

The effect of species on lacustrine $\delta^{18}\text{O}_{\text{diatom}}$ and its implication for palaeoenvironmental reconstructions

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Abstract

The oxygen isotope composition of diatom silica ($\delta^{18}\text{O}_{\text{diatom}}$) is increasingly being used to reconstruct climate from marine and lacustrine sedimentary archives. Though diatoms are assumed to precipitate their frustule in isotopic equilibrium with their surrounding water, it is unclear whether internal processes of a given species affect the fractionation of oxygen between the water and the diatom. We present $\delta^{18}\text{O}_{\text{diatom}}$ data from two diatom size fractions (3-38 μm and >38 μm) characterized by different species in a sediment core from Heart Lake, Alaska. Differences in $\delta^{18}\text{O}_{\text{diatom}}$ between the two size fractions varies from 0 - 1.2 ‰, with a mean offset of 0.01 ‰ (n = 20). Fourier transform infrared (FTIR) spectroscopy confirms our samples consist of pure biogenic silica (SiO_2) and $\delta^{18}\text{O}_{\text{diatom}}$ trends are not driven by contamination. The maximum offset is outside the range of error, but the mean is within analytical error of the technique (± 1.06 ‰), demonstrating no discernible species-dependent fractionation in $\delta^{18}\text{O}_{\text{diatom}}$. We conclude lacustrine $\delta^{18}\text{O}_{\text{diatom}}$ measurements offer a reliable and valuable method for reconstructing $\delta^{18}\text{O}_{\text{water}}$. Considering the presence of small offsets in our two records, we advise interpreting shifts in $\delta^{18}\text{O}_{\text{diatom}}$ only where the magnitude of change is greater than the combined analytical error.

Keywords: oxygen isotopes; diatom; lake; fractionation; palaeoclimate

Introduction

Diatoms are microscopic, unicellular algae with an external shell (frustule) composed of biogenic silica (opal, $\text{SiO}_2 \cdot n\text{H}_2\text{O}$) (Round *et al.* 2007). Distinct diatom communities exist due to variable ecological tolerances and optima (e.g. Moser *et al.* 1996; Battarbee *et al.* 2001) making them sensitive indicators of environmental change. Their siliceous frustules can be preserved over long timescales and are generally identifiable to the species level, enabling the reconstruction of past environments well beyond the instrumental record. The silica frustule comprises two layers: a tetrahedrally bonded silica layer (-Si-O-Si) which incorporates oxygen from the surrounding water during formation, and an outer hydrous layer (-Si-OH) which continues to exchange oxygen with the surrounding water following frustule formation (Leng and Swann, 2010). By isolating and extracting oxygen from the internal silica, the isotopic composition of the diatom ($\delta^{18}\text{O}_{\text{diatom}}$) can be determined and used to reconstruct past changes in climate (Leng and Barker, 2006).

The $\delta^{18}\text{O}_{\text{diatom}}$ value of a diatom frustule depends on the ambient water temperature and isotopic composition of the water ($\delta^{18}\text{O}_{\text{water}}$) in which the diatom grows (Labeyrie, 1974; Juillet-Leclerc and Labeyrie, 1987). By assuming $\delta^{18}\text{O}_{\text{diatom}}$ fractionates in isotopic equilibrium with this surrounding water, stratigraphic shifts in $\delta^{18}\text{O}_{\text{diatom}}$ can be used to infer changes in climate over time. The aspect of climate captured by $\delta^{18}\text{O}_{\text{diatom}}$ largely depends on local hydrology, climate, water residence time and seasonality of diatom growth. Previous studies have interpreted lacustrine $\delta^{18}\text{O}_{\text{diatom}}$ records to reflect changes in the precipitation/evaporation balance (P/E) (Rioual *et al.* 2001; Lamb *et al.* 2005), shifts in moisture source and regime (Shemesh *et al.* 2001a, b; Rosqvist *et al.* 2004; Jones *et al.* 2004; Leng *et al.* 2005; Schiff *et al.* 2009), changes in the amount and seasonality of precipitation (Barker *et al.* 2001; Morley *et al.* 2005; Mackay *et al.* 2013), and, to some extent, changes in temperature (Juillet-Leclerc and Labeyrie, 1987; Shemesh *et al.* 1995; Brandriss *et al.* 1998; Moschen *et al.* 2005). The oxygen isotope composition of marine diatoms has also been used to reconstruct palaeoceanographic conditions, whereby $\delta^{18}\text{O}_{\text{diatom}}$ is interpreted to reflect changes in sea surface temperature and surface salinity (e.g. Shemesh *et al.* 1992; Sancetta *et al.* 1985), sea ice extent and melt-water events (e.g. Shemesh *et al.* 1995; Swann *et al.* 2013; Pike *et al.* 2013), as well as changes in climate-ocean dynamics and ocean productivity (e.g. Juillet-Leclerc

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3 and Schrader, 1987; Swann *et al.* 2006; Romero *et al.* 2011). $\delta^{18}\text{O}_{\text{diatom}}$ is therefore
4 increasingly being used as an indicator of palaeoclimate from both marine and
5 terrestrial sedimentary archives (Leng and Barker, 2006; Swann and Leng, 2009).
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10 Following the development of the technique in the 1970's and 1980's (Labeyrie,
11 1974; Labeyrie and Juillet, 1982), efforts over the last decade have significantly
12 advanced our understanding of $\delta^{18}\text{O}_{\text{diatom}}$ fractionation, as well as addressed
13 problematic issues of sample contamination and silica maturation (see Leng and
14 Henderson, 2013 for a review). As diatoms are ubiquitous in most aquatic
15 environments, $\delta^{18}\text{O}_{\text{diatom}}$ data are particularly valuable in the absence of other
16 available proxies for isotopic analysis such as authigenic carbonates and carbonate-
17 based microfossils (e.g. foraminifera, ostracods). Examples of such environments
18 include oceans and lakes in high latitudes where carbonates are often limited or
19 absent (e.g. Rosqvist *et al.*, 1999, 2004; Hu and Shemesh, 2003; Schiff *et al.*, 2009;
20 Jones *et al.*, 2004; Swann *et al.*, 2010).
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29 30 **Vital effects on $\delta^{18}\text{O}_{\text{diatom}}$ fractionation**

31 Diatoms are generally assumed to precipitate their frustules in isotopic equilibrium
32 with the surrounding water ($\delta^{18}\text{O}_{\text{water}}$), and any biological fractionation is presumed to
33 be constant. Furthermore, there is a growing consensus that fractionation between
34 $\delta^{18}\text{O}_{\text{diatom}}$ and $\delta^{18}\text{O}_{\text{water}}$ is in the order of *c.* $-0.2\text{‰}/^{\circ}\text{C}$ (e.g. Brandriss *et al.* 1998;
35 Moschen *et al.* 2005; Dodd & Sharp, 2010) enabling changes in $\delta^{18}\text{O}_{\text{diatom}}$ to be
36 quantified over time. However, the $\delta^{18}\text{O}_{\text{diatom}}$ composition of a diatom frustule often
37 differs from that predicted by thermodynamics; an offset often referred to as a 'vital
38 effect' (or disequilibrium effect) (Leng and Barker, 2006). Such vital effects are well
39 documented and better understood in biogenic carbonates such as foraminifera in
40 the marine environment (Shackleton *et al.* 1973; Erez, 1978), and ostracods in the
41 lacustrine environment (Xia *et al.* 1997; Wetterich *et al.* 2008). In these instances,
42 vital effects may even serve as a valuable palaeoenvironmental indicator, for
43 example to reconstruct ocean thermal stratification (e.g. Fairbanks and Wiebe, 1980;
44 Ravelo and Fairbanks, 1992; Mulitza *et al.* 1997) or vertical temperature gradients
45 (Tedesco *et al.* 2007; Steph *et al.* 2009) due to the varying depths and temperatures
46 occupied by different species. However, the $\delta^{18}\text{O}$ of foraminiferal calcite is not only
47 controlled by the physical and chemical properties of the water column (e.g.
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3 $\delta^{18}\text{O}_{\text{water}}$, temperature, depth), but also by 'species-specific' vital effects; internal
4 processes such as calcification rate (Ortiz *et al.* 1996), symbiont photosynthesis
5 (Spero and Lea, 1993) and respiration (Wolf-Gladrow *et al.* 1999) of a given species
6 which are known to affect isotope fractionation. Some studies have also
7 demonstrated offsets can be found between the $\delta^{18}\text{O}$ of ostracods valves at different
8 stages of growth (e.g. juvenile vs. adult) (von Grafenstein *et al.* 1999; Keatings *et al.*
9 2002).

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16 Similarly, $\delta^{18}\text{O}$ vital effects in diatoms could result from species-specific fractionation,
17 with a varying fractionation factor due to species dependent $\delta^{18}\text{O}$ uptake in
18 equilibrium conditions, or a shortage or excess supply in non-equilibrium conditions
19 (Chapligin *et al.* 2012). The internal processes within the frustule could theoretically
20 alter $\delta^{18}\text{O}_{\text{diatom}}$ relative to the isotopic signal of the surrounding water, by an amount
21 that could vary from species to species. However, unlike biogenic carbonates, these
22 effects are poorly understood and much harder to observe, their small size (2 – 200
23 μm) making it difficult to isolate individual diatom species for isotope analysis.
24 Isotope measurements are typically made on samples containing many tens of
25 different diatom species. The possibility different diatom species fractionate oxygen
26 differently is therefore problematic, especially as the abundance of different species
27 changes through time downcore, and it is not possible to analyse the same species
28 in every sample. A number of studies have found no evidence for vital effects
29 between individual diatom species (e.g. Sancetta *et al.* 1985; Schmidt *et al.* 2001;
30 Moschen *et al.* 2005). When small (0.2 – 0.6‰) offsets have been observed between
31 different species, they are usually within the range of analytical reproducibility (e.g.
32 Shemesh *et al.* 1995; Brandriss *et al.* 1998). More recently, however, an offset of c.
33 3.0 - 3.5‰ was suggested for two size fractions of marine diatoms in the NW Pacific
34 (Swann *et al.* 2007; 2008). A potential species-driven offset has considerable
35 implications for the applicability of $\delta^{18}\text{O}_{\text{diatom}}$ as a palaeoclimatic proxy in the marine
36 environment, particularly as this magnitude of variation equates to a 15 – 16°C
37 temperature difference. In the NW Pacific, these offsets were attributed to size-
38 related effects associated with larger diatoms (>75 μm) (Swann *et al.* 2007),
39 although the results were largely inconclusive.
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3 Similar to biogenic carbonates, the habitat in which a diatom grows (e.g. benthic vs.
4 planktonic; water depth) may also affect its isotope composition. For example the
5 $\delta^{18}\text{O}$ of lake water may become more negative with increasing depth if lighter
6 isotopes are preferentially evaporated at the surface (e.g. von Grafenstein *et al.*
7 1999). In addition, water temperature decreases with depth and may also influence
8 the oxygen isotope fractionation of diatom silica (Juillet-Leclerc and Labeyrie, 1987;
9 Shemesh *et al.* 1992; Brandriss *et al.* 1998; Moschen *et al.* 2005), which must be
10 considered when interpreting records of $\delta^{18}\text{O}_{\text{diatom}}$. Any offset between diatom
11 species could also derive from the timing of the diatom bloom in the water column
12 and whether the $\delta^{18}\text{O}_{\text{diatom}}$ signal captured has a seasonal component (e.g. spring
13 vs. autumn; temperature and precipitation). The interpretation of $\delta^{18}\text{O}_{\text{diatom}}$ from any
14 sedimentary record therefore requires an understanding of the external processes
15 affecting $\delta^{18}\text{O}_{\text{diatom}}$ at a particular site, such as climate, water inflow and outflow, lake
16 residence time and diatom ecology.
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28 Here, we examine the issue of species-dependent vital effects in $\delta^{18}\text{O}_{\text{diatom}}$ using
29 lacustrine sediment material from Heart Lake on Adak Island, part of the Aleutian
30 Island chain in southwest Alaska. By analysing the $\delta^{18}\text{O}_{\text{diatom}}$ value of two size
31 fractions containing different diatom species, we investigate whether species specific
32 fractionation of oxygen is evident in a lacustrine environment.
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38 **Study Site**

39 Heart Lake, located on Adak Island in the NW Pacific (51.87°N, 176.63°W; Fig.1), is
40 a small (<0.25 km²) freshwater, through-flow lake. Separating the Bering Sea to the
41 north and the Pacific Ocean to the south, the island experiences a cool, wet and
42 windy maritime climate (Rodionov *et al.* 2005). Mean precipitation in January and
43 July is 150.4 mm and 71.6 mm, respectively (1942-1996) (NOAA, 2013). The mean
44 annual $\delta^{18}\text{O}$ of precipitation ($\delta^{18}\text{O}_{\text{precip}}$) is -8.80‰, varying slightly between January
45 (-9.41‰) and July (-8.93‰) (IAEA, 2013). The correlation between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of
46 available precipitation data from Adak (1962-1973) (IAEA, 2013) defines a local
47 meteoric water line (LMWL) where $\delta^2\text{H} = 6.86 \delta^{18}\text{O} - 3.26$ (Fig.2). The $\delta^{18}\text{O}$ of
48 surface lake water samples collected in summer 2009 and 2010 are -9.75‰ and -
49 9.24‰, and are similar to modern-day $\delta^{18}\text{O}_{\text{precip}}$ values. Therefore, evaporation of
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3 surface waters has very little influence on the $\delta^{18}\text{O}$ of the lake and suggests that the
4 $\delta^{18}\text{O}$ of Heart Lake reflects $\delta^{18}\text{O}_{\text{precip}}$ (rain and snowfall) received by the lake.
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8 **Methodology**

9 *Sample Material*

10 A 5.5 m-long sediment core (AS-10-1D) was recovered from Heart Lake in July
11 2010. A GPS-enabled sonar depth recorder guided bathymetric profiling, and the
12 core site was selected adjacent to the deepest part of the basin at 7.6 m-depth. The
13 core was recovered using a percussion corer operated from a floating platform. The
14 core was split, photographed and described (Krawiec, 2013), with 20 samples taken
15 downcore for $\delta^{18}\text{O}_{\text{diatom}}$ and diatom species analysis. The samples range in age from
16 8.0 to 1.0k cal a BP based on a best-fit age-depth model constructed using AMS ^{14}C
17 ages and correlated tephra ages from nearby Andrew Lake (Krawiec *et al.* 2013).
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26 *Sample Preparation*

27 Samples for $\delta^{18}\text{O}_{\text{diatom}}$ were prepared using a stepwise process of chemical
28 digestion, differential settling, sieving and heavy liquid separation based on Morley *et*
29 *al.* (2004). Sediment samples were treated with 30% H_2O_2 at 90°C until reactions
30 ceased (to remove organic material), before using 5% HCl to eliminate any
31 carbonates. Following differential settling, all samples were centrifuged in sodium
32 polytungstate ($3\text{Na}_2\text{WO}_4 \cdot 9\text{WO}_3 \cdot \text{H}_2\text{O}$) (SPT) heavy liquid at 2500 rpm for 20 minutes,
33 resulting in the separation and suspension of diatoms from the heavier detrital
34 residue. This process was repeated three times using specific gravities of 2.50, 2.30
35 and 2.25 g ml^{-1} . After the final SPT separation, samples were washed five times at
36 1500 rpm for 5 minutes to remove any remaining SPT. Purified diatom samples were
37 then sieved at 38 μm and 3 μm , resulting in two size fractions of 3–38 μm and >38
38 μm for $\delta^{18}\text{O}_{\text{diatom}}$ analysis. The < 3 μm fraction was discarded as it was too small (~1
39 mg) to be analysed. The remaining fractions (3-38 and >38 μm) of the sample were
40 then treated with an additional stage of 30% H_2O_2 at 70°C for one week and
41 centrifuge washed to ensure no traces of organic matter remained.
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54 Sub-samples of the purified diatom material were retained and mounted on cover
55 slips using Naphrax®. The samples were visually inspected for contamination before
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3 diatom assemblage analysis using light microscopy (300 frustules per sample).
4 Standard diatom preparation and analysis (Battarbee *et al.* 2001) was also
5 performed on the unprocessed lake sediment to identify the diatom assemblages
6 species present and determine whether any species were lost during preparation for
7 diatom isotope analysis.
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11 12 13 *Contamination Assessment*

14 Fourier transform infrared spectroscopy (FTIR) was used as a rapid, non-destructive
15 means to assess the chemical composition of biogenic silica within our samples
16 (Leng *et al.* 2009), and ultimately sample purity (see Swann and Patwardham, 2011).
17 Sixteen purified diatom samples, together with the within-run laboratory diatom
18 standard (BFC_{mod}) were analysed using FTIR (Fig. 3). FTIR produces energy
19 absorption spectra of a sample over a range of wavelengths. Individual absorption
20 peaks correspond to specific chemical bonds and compounds, with pure silica
21 displaying peaks in two distinct regions at $>2500\text{ cm}^{-1}$ (hydroxyl) and $<1300\text{ cm}^{-1}$
22 (silica) (Fidalgo and Ilharco, 2001; Patwardhan *et al.* 2006; Swann and Patwardhan,
23 2011). FTIR analyses of all purified diatom isotope samples indicate peaks
24 corresponding to the BFC_{mod} standard, known to represent clean, fossilised
25 diatomite (Fig.3). Spectral deviation from the standard would indicate additional
26 compounds within the sample and contamination by non-diatom components
27 (Swann and Patwardhan, 2011). Peaks centered at $\sim 450\text{ cm}^{-1}$, $\sim 800\text{ cm}^{-1}$ and ~ 1100
28 cm^{-1} indicate pure silica and suggest that the samples comprise purely diatoms.
29 Additionally, scanning electron microscope (SEM) imaging was used to check
30 sample purity prior to oxygen isotope analysis (Fig.4).
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45 *Oxygen Isotope Analysis*

46 Purified diatom samples were analysed for $\delta^{18}\text{O}_{\text{diatom}}$ at the NERC Isotope
47 Geosciences Laboratory (UK) using a step-wise fluorination method outlined in Leng
48 and Barker (2006). The outer hydrous layer of the diatom, known to freely exchange
49 isotopically with water (e.g. Juillet-Leclerc and Labeyrie, 1987), was removed in a
50 pre-fluorination stage using a BrF_5 reagent at low temperature. This was followed by
51 a full reaction at high temperature to liberate oxygen that was converted to CO_2
52 (Clayton and Mayeda, 1963) and measured for $\delta^{18}\text{O}_{\text{diatom}}$ using a MAT 253 dual-inlet
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3 mass spectrometer. All $\delta^{18}\text{O}$ values were converted to the VSMOW scale using the
4 within-run laboratory standard BFC_{mod} , and are reported here in per mil (‰).
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8 **Results**

9 *Diatom Assemblages*

10 Diatom frustules are well preserved in all 60 samples as indicated by SEM images
11 that show no evidence of valve dissolution (Fig. 4). The smaller size fraction (3-38
12 μm) is taxonomically diverse, with 120 diatom species identified. The flora is
13 dominated by seven species (*Psammothidium levanderi*, *Rossithidium pusillum*,
14 *Cyclotella rossii*, *Pseudostaurosira brevistriata*, *Staurosira construens*, *Stauroforma*
15 *exiguiformis* and *Staurosirella pinnata*) with a combined relative abundance of 76 %
16 across all samples (Fig. 4b). Subtle shifts in species assemblages are evident
17 among the samples, with the most notable transition at c. 320 cm depth, when the
18 abundance of *P. levanderi* and *C. rossii* decline markedly and the abundance of the
19 small *Staurosira*/*Staurosirella* species increasingly dominate. In the larger size
20 fraction (>38), 28 different diatom species were identified. The assemblages are
21 dominated by six species (*Didymosphenia geminata*, *Surirella robusta*, *Surirella*
22 *splendida* *Pinnularia turnerae*, *Rhopalodia gibba* and *Campylodiscus hibernicus*),
23 with a combined relative abundance of 85% across all samples (Fig.4a). The diatom
24 assemblage in this larger fraction is distinctly different from that of the smaller size
25 fraction; they are not merely larger specimens of the same species. The transition at
26 ~320 cm depth is also evident in the larger size fraction, with a marked increase in
27 *D.geminata*, *P.turnerae* and *R.gibba*, which replace *C.hibernicus* and *Surirella*
28 species. The unprocessed bulk sediment samples are composed of the same diatom
29 assemblages found in both the purified $\delta^{18}\text{O}_{\text{diatom}}$ size fractions (Fig.4c). Most notably
30 however, the larger >38 μm diatoms only represent ~1% of the relative abundance
31 across all bulk sediment samples.
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49 $\delta^{18}\text{O}_{\text{diatom}}$ Values

50 The $\delta^{18}\text{O}_{\text{diatom}}$ values from Heart Lake range from +28.8‰ to +33.4‰ (Table 1; Fig.
51 5). Comparing the two size fractions, $\delta^{18}\text{O}_{\text{diatom}}$ values differ by 0 – 1.2‰ ($r^2 = 0.75$, p
52 = <0.05), with a mean difference of -0.01‰. Duplicate analyses of $\delta^{18}\text{O}_{\text{diatom}}$ indicate
53 an analytical reproducibility (1σ) of $\pm 0.19\%$ for the smaller (3-38 μm) fraction, \pm
54 0.49‰ for the larger (> 38 μm) fraction, and $\pm 0.31\%$ for the BFC_{mod} laboratory
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3 diatom standard. All 20 pairs have $\delta^{18}\text{O}_{\text{diatom}}$ values within the combined analytical
4 uncertainty of $\pm 1.06\text{‰}$ (2σ) for the two size fractions. Neither fraction is consistently
5 more isotopically positive or negative relative to the other, and the two values are
6 similar down core, aside from two samples at 147.5 cm and 161.5 cm depth where
7 they diverge.
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13 The relationships between $\delta^{18}\text{O}_{\text{diatom}}$ and diatom assemblages were evaluated using
14 principal components analysis (PCA) (Fig.5b, c) (ter Braak and Prentice, 1988). A
15 PCA was applied to a correlation matrix based on the dominant diatom species in all
16 20 samples, in both size fractions. The stratigraphic changes are captured in the first
17 and second PCA axes, which account for 49.9% and 29.9% of variance in the small
18 size fraction (Fig.5b), and 55.3% and 17.0% in the large fraction (Fig. 5c).
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24 Discussion

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26 All 20 pairs of diatom size fractions have differences in $\delta^{18}\text{O}_{\text{diatom}}$ within the 2σ
27 uncertainty range of this technique. Furthermore, the mean difference between the
28 two data sets is close to zero ($\mu = -0.01\text{‰}$) and indicates the two $\delta^{18}\text{O}_{\text{diatom}}$ records
29 are not statistically different (Fig. 6).
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35 The diatom assemblages from the two size fractions are composed of entirely
36 different species, and the relative abundance of each species varies through time
37 (Fig. 4). The main growing season of diatoms identified in Heart Lake occurs in
38 spring following winter snow melt, when sediment and nutrient input to the lake is
39 high and temperatures begin to increase. While some species such as *D. geminata*
40 are known to reside in freshwaters all year round, the main bloom occurs in late
41 spring and summer (Whitton *et al.* 2009). *C. hibernicus*, which is dominant in the
42 lower section of the core, also blooms in both spring and autumn (Griffiths, 1923;
43 Ramrath *et al.* 1999). Autumn diatom blooms are typically caused by the breakdown
44 of summer stratification and entrainment of nutrients while there are still sufficient
45 light levels for growth (Round *et al.* 2007). Temperatures in Heart Lake are
46 consistently low year-round and the lake water is well mixed, meaning autumn
47 blooms are unlikely to occur. As a result, both size fractions of the diatom sample
48 capture the spring/summer $\delta^{18}\text{O}_{\text{diatom}}$ signal when their silica frustule is formed
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3 (Moschen *et al.* 2005), and this rules out possible 'seasonal-effects' on $\delta^{18}\text{O}_{\text{diatom}}$
4 values.
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8 Aside from the planktonic/tychoplanktonic species *C. rossii*, all of the dominant
9 diatom species in Heart Lake are generally benthic and occupy the same habitat and
10 pool of water ($\delta^{18}\text{O}_{\text{water}}$). Heart Lake is 7.6 m deep and is likely to be well mixed.
11 Given the similarity between the $\delta^{18}\text{O}$ value of precipitation and lake water at Heart
12 Lake, isotopic enrichment due to evaporation is insignificant. Any so-called water
13 column effect is likely only applicable to deeper lakes than Heart Lake, or within the
14 marine environment where there may be variations associated with different water
15 masses. We can therefore assume all pairs of diatom fractions analysed for
16 $\delta^{18}\text{O}_{\text{diatom}}$ formed their silica frustules under the same environmental conditions (i.e.
17 depth, temperature, $\delta^{18}\text{O}_{\text{water}}$). Even though there are subtle differences between
18 species habit, with some being solitary (i.e. *C. hibernicus*), some colonial (i.e. *D.*
19 *geminata*), others motile (i.e. *C. rossii*), attached to substrata (i.e. *R. gibba*) or a
20 combination of the above, these attributes appear insignificant given there is no
21 discernible difference in $\delta^{18}\text{O}_{\text{diatom}}$.
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33 Evidence of a size-related species effect on $\delta^{18}\text{O}_{\text{diatom}}$ has previously been
34 documented in the marine environment, although these results are rather
35 inconclusive. Swann *et al.* (2007) report more positive values of $\delta^{18}\text{O}_{\text{diatom}}$ in smaller
36 diatoms compared to larger ones; but further research suggested the opposite
37 (Swann *et al.* 2008). Diatom size is also inherently linked to growth rate, with most
38 diatoms exhibiting a gradual reduction in size/growth with increasing maturity and
39 successive cell division (Round *et al.* 2007). We cannot quantify the growth rate of
40 specific diatoms within our sediment record, but we note in each size fraction the
41 diameter of any given specie does not vary visibly (Fig. 4). While it would be
42 incorrect to assume growth rates are consistent across all species, there is no
43 evidence for a relationship between diatom size and the amount of fractionation in
44 our samples, with no one size fraction consistently more positive or negative in
45 $\delta^{18}\text{O}_{\text{diatom}}$ relative to the other (and within analytical error).
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56 Visual inspection of all samples by light microscopy and SEM imaging revealed no
57 obvious sign of contamination (e.g. SPT, minerals, tephra), which is further
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3 confirmed by FTIR analysis. As the fluorination process will liberate oxygen from any
4 oxygen-bearing mineral in the sample (Brewer *et al.* 2008), having ensured the
5 diatoms are clean and free from contaminant, we consider the $\delta^{18}\text{O}_{\text{diatom}}$ data to be
6 reliable.
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9 10 11 *Species-specific effects*

12 In the large size fraction (>38 μm), the diatom species *C.hibernicus* and *R.gibba*
13 show the strongest correlation with downcore variation in $\delta^{18}\text{O}_{\text{diatom}}$, in positive and
14 negative associations (i.e. with more positive $\delta^{18}\text{O}_{\text{diatom}}$, the abundance of
15 *C.hibernicus* increases and the abundance of *R.gibba* decreases) (Fig. 5c). In the
16 small (3-38 μm) diatom fraction, *C.rossii* and *S.construens* are most closely related
17 with $\delta^{18}\text{O}_{\text{diatom}}$, in positive and negative associations (Fig. 5b). The remaining
18 dominant diatom species, in both size fractions, appear unrelated to the $\delta^{18}\text{O}_{\text{diatom}}$
19 vector in the PCA, with several species being orthogonal to the $\delta^{18}\text{O}_{\text{diatom}}$ gradient (*D.*
20 *geminata*, *S. splendida*, *P. turnerae*, *P. brevistriata*, *R. pusillum*). (Fig. 5b, c). Given
21 only two different species drive ~50% of the variance in each size fraction, and there
22 is no discernible difference in the $\delta^{18}\text{O}_{\text{diatom}}$ signal from these two fractions, we
23 therefore find no evidence to suggest there is a species-driven effect controlling
24 $\delta^{18}\text{O}_{\text{diatom}}$. *C. hibernicus* disappears from Heart Lake after 254.5 cm, but there is no
25 evidence of a concurrent shift in the $\delta^{18}\text{O}_{\text{diatom}}$ record at this time. The data therefore
26 suggest stratigraphic shifts in diatom assemblages are ecological responses to
27 climatic and environmental changes, as well as in the $\delta^{18}\text{O}_{\text{diatom}}$ record, rather than
28 driving the isotopic signal through differences in species-specific fractionation.
29 Determining the precise environmental and ecological factors driving species
30 assemblages and changes to $\delta^{18}\text{O}_{\text{diatom}}$ is, however, beyond the scope of this paper.
31 In this study, samples were initially analyzed downcore to represent different
32 environmental conditions and assemblages to test for a possible species effects on
33 oxygen isotopes in diatoms.
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51 The diatom composition of the two size fractions analysed here represents different
52 species assemblages and are considered independent of each other. If species-
53 dependent vital effects were present, we would expect the $\delta^{18}\text{O}_{\text{diatom}}$ data for each
54 size fraction to deviate and be consistently offset from one another. While the data
55 do not establish whether diatoms precipitate their silica in isotopic equilibrium with
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lake water, they demonstrate different species of diatoms fractionate oxygen isotopes at a similar magnitude.

Conclusions

$\delta^{18}\text{O}$ from diatom silica is generally presumed to precipitate in isotopic equilibrium with the surrounding water, however the presence of species-dependent vital effects on fractionation has, until now, been unclear. Our $\delta^{18}\text{O}_{\text{diatom}}$ data from Heart Lake reveal only small differences (0 - 1.2‰, $n = 20$) between two size fractions containing different diatom species assemblages. Given all differences are within the combined analytical error of the technique, it suggests there is no species or size-related effects controlling fractionation of $\delta^{18}\text{O}_{\text{diatom}}$ and bulk $\delta^{18}\text{O}_{\text{diatom}}$ samples are suitable for investigating palaeoenvironmental change at Heart Lake.

Diatom species analyses of both the raw sediment and the purified diatom samples reveal some diatom species were lost during preparation for diatom oxygen isotopes. However, considering these species account for < 1% of overall abundance across all samples, we conclude the purified samples analysed for $\delta^{18}\text{O}_{\text{diatom}}$ are representative of the species found within the raw sediment. It is therefore advised samples for $\delta^{18}\text{O}_{\text{diatom}}$ follow the same rigorous preparation and analytical procedures employed here.

We conclude $\delta^{18}\text{O}_{\text{diatom}}$ measurements from lacustrine diatom silica are a reliable and valuable method for reconstructing past $\delta^{18}\text{O}_{\text{water}}$. As diatoms are found in nearly all aquatic environments, $\delta^{18}\text{O}_{\text{diatom}}$ records offer an important source of information in regions devoid of other proxies available for isotopic analysis (e.g. carbonates), such as in the high latitude regions. Considering the presence of small offsets in our two records, we advise interpreting shifts in $\delta^{18}\text{O}_{\text{diatom}}$ only where the magnitude of change is greater than the combined analytical error for those samples.

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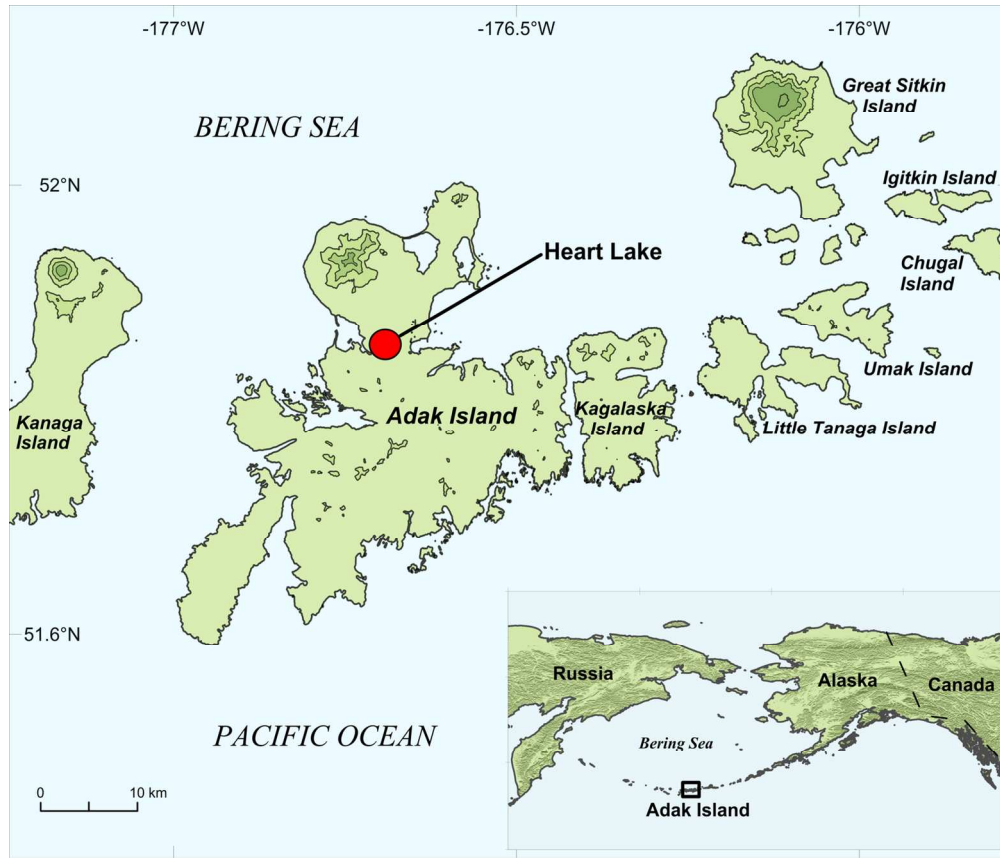


Figure 1. Location of Heart Lake on Adak Island, SW Alaska
71x60mm (600 x 600 DPI)

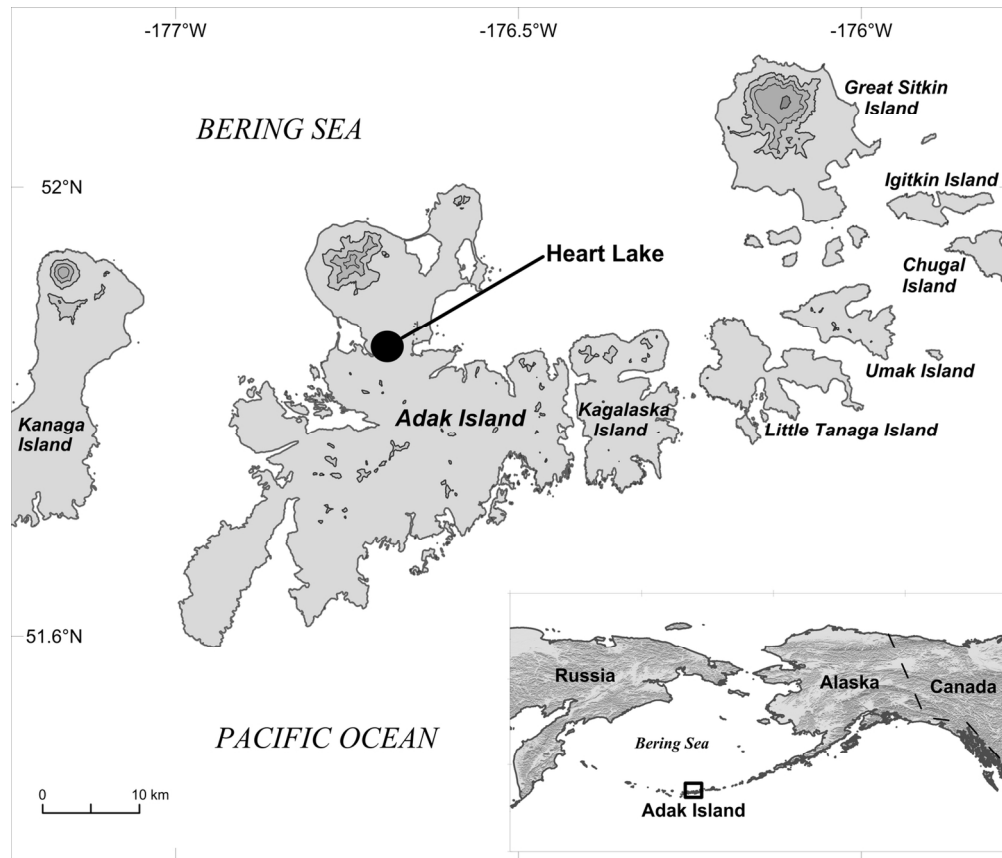


Figure 1. Location of Heart Lake on Adak Island, SW Alaska
71x60mm (600 x 600 DPI)

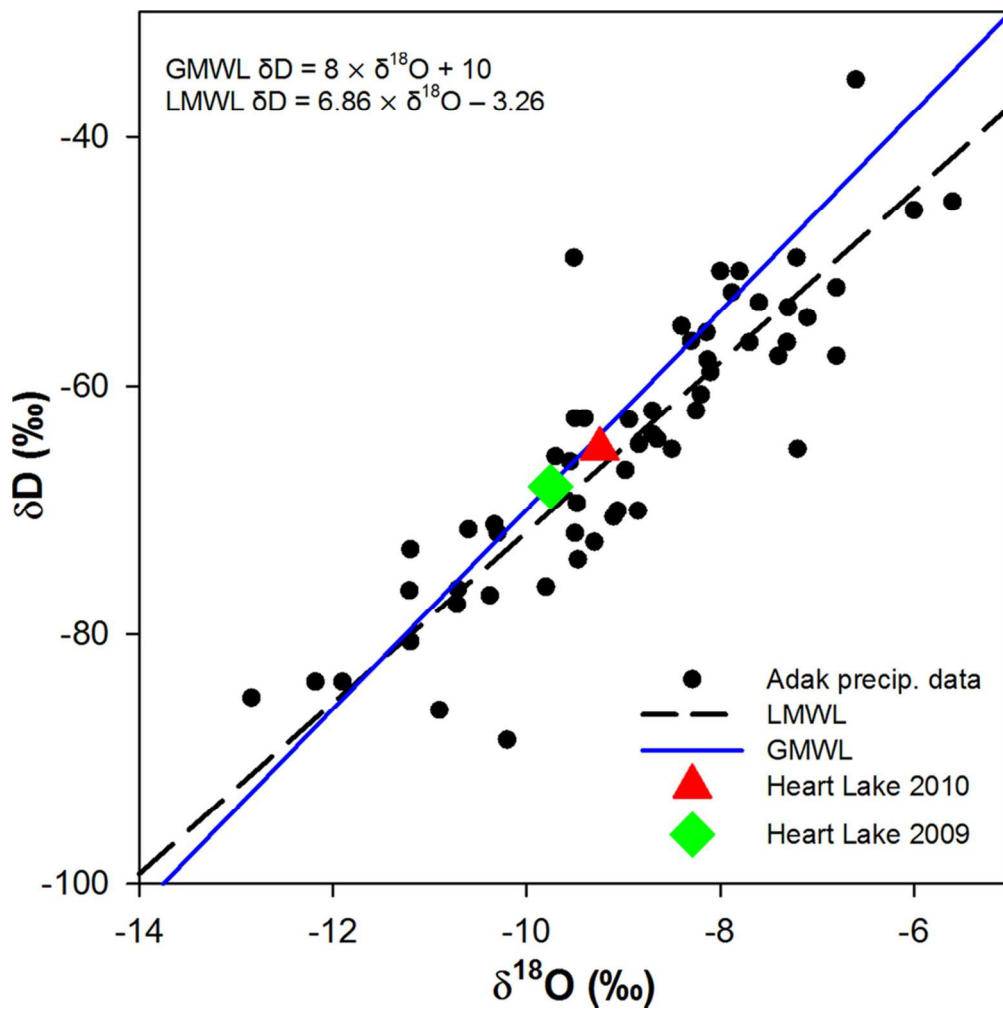


Figure 2. Heart Lake surface water $\delta^{18}O$ (2009 and 2010) on the local meteoric water line (LMWL) and the global meteoric water line (GMWL). LMWL data are derived from Adak precipitation samples collected by the Global Network of Isotopes in Precipitation (GNIP)
83x83mm (300 x 300 DPI)

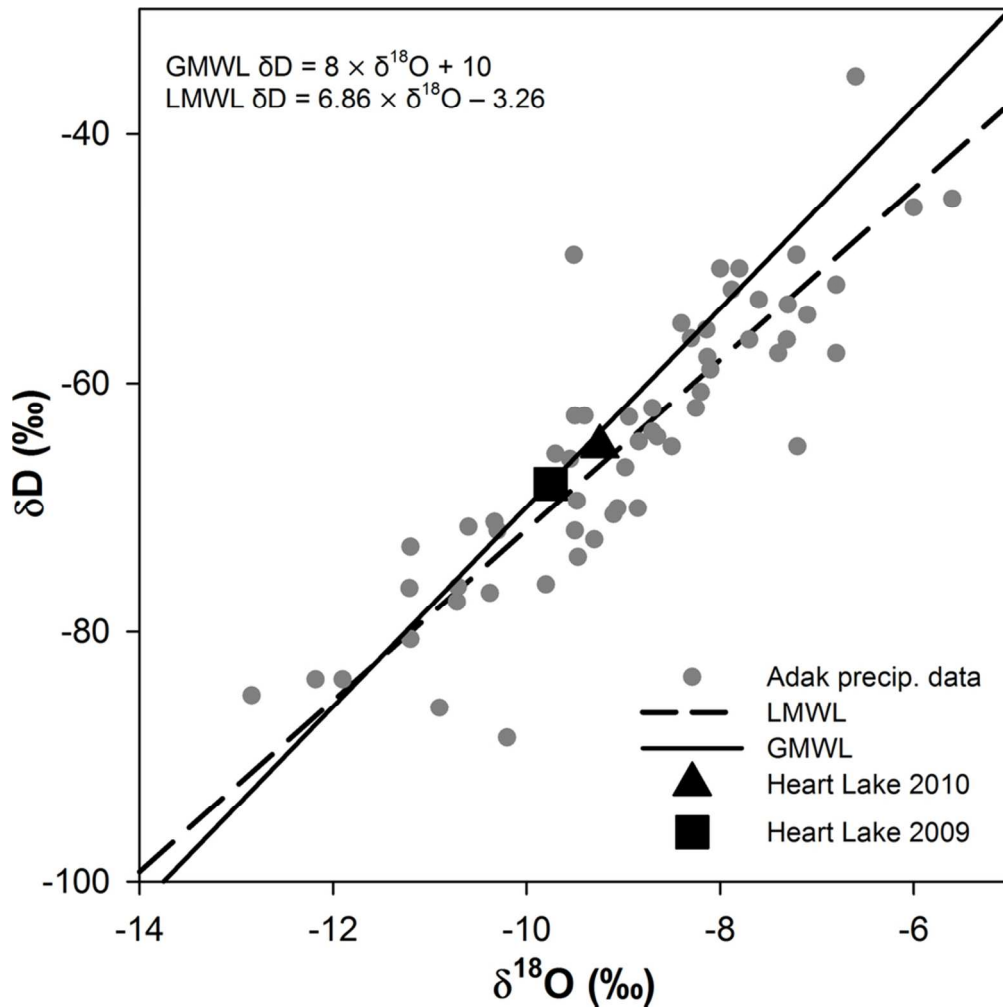


Figure 2. Heart Lake surface water $\delta^{18}O$ (2009 and 2010) on the local meteoric water line (LMWL) and the global meteoric water line (GMWL). LMWL data are derived from Adak precipitation samples collected by the Global Network of Isotopes in Precipitation (GNIP)
 83x83mm (300 x 300 DPI)

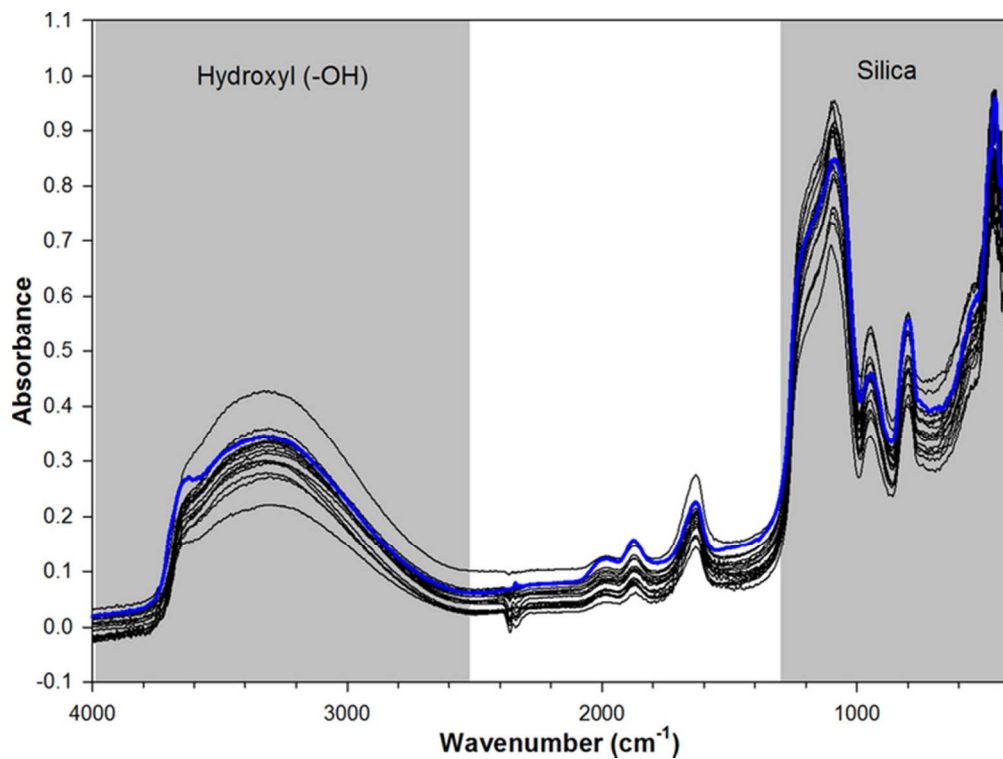


Figure 3. Fourier Transform Infrared Spectra (FTIR) of purified Heart Lake diatom samples (black) and the BFC_{mod} diatom standard (blue) composed of pure diatomite. The grey shaded areas indicate the separate hydroxyl (-OH) and silica components
62x46mm (300 x 300 DPI)

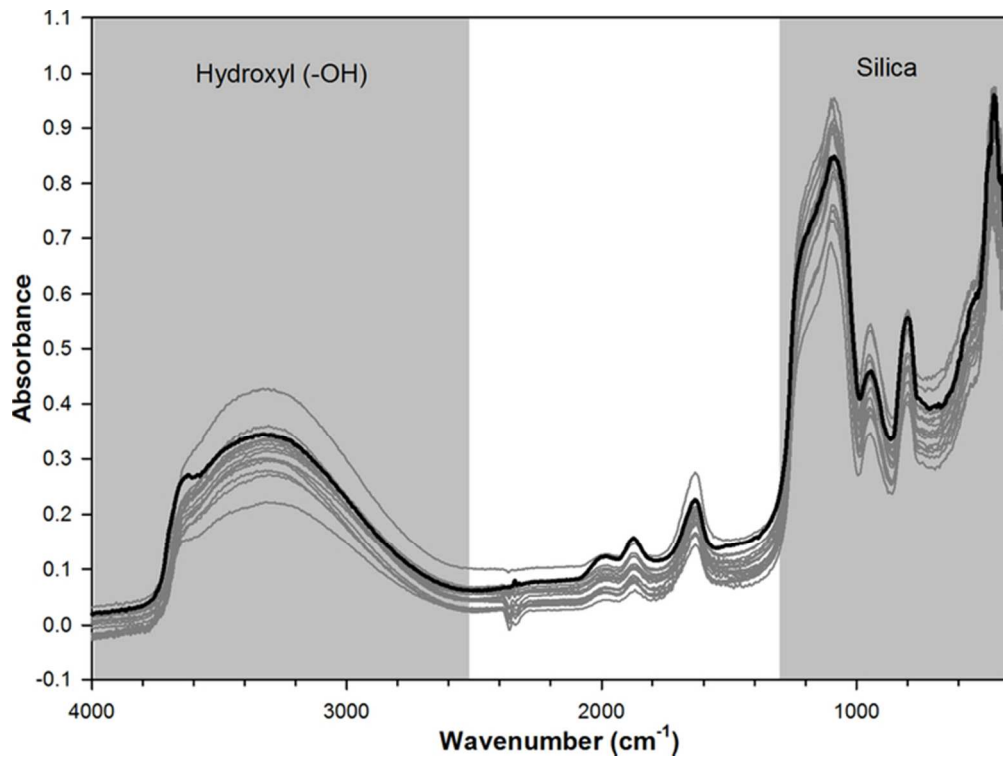


Figure 3. Fourier Transform Infrared Spectra (FTIR) of purified Heart Lake diatom samples (grey) and the BFC_{mod} diatom standard (black) composed of pure diatomite. The grey shaded areas indicate the separate hydroxyl (-OH) and silica components
62x46mm (300 x 300 DPI)

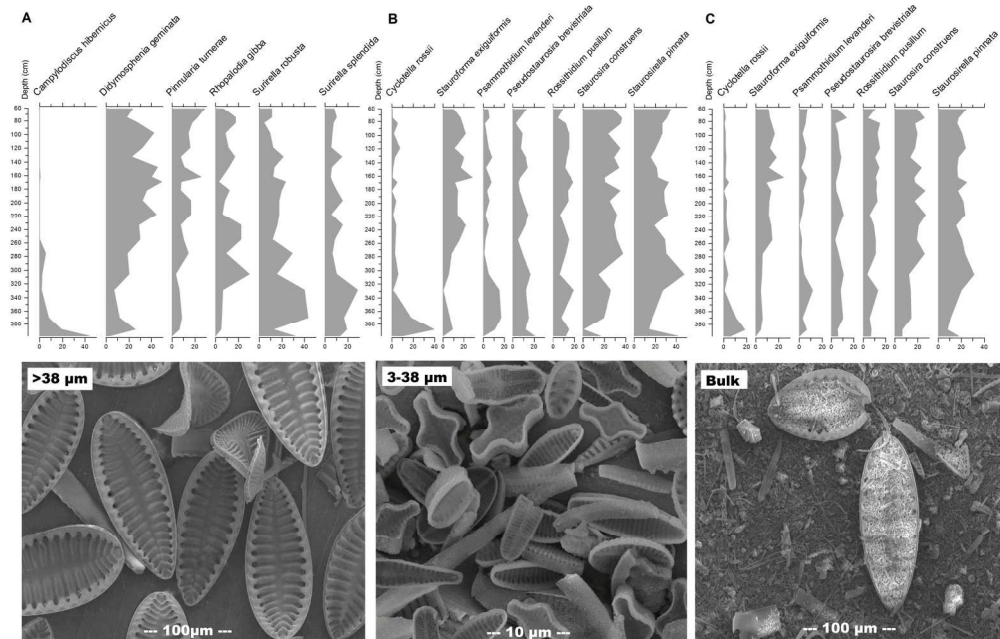


Figure 4. Stratigraphic changes in Heart Lake dominant diatom assemblages in the (A) large >38 μm (B) small 3-38 μm and (C) bulk (unprocessed) sediment diatom size fractions. Corresponding SEM images of selected diatom species are presented below each graph
111x70mm (600 x 600 DPI)

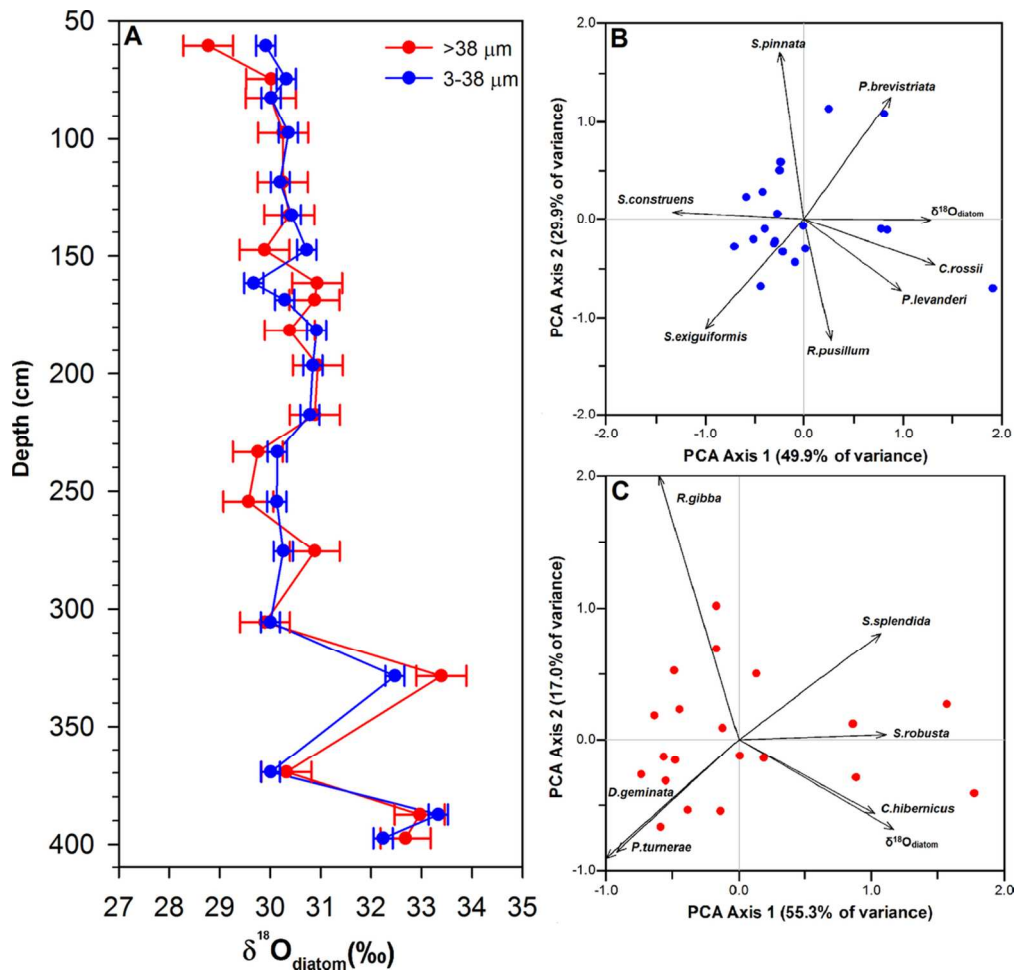


Figure 5. (A) Heart Lake $\delta^{18}\text{O}_{\text{diatom}}$ records from diatom size fractions of 3-38 μm (blue) and >38 μm (red) with analytical error. Principal components analysis of Heart Lake $\delta^{18}\text{O}_{\text{diatom}}$ and dominant diatom assemblages are displayed in the biplots showing the (B) 3-38 μm size fraction, and (C) the >38 μm size fraction
112x107mm (300 x 300 DPI)

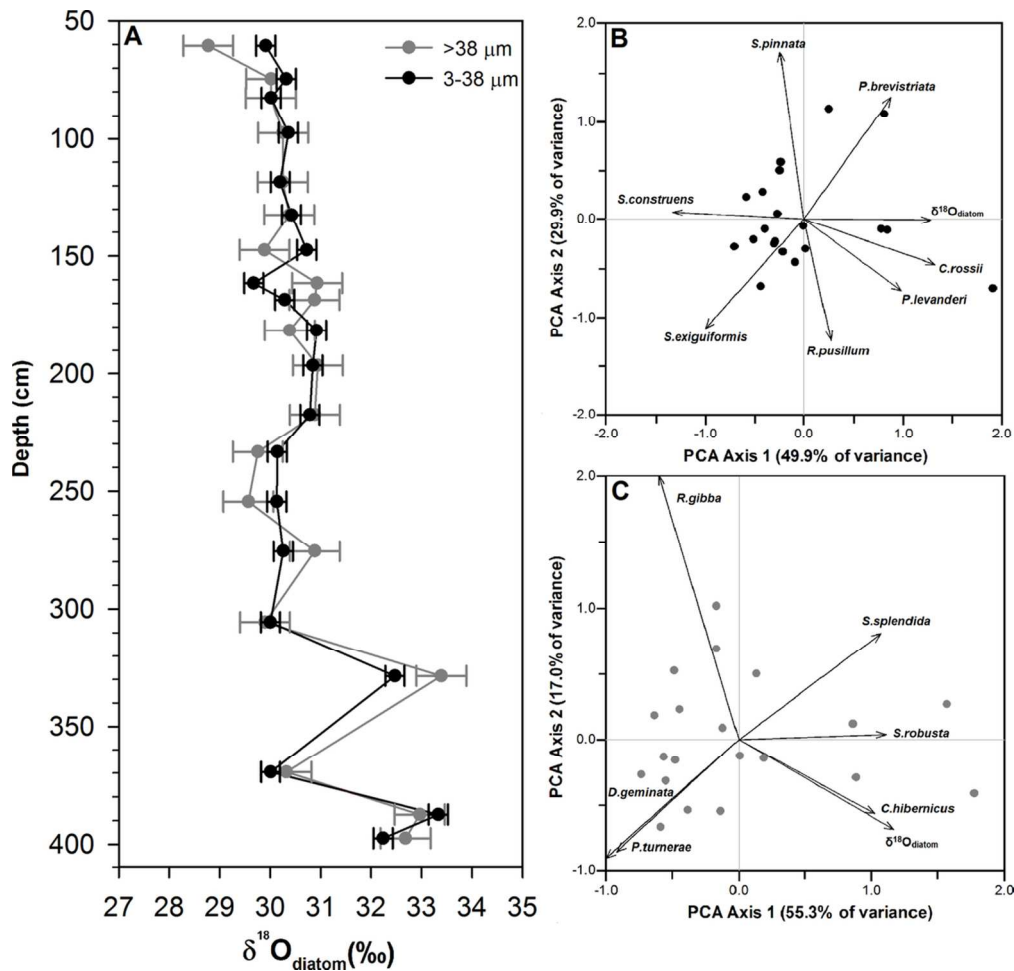


Figure 5. (A) Heart Lake $\delta^{18}\text{O}_{\text{diatom}}$ records from diatom size fractions of 3-38 μm (black) and >38 μm (grey) with analytical error. Principal components analysis of Heart Lake $\delta^{18}\text{O}_{\text{diatom}}$ and dominant diatom assemblages are displayed in the biplots showing the (B) 3-38 μm size fraction, and (C) the >38 μm size fraction
 112x107mm (300 x 300 DPI)

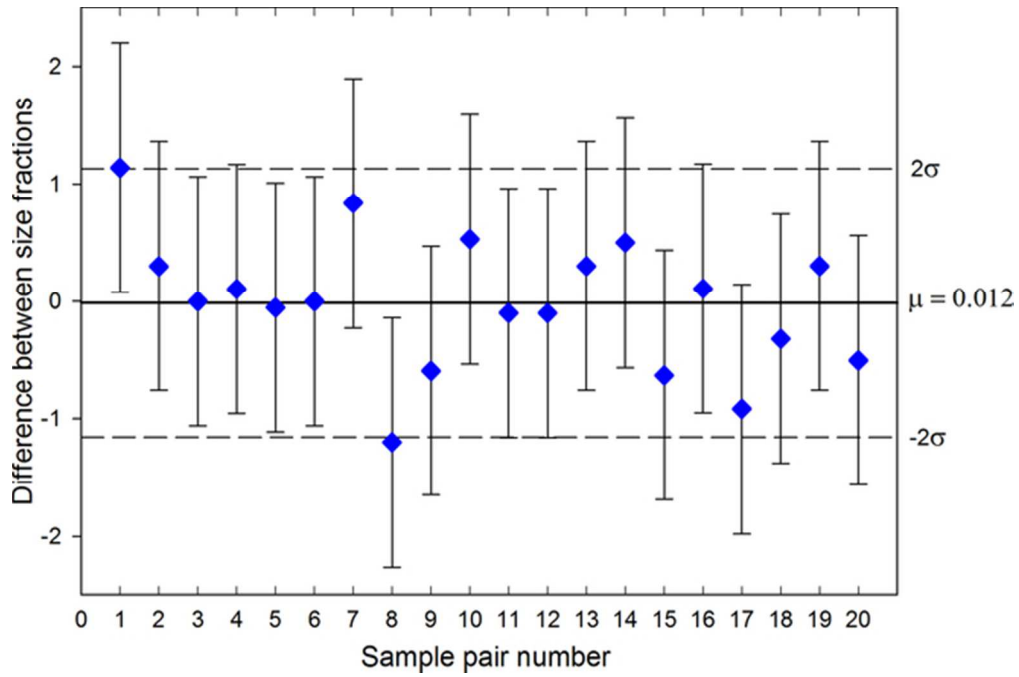


Figure 6. Difference in $\delta^{18}\text{O}_{\text{diatom}}$ between 20 pairs of different diatom size fractions from Heart Lake. Solid line indicates the mean difference between the two data sets, dashed lines represent the 2σ uncertainty range of the technique used
55x36mm (300 x 300 DPI)

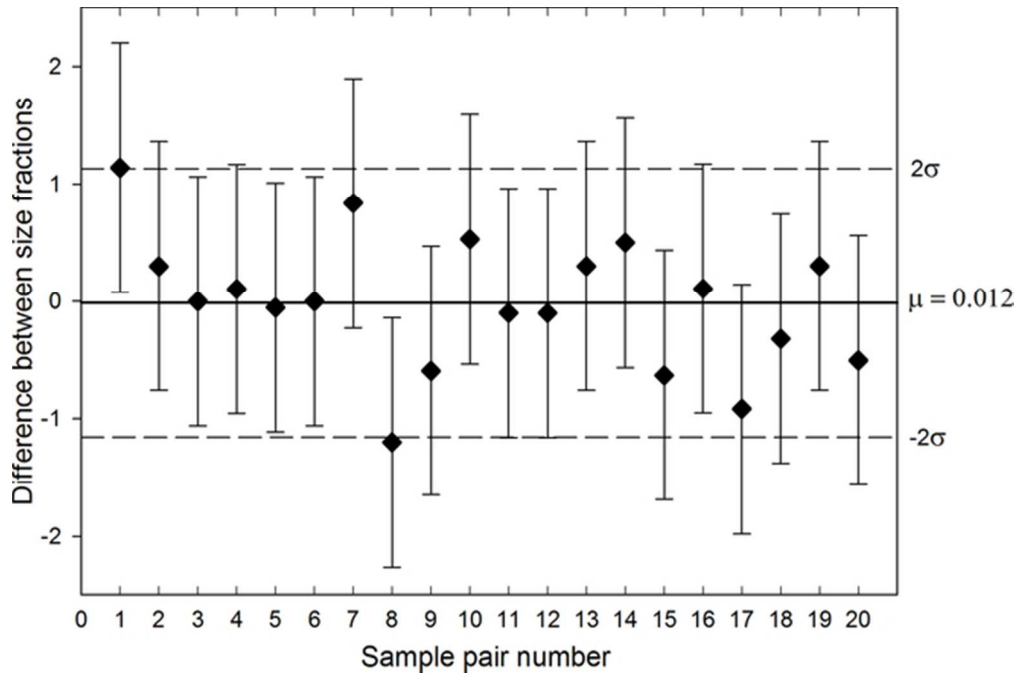


Figure 6. Difference in $\delta^{18}\text{O}_{\text{diatom}}$ between 20 pairs of different diatom size fractions from Heart Lake. Solid line indicates the mean difference between the two data sets, dashed lines represent the 2σ uncertainty range of the technique used
55x36mm (300 x 300 DPI)