Zhao, Y., Liu, W., Liu, Z., 2016.

662   Environmental controls on long-chain alkenone occurrence and compositional patterns in

663   lacustrine sediments, northwestern China. Organic Geochemistry 91, 43–53.

664 Stainton, M.P., Capel, M.J., Armstrong, F.A.J., 1977. The chemical analysis of freshwater.

665   2nd ed. Environment Canada Miscellaneous Special Publication 25.

666 Sun, Q., Chu, G., Li, S., Lü, C., Zheng, M., 2004. Long-chain alkenones in sulfate lakes and

667   its paleoclimatic implications. Chinese Science Bulletin 49, 2082–2086.

668   Sun, Q., Chu, G., Liu, G., Li, S., Wang, X., 2007. Calibration of alkenone unsaturation index

669   with growth temperature for a lacustrine species, *Chrysotila lamellosa* (Haptophyceae).

670   Organic Geochemistry 38, 1226–1234.

671   Takahashi, H., Kopriva, S., Giordano, M., Saito, K., Hell, R., 2011. Sulfur assimilation in

672   photosynthetic organisms: Molecular functions and regulations of transporters and

673   assimilatory enzymes. Annual Review of Plant Biology 62, 157–184.

674   Taranu, Z.E., Gregory-Eaves, I., Steele, R.J., Beaulieu, M., Legendre, P., 2017. Predicting

675   microcystin concentrations in lakes and reservoirs at a continental scale: A new

676   framework for modelling an important health risk factor. Global Ecology and

677   Biogeogeography 26, 625–637.

678   Theissen, K.M., Zinniker, D.A., Moldowan, J.M., Dunbar, R.B., Rowe, H.D., 2005.

679   Pronounced occurrence of long-chain alkenones and dinosterol in a 25,000-year lipid

680   molecular fossil record from Lake Titicaca, South America. Geochimica et Cosmochimica

681   Acta 69, 623–636.

682   Theroux, S., D'Andrea, W.J., Toney, J.L., Amaral-Zettler, L., Huang, Y., 2010. Phylogenetic

683   diversity and evolutionary relatedness of alkenone-producing haptophyte algae in lakes:

684   Implications for continental paleotemperature reconstructions. Earth and Planetary Science

685   Letters 300, 311–320.

686   Theroux, S., 2012. Diversity and Ecology of Lacustrine Haptophyte Algae: Implications for

687   Paleothermometry. PhD Thesis, Brown University.

688   Theroux, S., Toney, J.L., Amaral-Zettler, L., Huang, Y., 2013. Production and temperature

689   sensitivity of long chain alkenones in the cultured haptophyte *Pseudoisochrysis paradoxa*.

690   Organic Geochemistry 62, 68–73.

691 Thiel, V., Jenisch, A., Landmann, G., Reimer, A., Michaelis, W., 1997. Unusual distributions

692 of long-chain alkenones and tetrahymanol from the highly alkaline Lake Van, Turkey.

693 Geochimica et Cosmochimica Acta 61, 2053–2064.

694 Toney, J.L., Huang, Y., Fritz, S.C., Baker, P.A., Grimm, E., Nyren, P., 2010. Climatic and

695 environmental controls on the occurrence and distributions of long-chain alkenones in

696 lakes of the interior United States. Geochimica et Cosmochimica Acta 74, 1563–1578.

697 Toney, J.L., Leavitt, P.R., Huang, Y., 2011. Alkenones are common in prairie lakes of

698 interior Canada. Organic Geochemistry 42, 707–712.

699 Toney, J.L., Theroux, S., Andersen, R.A., Coleman, A., Amaral-Zettler, L., Huang, Y., 2012.

700 Culturing of the first 37:4 predominant lacustrine haptophyte: Geochemical, biochemical,

701 and genetic implications. Geochimica et Cosmochimica Acta 78, 51–64.

702 Volkman, J.K., Eglinton, G., Corner, E.D.S., Forsberg, T.E.V., 1980. Long-chain alkenes and

703 alkenones in the marine coccolithophorid *Emiliania huxleyi*. Phytochemistry 19, 2619–

704 2622.

705 Volkman, J.K., Barrett, S.M., Blackburn, S.I., Sikes, E.L., 1995. Alkenones in *Gephyrocapsa*

706 *oceanica*: Implications for studies of paleoclimate. Geochimica et Cosmochimica Acta 59,

707 513–520.

708 Wetzel, R.G, 2001. Limnology. Lake and river ecosystems. 3rd ed. Academic Press: 1006 pp.

709 Whittingham, M.J., Stephens, P.A., Bradbury, R.B., Freckleton, R.P., 2006. Why do we still

710 use stepwise modelling in ecology and behaviour? The Journal of Animal Ecology 75,

711 1182–1189.

712 Zhao, J., An, C., Longo, W.M., Dillon, J.T., Zhao, Y., Shi, C., Chen, Y., Huang, Y., 2014.

713 Occurrence of extended chain length C_{41} and C_{42} alkenones in hypersaline lakes. Organic

714 Geochemistry 75, 48–53.

715 Zheng, Y., Huang, Y., Andersen, R.A., Amaral-Zettler, L.A., 2016. Excluding the di-
716   unsaturated alkenone in the U^K_{37} index strengthens temperature correlation for the
717   common lacustrine and brackish-water haptophytes. Geochimica et Cosmochimica Acta
718   175, 36–46.

719 Zink, K.-G., Leythaeuser, D., Melkonian, M., Schwark, L., 2001. Temperature dependency of
720   long-chain alkenone distributions in recent to fossil limnic sediments and in lake waters.
721   Geochimica et Cosmochimica Acta 65, 253–265.

722 Zuur, A., A.A.S., Ieno, E., 2012. Zero inflated model and generalized linear mixed models
723   with R. Highland Statistics Ltd.

724

725 **Figure captions**

726

727 Fig. 1. Map of the southern Saskatchewan, Canada showing the location of the studied lakes.
728 Individual lakes are plotted as "+" (no LCAs) or circles scaled to LCA concentration (μg
729 $gTOC^{-1}$). The different LCA profiles found in this study are indicated by a colour code
730 (white: high %$C_{37:4}$; grey: $C_{37:3}$-dominated; black: C_{38}-dominated).

731

732 Fig. 2. Signature sedimentary LCA profiles for the Canadian lakes. The profile with high (>
733 60%) %$C_{37:4}$ (A) is observed in the great majority of the studied lakes, while the $C_{37:3}$-
734 dominated (B) and C_{38}-dominated (C) profiles are only found in five lakes and a single lake,
735 respectively. Four freshwater/oligohaline lakes also exhibit profile typical of Group I
736 haptophyte (D), with the presence of tri-unsaturated isomers.

737

738 Fig. 3. Probability of LCA occurrence, showing (A)–(D) logistic (binomial model) regression
739 plots, (E) mosaic plot of LCA occurrence *vs.* thermal regime for raw data (left) and model

740   fitted values (right), and (F) the shared and independent fraction of variance explained by

741   each predictor in the final LCA model. Shaded areas show 95% CIs (confidence intervals)

742   and ticks on the upper and lower axes indicate original observations. Statistics (r-square, p

743   value) for the regression plots (A)-(D) are presented in the supplementary Table S2.

744

745   Fig. 4. Classification tree of LCA occurrence. Splits indicate thresholds for occurrence at

746   particular environmental values. Bars at each node show proportions of observations where

747   LCAs are present (dark grey) or absent (light grey).

748

749   Fig. 5. LCA concentration as a function of selected significant environmental variables,

750   showing (A)-(C) scatter plots and fitted regression lines (lognormal model), (D) boxplot of

751   LCA concentration as a function of thermal regime, and (E) the shared and independent

752   fraction of variance explained by each predictor in the final LCA model. Shaded areas show

753   95% Cis and ticks on the upper and lower axes indicate original observations. Error bars in

754   (D) indicate model fitted values and 95% Cis (confidence intervals). Statistics (r-square, p

755   value) for the scatter plots (A)-(C) are presented in the supplementary Table S2.

756

757   Fig. 6. Regression tree of LCA occurrence. Splits indicate thresholds for occurrence at

758   particular environmental values. Boxplots summarise LCA concentration for observations in

759   that node. Note that boxplot scale is in $\log_{10}$ units ($\log_{10}$ of LCA concentration in µg/gTOC).

760

761   Table 1. Location, characteristics and organic geochemistry data for the studied Canadian

762   lakes. N.D. = not determined.

Lake Name	Lat. (°N)	Long. (°W)	Stratif. [a]	Depth (m)	Salinity (g/L)	Temp. (°C)	pH	SCR [b]	TOC (%)	LCAs (µg/gTOC)	LCAs (µg/gsed)	%C$_{37:4}$ [c]	C$_{37}$/C$_{38}$ [d]	RIK$_{37}$ [e]	U$^K_{37}$ [f]	U$^{K'}_{37}$ [g]	U$^{K''}_{37}$ [h]
LCAs present																	
Alsask	51.34	109.88	N	3.2	74.8	19.6	9.35	17.7	3.21	135	4.33	29.6	1.59	1.00	-0.18	0.16	0.67
Antelope	50.28	108.39	N	7.7	7.87	21.4	9.40	91.1	7.89	799	63.0	70.5	5.17	1.00	-0.66	0.17	0.26
Arthur	52.56	105.44	T, M	6.7	8.29	15.1	8.88	N.D.	8.71	3296	287	69.9	2.83	1.00	-0.64	0.19	0.26
Bitter	50.10	109.79	N	0.9	15.1	19.1	8.69	510	2.15	609	13.1	46.9	1.28	1.00	-0.33	0.25	0.46
Blackstrap	51.79	106.44	N	7.9	0.43	19.1	8.84	47.2	8.22	26.9	2.21	52.0	2.59	1.00	-0.46	0.12	0.45
Bullkin	49.25	105.07	N	0.7	2.01	19.4	9.06	25.5	1.24	49.8	0.62	76.5	2.57	1.00	-0.70	0.26	0.18
Charron	52.40	104.31	T	8.8	4.9	16.9	10.5	125	14.6	927	135	58.2	2.82	1.00	-0.50	0.19	0.37
Clair	51.99	104.06	N	3.0	0.78	22.1	9.20	25.3	8.33	23.9	2.00	55.8	N.D.	1.00	-0.51	0.11	0.41
Coldspring	52.35	108.59	N	2.4	5.86	19.1	8.62	88.3	5.03	178	8.95	73.0	3.50	1.00	-0.62	0.40	0.18
Deadmoose	52.30	105.17	T, M	15.1	12.3	12.6	8.87	99.8	8.24	383	31.6	53.3	1.73	1.00	-0.41	0.27	0.39
Dewey	50.58	107.07	N	2.3	8.54	18.8	8.40	216	7.87	144	11.4	38.2	2.14	1.00	-0.26	0.19	0.57
Diefenbaker	51.10	106.62	N	28.5	0.22	19.3	8.27	19.8	3.11	87.6	2.72	52.1	1.38	0.47	-0.48	0.17	0.28
Eaglehill	52.36	108.97	N	1.1	2.64	18.7	9.03	1.92	4.61	37.5	1.73	79.5	N.D.	1.00	-0.76	0.17	0.18
Farewell	52.47	109.38	N	0.4	3.57	22.4	9.40	13.4	1.45	92.4	1.34	45.5	N.D.	1.00	-0.34	0.22	0.48
Fife	49.22	105.86	N	2.4	1.47	19.9	9.06	4.31	2.88	10.4	0.30	72.4	2.56	1.00	-0.63	0.33	0.20
Fishing	51.82	103.47	N	11.5	1.31	20.1	9.17	66.2	12.3	39.9	4.90	50.9	1.29	0.44	-0.45	0.23	0.27
Freefight	50.40	109.11	T, M	15	40.8	17.6	8.33	89.2	1.14	135	1.54	46.3	1.63	1.00	-0.29	0.32	0.44
Greenwater	52.52	103.50	N	7.5	0.33	20.1	8.23	153	17.2	2.48	0.43	43.3	N.D.	1.00	-0.23	0.36	0.46
Humboldt	52.14	105.12	N	6.3	0.84	20.8	9.13	31.8	9.89	23.7	2.34	55.5	7.05	1.00	-0.53	0.06	0.43
Junction	49.96	109.51	N	9.0	0.66	19.3	8.92	57.9	3.49	148	5.17	N.D.	N.D.	1.00	N.D.	N.D.	N.D.
Katepwa	50.71	103.65	T	22.4	0.8	15.6	8.29	237	6.38	14.7	0.94	47.7	6.35	1.00	-0.43	0.09	0.50
Kenosee	49.82	102.30	N	7.4	1.32	19.1	8.79	26.6	8.92	82.7	7.38	62.2	6.33	1.00	-0.59	0.08	0.36
L. Manitou	51.74	105.52	N	6.7	44.4	19.6	9.15	475	13.1	5063	662	55.6	2.24	1.00	-0.46	0.21	0.39
Lenore SB	52.45	105.00	T	9.1	4.43	21.1	9.38	39.9	16.1	143	22.9	39.4	0.88	0.54	-0.29	0.28	0.41
Little Manitou	52.62	109.62	N	3.8	3.62	21.5	9.19	4.56	13.1	50.1	6.55	48.7	4.00	0.49	-0.43	0.22	0.31
Luck	51.07	107.10	N	0.8	4.55	18.2	9.64	47.7	3.00	27.5	1.91	66.7	1.99	1.00	-0.57	0.28	0.26
Madge	51.65	101.63	N	12.3	0.38	19.4	8.36	235	8.61	11.0	0.95	51.3	1.66	1.00	-0.42	0.20	0.43
Manitou	52.76	109.75	T	15.9	29.1	8.75	9.56	13.2	8.37	27,575	2308	66.4	2.14	1.00	-0.59	0.22	0.28
Mason	50.36	109.35	N	1.9	4.97	19.3	9.25	21.2	5.02	12.4	0.62	72.3	3.04	1.00	-0.61	0.41	0.19

32

McLean	52.62	109.37	N	6.5	46.2	21.1	9.27	552	1.71	77.9	1.33	44.2	1.63	1.00	-0.39	0.10	0.53
Middle	52.56	105.19	T	10.0	5.14	16.8	8.93	88.7	6.40	11,522	737	72.9	1.29	1.00	-0.68	0.18	0.23
Montague	49.47	105.83	N	1.4	1.07	19.6	9.06	15.6	1.38	135	1.87	72.9	2.58	1.00	-0.67	0.22	0.22
Old Wives	50.10	105.99	N	1.7	1.51	18.9	9.29	6.78	0.10	17.9	0.02	N.D.	N.D.	1.00	N.D.	N.D.	0.33
Opuntia	51.81	108.57	N	1.6	2.98	21.4	9.02	35.6	2.41	603	14.5	75.8	7.73	1.00	-0.73	0.12	0.22
Porter	52.19	106.29	N	4.0	2.78	19.2	8.48	5.31	4.62	819	37.8	66.9	2.56	1.00	-0.59	0.25	0.27
Quill	51.86	104.37	N	6.4	7.29	19.1	8.78	189	8.05	383	30.8	65.1	4.13	1.00	-0.51	0.41	0.24
Rabbit	52.61	107.02	S	6.5	4.23	19.3	9.21	80.5	11.8	72.6	8.60	70.0	4.49	1.00	-0.64	0.19	0.26
Redberry	52.62	107.02	T	14.8	8.61	12.1	9.09	22.8	4.40	11,143	490	69.4	2.15	1.00	-0.64	0.16	0.27
Reesor	49.66	110.11	N	5.0	0.14	18.9	8.48	N.D.	9.15	10.3	0.41	72.5	1.64	1.00	N.D.	N.D.	0.28
Richmond	52.01	108.02	N	1.8	10.0	18.8	8.62	3634	12.1	73.3	8.88	34.1	0.38	1.00	-0.24	0.16	0.62
Shannon	52.65	105.43	T	11.2	1.67	15.8	9.06	53.4	9.53	445	42.4	61.6	2.80	1.00	-0.55	0.18	0.34
Shoe	49.72	105.36	N	3.8	25.1	19.9	8.90	266	6.94	110	7.64	41.4	1.71	1.00	-0.28	0.23	0.52
Sink	52.44	109.19	N	2.8	2.83	20.9	8.66	12.2	14.7	175	25.8	79.2	2.78	1.00	-0.76	0.18	0.18
Snakehole	50.50	108.49	N	2.4	50.2	23.2	8.96	N.D.	4.16	191	7.96	45.6	1.97	1.00	-0.38	0.13	0.51
Springwater	52.01	108.39	N	3.1	6.56	22.4	8.62	47.5	4.40	797	35.1	37.5	2.17	1.00	-0.21	0.26	0.55
Success	50.49	108.02	T, M	17.4	34.9	11.3	8.62	43.4	4.66	94.2	4.39	45.1	1.50	1.00	-0.30	0.28	0.47
Sufferin	52.64	109.90	N	8	0.22	19.7	8.18	N.D.	12.7	31.8	4.03	37.3	1.64	1.00	-0.28	0.14	0.59
Summercove	49.32	107.04	N	5	0.98	20.0	8.97	13.1	5.24	31.5	1.65	67.8	6.97	1.00	-0.65	0.09	0.30
Sunny	52.02	108.52	T, M	15	11.6	8.63	8.50	43.3	7.94	3143	249	63.5	2.27	1.00	-0.56	0.21	0.31
Tramping	52.03	108.82	N	3.4	11.6	23.7	9.14	121	12.6	99.3	12.5	67.3	0.49	1.00	-0.63	0.13	0.30
Unknown 1	51.55	104.27	N	1.8	1.27	21.7	8.73	N.D.	5.24	53.3	2.79	69.8	0.93	1.00	-0.60	0.32	0.23
Van Scoy	52.08	107.55	N	0.9	6.44	20.6	8.82	N.D.	7.03	17.6	1.24	28.9	1.40	1.00	-0.05	0.33	0.62
Wakaw	52.66	105.60	T	10.3	1.5	19.7	8.54	5.06	12.2	132	16.1	68.6	N.D.	1.00	-0.67	0.05	0.30
Waldsea	52.26	105.18	T, M	19.9	12.8	9.85	8.45	165	8.51	24,494	2084	71.9	2.81	1.00	-0.67	0.16	0.25
White Heron	51.89	109.05	N	0.8	1.88	21.9	8.94	12.7	6.46	29.5	1.91	67.7	N.D.	1.00	-0.62	0.17	0.28
Willow Bunch	49.45	105.46	N	1.1	2.58	21.1	9.16	19.1	1.30	108	1.40	76.8	3.37	1.00	-0.71	0.27	0.18
Wolverine	52.03	105.22	N	3.1	0.8	21.2	8.93	2.05	12.5	21.2	2.65	76.9	4.31	1.00	-0.67	0.44	0.14
X5	52.67	110.08	T	4.1	13.6	17.9	9.04	20.8	12.2	6064	741	65.1	2.91	1.00	-0.59	0.18	0.31

LCAs absent

Adrian	49.69	102.87	N	1.2	0.79	19.8	9.13	1.98	5.37	x	x	x	x	x	x	x	x
Alioth	52.51	109.55	N	0.6	13.9	21.2	10.3	179	9.14	x	x	x	x	x	x	x	x

Annie Laurie	51.97	102.67	N	1.3	0.25	22.1	8.78	23.3	20.0	x	x	x	x	x	x	x	x
Barber	51.37	107.66	N	1.6	1.26	20.4	8.57	34.1	3.87	x	x	x	x	x	x	Barber	x
Buffalo Pound	50.61	105.43	N	4.1	0.31	22.2	8.85	1.27	4.94	x	x	x	x	x	x	x	x
Chapleau	50.18	103.34	N	1.4	0.41	22.1	9.00	15.3	9.75	x	x	x	x	x	x	x	x
Clearwater	50.87	107.93	N	7.0	0.68	19.1	9.18	15.8	9.27	x	x	x	x	x	x	x	x
Crooked	50.61	102.72	N	10.2	0.82	21.0	8.72	49.8	5.70	x	x	x	x	x	x	x	x
Cutbank	50.53	107.00	S	2.4	2.41	20.4	9.13	23.1	5.87	x	x	x	x	x	x	Cutbank	x
Cypress	49.47	109.47	N	5.6	0.3	19.1	8.82	115	2.85	x	x	x	x	x	x	x	x
Deep	50.42	103.68	N	2.2	1.53	21.8	8.88	62.9	6.56	x	x	x	x	x	x	Deep	x
Edouard	52.38	104.35	N	5.6	0.34	19.9	11.1	98.6	13.4	x	x	x	x	x	x	x	x
Elkwater	49.67	110.29	N	7.9	0.18	18.7	8.65	67.6	4.25	x	x	x	x	x	x	x	x
Eyebrow	50.96	106.19	N	0.9	0.21	17.9	8.36	N.D.	1.34	x	x	x	x	x	x	x	x
Good Spirt	51.55	102.67	S	5.2	0.47	19.8	8.62	1.22	11.2	x	x	x	x	x	x	x	x
Gouverneur	49.76	107.65	N	7.2	1.38	19.8	8.73	35.9	2.37	x	x	x	x	x	x	x	x
Grassy	51.24	104.63	N	2.0	0.64	21.2	8.98	9.40	12.6	x	x	x	x	x	x	x	x
Gull	50.11	108.50	N	5.0	2.04	20.1	8.96	38.3	N.D.	x	x	x	x	x	x	Gull	x
Handsome	50.49	107.38	N	1.9	2.19	18.4	9.04	28.1	6.89	x	x	x	x	x	x	x	x
Harris	49.82	109.50	N	5.1	0.35	18.9	8.83	133	0.86	x	x	x	x	x	x	x	x
Horse	51.39	103.56	N	3.3	0.72	20.7	8.78	8.20	22.1	x	x	x	x	x	x	Horse	x
Island	49.13	108.23	N	2.0	0.18	21.7	8.87	7.31	6.62	x	x	x	x	x	x	x	x
Kindersley	51.42	109.10	N	1.6	0.42	17.6	9.66	37.7	2.02	x	x	x	x	x	x	Kindersley	x
Kipabiskau	52.57	104.18	N	6.8	0.78	21.7	8.90	N.D.	16.6	x	x	x	x	x	x	x	x
Lac Pelletier	49.99	107.94	N	8.8	0.41	20.9	9.46	1.04	12.1	x	x	x	x	x	x	x	x
Last Mountain	51.10	105.26	N	18.5	1.05	19.2	8.84	1.80	9.25	x	x	x	x	x	x	Last	x
Lenore	52.51	104.97	T	10.1	2.26	17.7	8.97	168	7.77	x	x	x	x	x	x	x	x
Lewis Creek South	51.44	105.53	N	1.9	1.36	21.1	8.74	N.D.	8.21	x	x	x	x	x	x	Lewis	x
Lonetree	49.08	108.26	N	4.0	0.19	20.2	9.05	7.09	7.32	x	x	x	x	x	x	x	x
Macklin	52.32	109.95	N	2.8	0.8	20.2	8.38	N.D.	12.3	x	x	x	x	x	x	x	x
McDonald	49.19	103.24	N	14.5	0.25	20.0	8.48	N.D.	2.60	x	x	x	x	x	x	x	x
Newton	49.32	107.83	N	4.2	0.38	20.6	8.95	32.0	1.54	x	x	x	x	x	x	x	x
Nickle	49.59	103.78	N	5.7	0.25	19.2	8.17	37.6	2.27	x	x	x	x	x	x	Nickle	x
Pasqua	50.79	103.94	T	12.5	0.77	21.2	8.78	23.7	5.59	x	x	x	x	x	x	x	x
Peter	51.46	104.91	N	0.5	2.78	22.4	9.49	165	12.6	x	x	x	x	x	x	x	x

Pike	51.90	106.82	N	2.5	0.26	19.6	8.66	2.47	12.8	x	x	x	x	x	x	x	x
Reflex	52.66	110.01	N	2.8	102	22.2	9.40	0.95	4.22	x	x	x	x	x	x	x	x
Round	50.54	102.37	N	10.4	0.83	21.4	8.82	26.6	4.31	x	x	x	x	x	x	x	x
Shrimp	51.59	108.45	N	3.0	0.41	22.6	9.05	23.9	11.8	x	x	x	x	x	x	x	x
Snipe	51.23	108.86	N	1.2	1.67	18.6	8.85	35.4	3.40	x	x	x	x	x	x	x	x
Stony Beach	50.50	105.19	S	2.7	0.35	22.1	9.53	1.08	6.00	x	x	x	x	x	x	x	x
Thackeray	52.54	108.69	N	1.6	1.17	19.6	9.42	24.7	8.17	x	x	x	x	x	x	Thackeray	x
Thomson	49.77	106.60	N	8.3	1.01	20.3	8.87	40.0	N.D.	x	x	x	x	x	x	x	x
Unknown 3	50.65	108.97	N	0.1	57.8	21.5	8.57	N.D.	0.23	x	x	x	x	x	x	Unkn	x
Vincent	50.22	108.94	N	0.1	49.9	22.0	9.42	N.D.	2.61	x	x	x	x	x	x	Vin	x
WhiteBear	49.77	102.26	N	8.5	2.03	19.2	8.67	16.3	7.75	x	x	x	x	x	x	x	x
X3	51.94	107.58	N	0.6	3.08	19.8	9.76	457	7.34	x	x	x	x	x	x	x	x
York	51.16	102.48	N	3.0	1.09	22.8	8.97	21.6	14.2	x	x	x	x	x	x	York	x

[a] Stratification: none (N), stratified (S), thermal (T), meromictic (M)
[b] SCR: Sulfate to Carbonate Ratio (Toney et al., 2010)
[c] $\%C_{37:4} = 100*([C_{37:4}]/[C_{37} \text{ LCAs}])$ (Rosell-Melé, 1998)
[d] $C_{37}/C_{38} = [C_{37} \text{ LCAs}]/[C_{38} \text{ LCAs}]$ (Volkman et al., 1995)
[e] $RIK_{37} = [C_{37:3a}]/[C_{37:3a} + C_{37:3b}]$ (Longo et al., 2016)
[f] $U^{K}_{37} = [C_{37:2} - C_{37:4}]/[C_{37:2} + C_{37:3} + C_{37:4}]$ (Brassell et al., 1986)
[g] $U^{K'}_{37} = [C_{37:2}]/[C_{37:2} + C_{37:3}]$ (Brassell et al., 1986)
[h] $U^{K''}_{37} = [C_{37:3}]/[C_{37:3} + C_{37:4}]$ (Zheng et al., 2016)

Fig. 1

LCA concentration (μg/g TOC)

+ 0
∘ 0-10
∘ 10-100
○ 100-1000
◯ > 1000

LCA profile

○ High %$C_{37:4}$
◉ $C_{37:3}$ dominated
● C_{38} dominated

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6