

# Developing a methodology for carbon isotope analysis of lacustrine diatoms

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## **ABSTRACT**

Stable isotope analysis of sedimentary carbon in lakes can help reveal changes in terrestrial and aquatic carbon cycles. A method based on a single, photosynthetic organism, where host effects are minimised, should offer more precision than carbon isotope studies of bulk lake sediments. Here we systematically develop a method for use on fossil lacustrine diatom frustules, adapted from previous studies in marine environments. A step-wise cleaning experiment on diatomaceous lake sediments from Lake Challa, Mt, Kilimanjaro, was made to demonstrate the necessary treatment stages to remove external sedimentary carbon. Changes in soluble carbon compounds during these cleaning experiments were measured using GC/MS. Mass spectrometry methods were refined to measure the small percentage carbon of these samples and details of these methods are presented. Samples of cleaned diatoms containing <1% carbon yielded robust results. Carbon isotope analysis of diatom samples containing different species mixtures were performed and showed suggestive differences, although the effects lay within current experimental error and require further work. Unlike work on oxygen and silicon isotopes from diatom frustules, mineral contamination had no discernable impact on the diatom carbon isotope ratios from these sediments. The range of values found in the lakes investigated thus far can be interpreted with reference to the supply and nature of carbon from the catchment as well as demand generated from lake primary productivity.

## INTRODUCTION

Understanding carbon cycling through lake basins is of fundamental importance to global-change science<sup>[1, 2]</sup>, yet few methods are available with which to reconstruct lacustrine dissolved carbon concentrations<sup>[3]</sup>. Isotopic methods offer the potential to trace modern and palaeoenvironmental carbon pathways from the fractionation of  $^{12}\text{C}$  and  $^{13}\text{C}$  according to metabolic and geochemical processes<sup>[4, 5]</sup>. Carbon isotope analysis of sedimentary organic matter ( $\delta^{13}\text{C}_{\text{bulk}}$ ) has been frequently used to analyse changes in particulate carbon inputs to lake sediments<sup>[6, 7]</sup>. In most cases, the interpretation of this signal rests on the ability to identify the dominant source of carbon contained within the organic fraction of the sediments and the extent to which the carbon has been processed through lake ecosystems. In some instances it has been possible (with the addition of Carbon/Nitrogen, pollen and grass epidermis data) to distinguish material from terrestrial  $\text{C}_3$  or  $\text{C}_4$  plants, although carbon from macrophytes and especially algae also contribute a significant fraction of this signal in many lake sediments<sup>[8]</sup>. To identify better the provenance of the organic matter, compound-specific carbon isotope analysis of terrestrial and macrophyte fractions has been undertaken for some lakes<sup>[9-11]</sup> but diagnostic carbon compounds synthesized by specific algal groups have not been forthcoming<sup>[12]</sup>. Productivity changes also influence fractionation, since algae preferentially use the lighter isotope as it is energetically more efficient. Increased productivity leads to higher  $\delta^{13}\text{C}_{\text{bulk}}$  values<sup>[13]</sup> provided carbon supply does not change, for example, the bulk value may be strongly influenced by organic detritus produced by floating and submerged aquatic macrophytes, which may be very abundant in shallow lakes<sup>[14]</sup>. For these reasons, the use of  $\delta^{13}\text{C}_{\text{bulk}}$  is a useful but a blunt tool for the reconstruction of lake carbon cycles and new methods are needed.

To overcome the problem of the heterogeneous nature of sedimentary organic carbon, and of an isotope signature brought about through a multitude of possible fractionation processes, a method based on a single ubiquitous organism that uses dissolved carbon (dissolved inorganic carbon, DIC) for photosynthesis offers great potential for revealing changes in the lacustrine carbon cycle. Specifically, we propose that organic matter preserved within diatom frustules can be analysed for carbon isotopes ( $\delta^{13}\text{C}_{\text{diatom}}$ ) in lacustrine sediments, as has previously been shown in marine environments<sup>[15] [16]</sup>. Diatom frustules contain proteins (pleuralins, silaffins and long chain polyamines) that are central to silica sequestration to form the frustule and are then entombed within the silica cell wall structure<sup>[17-19]</sup>. Analyses of  $\delta^{13}\text{C}_{\text{diatom}}$  in Southern Ocean cores<sup>[20-22]</sup> highlight the feasibility and applicability of this technique in palaeoceanography. However, this

method has not yet been applied to lake sediments where more numerous carbon sources pose significant challenges for the interpretation of the palaeoenvironmental record.

Isotope ratio mass spectrometry of materials containing small concentrations of carbon is highly dependent on sample preparation and treatment. This paper describes a methodology for the extraction of diatoms from lake sediments and analytical considerations for carbon isotope analysis. It explores the issue of sample cleanliness, a particular problem for diatom extraction from sediments where the frustules are mixed with non-diatomaceous organic matter and carbonates, and the steps needed to produce robust data. We also test the sensitivity of the method to species composition, diatom breakage and contamination by non-diatomaceous materials.

## MATERIALS AND METHODS

### *Lake sediments*

Sediment contained in cores from Lake Challa, a permanently stratified crater lake near Mt. Kilimanjaro in equatorial East Africa, formed the basis of these experiments. This site was chosen because down-core reconstructions of  $\delta^{13}\text{C}_{\text{diatom}}$  [23] were to be undertaken in connection with a multidisciplinary palaeolimnological study on this lake [24]. Importantly, the sediments are highly enriched in diatoms (40-60 wt% BSi) and composed of only two principal taxa; *Gomphocymbella* spp. and *Nitzschia paleacea*. Two forms of *Gomphocymbella* were identified although these have not been formally named. For the purpose, of this paper we refer only to the genus *Gomphocymbella* as these forms are morphologically very similar and are both found in the lake today suggesting they have similar ecological affinities (Figure 1a). The high concentration of fossil diatoms and the dominance of these two taxa make Lake Challa an excellent experimental site from which to develop the  $\delta^{13}\text{C}_{\text{diatom}}$  method in lakes.

### *Staged cleaning experiments*

A framework for staged cleaning experiments was devised to examine the extent of treatment required for removal of sedimentary organic matter external to the diatom frustules, leaving only silica-bound carbon for isotope analysis. The raw sediment used in the experiment contained 3.5% C and its  $\delta^{13}\text{C}$  was  $-27.9\text{‰}$ . Standard methods for the removal of carbonates (10% HCl heated at approximately 80°C for 2h) and organic matter (30%  $\text{H}_2\text{O}_2$  heated at approximately 80°C for 7.5h) were carried out before a stronger oxidising agent (concentrated  $\text{HNO}_3$  heated at approximately

80°C for 1h) was used as suggested by Crosta et al. [15]. Further prolonged treatment in H<sub>2</sub>O<sub>2</sub> for up to 60 hours verified that all organic and inorganic carbon, external to the diatom frustules, had been removed and a plateau of percentage organic carbon was reached. At each stage of the treatment process a sample was taken, rinsed in ultrapure (0.055 µS cm<sup>-1</sup>) deionised water and dried to produce 10-20 mg of diatom frustules for analysis.

### *Isotope analysis*

The total organic carbon (%TOC) content and δ<sup>13</sup>C values of the diatom organic matter were analyzed using an online system comprised of a Costech ECS4010 elemental analyser (Costech, Milan, Italy), a Costech chromatography software package (EAS 1.7), a VG TripleTrap, and a VG Optima mass spectrometer (now IsoPrime, Cheadle Hulme, UK). The samples were weighed into Sn capsules and sequentially dropped into a furnace at 1020°C under a continuous flow of helium carrier gas. A pulse of oxygen gas promoted an exothermal flash oxidation of the Sn and the gases produced were further oxidised by chromium and cobaltous oxide in the lower part of the furnace. After removal of excess oxygen and water (by passage through hot copper and magnesium perchlorate), the remaining N<sub>2</sub> and CO<sub>2</sub> then pass through a GC column and by a thermal conductivity detector. This generated an electrical signal proportional to the concentrations of N<sub>2</sub> and CO<sub>2</sub> present in the helium stream. The Costech EAS software station acquired and evaluated this information, producing %N and %C data for the sample. In the case of diatoms, %N is usually at baseline so that data were not reported. By analysing a standard sample of low %C under the same operating conditions it was possible to calibrate the instrument and quantify the content of C of the diatom samples. The helium stream then carried the CO<sub>2</sub> through a trap at -90°C (for complete removal of water), before reaching the Triple Trap held at -196°C. Here the CO<sub>2</sub> was frozen, allowing any N<sub>2</sub> and helium to vent to the atmosphere. The Triple Trap was then evacuated before warming the CO<sub>2</sub> trap and expanding the sample CO<sub>2</sub> into the inlet of the Optima mass spectrometer. We then used a secondary cryogenic trap in the mass spectrometer, positioned directly in front of the inlet which introduced the CO<sub>2</sub> closer to the source, thus allowing a sufficient pressure of CO<sub>2</sub> even though the volume was very small. The Optima mass spectrometer has triple collectors allowing simultaneous monitoring of CO<sub>2</sub> ion beams at  $m/z = 44, 45$  and  $46$ ; and a dual-inlet allowing rapid comparison of sample CO<sub>2</sub> compared with a reference CO<sub>2</sub>. The 45/44 ratios were converted to <sup>13</sup>C/<sup>12</sup>C ratios after correction for common ion effects ('Craig' correction). The system performed an automated run of up to 30 diatom samples at a time because ash builds up in the reaction tube and needed to be removed to enable efficient combustion (Figure 1b and 1c). Each run contained 10 replicates of the laboratory primary standard (BROC, a sample of *Brassica*

*oleracea* (broccoli) grown in Nottingham University field trials at Sutton Bonnington.) and a secondary low %C standard (SOILB an International soil standard from LECO corporation, USA). From knowledge of the laboratory standard's  $\delta^{13}\text{C}$  value versus VPDB (Vienna Pee Dee Belemnite) calibrated to reference samples NBS-19 and NBS-22, held and distributed by the International Atomic Energy Agency in Vienna, the  $^{13}\text{C}/^{12}\text{C}$  ratios of the unknown samples were converted to  $\delta^{13}\text{C}$  values versus VPDB. Typical errors for standard materials are 0.1‰ ( $\delta^{13}\text{C}$ ) and 0.1 (%C).

### *Species separation and breakage*

Three cleaned samples were put through gravitational Split Thin Flow Fractionation (SPLITT), which utilises the different densities and hydrodynamic properties of diatoms to produce two distinct end fractions [25, 26]. In this case, one end fraction comprised predominantly *Gomphocymbella* spp. and the other was mainly *Nitzschia paleacea* (Figure 1d). These samples were each split into three replicates, giving nine samples in total. They were then dried and analysed for %C and  $\delta^{13}\text{C}_{\text{diatom}}$  as outlined above. In order to evaluate whether the degree of frustule breakage produced differences in the preservation or liberation of the carbon inside, three treated samples were sieved at 20  $\mu\text{m}$  yielding a <20  $\mu\text{m}$  fraction largely composed of broken fragments and a >20  $\mu\text{m}$  fraction consisting predominantly of whole diatoms (Table 1). The samples were dried and analysed for %C and  $\delta^{13}\text{C}$  as above.

### *Organic geochemical analyses*

To examine which solvent-soluble carbon compounds were removed during the staged cleaning experiments, combined gas chromatography-mass spectrometry (GC/MS) was undertaken. Five dried diatom samples (of about 3g), from different stages of the treatment process were crushed and agitated in hexane for 10mins in a sonic bath (Camlab, Cambridge, 300W). The samples were allowed to settle and the supernatant decanted and reduced to 2mL in a stream of  $\text{N}_2$  gas. A procedural blank (PB) was treated in exactly the same way. Gas chromatography-Mass Spectrometry was performed using a Varian (Varian, Oxford) CP-8400 autosampler fitted to a Varian CP-3800 gas chromatography coupled with a Varian 1200L triple quadrupole mass spectrometer (Electron ionization at 70eV; full scan mode ( $m/z$  47-600); scan time 0.5 sec; detector voltage 1500V; source temperature 250°C). The GC was fitted with a Varian VF-5MS column, 60m x 0.32mm x 0.25 $\mu\text{m}$ . The GC was temperature programmed from 60°C (5min isothermal) to 320°C

(10min isothermal) at 10°C / min. Injection (2µL) was split-less for the first 0.70 min and 1:20 split thereafter. The injector temperature was 300°C.

## RESULTS

### *Staged cleaning experiment and organic geochemistry*

Treatment in HCl resulted in a very small percent carbon increase to 3.6% which suggests that the acid removed carbonate compounds and perhaps mineral oxides that contribute to the non-carbon mass of the sample (Figure 2). The slight decrease in  $\delta^{13}\text{C}$  to  $-28.4\text{‰}$  is consistent with the removal of carbonates, which have high  $\delta^{13}\text{C}$  values. A large change in %C or  $\delta^{13}\text{C}$  was not expected as carbonates are only found in trace amounts in this unit of the Challa sediment sequence (B. Plessen, GFZ Potsdam, personal communication). The result of organic geochemical analyses supports this explanation as there was little change in the distribution of the *n*-alkanes (Figure 4). Although for these samples treatment in HCl resulted in little change, the removal of carbonates was an important step, especially as the final  $\delta^{13}\text{C}_{\text{diatom}}$  measurements were made on very small samples of diatom carbon and would be susceptible to contamination by even minute amounts of inorganic carbon.

Treatment with  $\text{H}_2\text{O}_2$  significantly reduced the TOC content to just 0.7% and resulted in a negative  $\delta^{13}\text{C}$  shift to  $-33.6\text{‰}$  (Figure 2). These changes are probably due to the result of organic matter (aquatic and terrestrial), external to the diatom frustules, being oxidised from the sediments. Soil organic matter from the Lake Challa catchment is derived from a mix of  $\text{C}_3$  and  $\text{C}_4$  plants and has a  $\delta^{13}\text{C}$  value of  $-19\text{‰}$ , therefore the reduction in  $\delta^{13}\text{C}$  is likely to be the removal of terrestrial material with a relatively high  $\delta^{13}\text{C}$  signature. Next, the treatment in nitric acid further reduced TOC to values of 0.1%. As  $\text{HNO}_3$  is a stronger oxidising agent than  $\text{H}_2\text{O}_2$  any persistent organic matter external to the diatom frustules should have been lost at this stage. This is supported by the gas chromatograms (Figure 3) which show a significant change in the carbon compounds present in the samples after  $\text{H}_2\text{O}_2$  and  $\text{HNO}_3$  treatment; in particular the loss of  $\text{C}_{27}\text{-C}_{29}$  chains and some shorter chains. A fall in  $\delta^{13}\text{C}$  (to  $-31.3\text{‰}$ ) indicates either that a carbon source with a higher isotopic composition to the diatoms has been removed, perhaps a lipid fraction<sup>[27]</sup> or potentially that an attack on internal diatomaceous carbon had occurred due to the harsh treatment method<sup>[28]</sup>. In support of the former, it has been argued that HCl and  $\text{H}_2\text{O}_2$  do not efficiently remove labile organic matter from diatom frustules and stronger oxidising agents such as  $\text{HClO}_4$  and  $\text{HNO}_3$  are needed<sup>[15]</sup>. The presence of organic matter observed through optical microscopy prior to treatment in nitric acid also highlighted the need for powerful oxidising agents<sup>[23]</sup>.

Further prolonged treatments in H<sub>2</sub>O<sub>2</sub> resulted in a fluctuation of %C and  $\delta^{13}\text{C}$  around a plateau. The gas chromatograms show slight increases in short-chain carbon compounds during this stage, which is contrary to what would be expected from the oxidation process. One explanation is that etching of the diatom silica during the HNO<sub>3</sub> stage enabled carbon to be leached from the diatom silica structure. These results highlight the need to achieve a balance between removing all external sedimentary carbon and degrading internal diatom organic material that provides the basis for the measurement of  $\delta^{13}\text{C}_{\text{diatom}}$ .

### *Species and breakage effects*

The samples prepared to test the effect of species composition (Figure 1d) indicate that the higher the *Nitzschia paleacea* : *Gomphocymbella* ratio, the lower the measured  $\delta^{13}\text{C}$  value (Figure 4). This relationship holds in terms of percentage frustule counts and even more strongly when calculated using the volume of these diatoms, since *Gomphocymbella* frustules have 24 times the volume of the *Nitzschia* spp. However, the results are within the standard deviation of these samples and are not statistically significant, probably due to the very small sample sizes that may have contributed to the scatter of the data.

The result of the breakage experiment showed that broken diatoms were on average 1.3‰ lower in  $\delta^{13}\text{C}$  than the sample with intact valves, although again this falls within the standard deviation. The depletion of  $\delta^{13}\text{C}_{\text{diatom}}$  is consistent with a loss of <sup>13</sup>C- rich amino acids observed in other studies [29, 30]. In the absence of a statistically significant difference, it seems that carbon preserved within the diatom silica cell structure is effectively protected even in physically broken valves. This is consistent with strong bonds being formed between the amino acids and diatom silica.

## **DISCUSSION**

### *Comparison of %C and $\delta^{13}\text{C}_{\text{diatom}}$*

These experiments point to a relationship between the degree of cleaning and the  $\delta^{13}\text{C}_{\text{diatom}}$  due to labile organic matter, with a higher isotopic signature than the diatoms, still being present before HNO<sub>3</sub> oxidation. It is therefore important to identify the point below which  $\delta^{13}\text{C}_{\text{diatom}}$  can be considered independent of the degree of cleaning. Figure 5 shows further measurements of



$\delta^{13}\text{C}_{\text{diatom}}$  made on Lake Challa sediment, plotted against organic carbon content of cleaned diatom frustules from the same samples [23]. There is a negative relationship ( $r^2 = 0.38$ ) between %C measured on the cleaned diatoms and  $\delta^{13}\text{C}_{\text{diatom}}$  driven by a small number of samples that retained in excess of 1% carbon. This suggests that samples with >1% carbon may still contain carbon which is external to the diatom frustules. This material could well be derived from diatom cellular components or extracellular membranes but it would be best practice to avoid material other than that present within the frustule silica itself. However, it is possible that the amount of carbon in the frustules varies according to environmental conditions and perhaps species. For example, one study [31] has suggested that when sea water contained more iron, marine diatoms reduced their consumption of silica relative to carbon leading to the hypothesis that changes in %C<sub>diatom</sub> during glacial-interglacial cycles were the result of changing nutrient concentrations [31].

#### *Sample size, composition and reproducibility*

It is essential to work with near-pure diatom samples independent of external carbon influences even though this means that the organic carbon to be analysed typically comprised only 0.1-1% of the cleaned biogenic silica. At this low carbon concentration, analytical errors are magnified and sample reproducibility is brought into question. Results from replicate subsamples of the initial raw samples from these experiments had a standard deviation of 0.2‰ and this increased to 0.8‰ following removal of external sample carbon  $\text{H}_2\text{O}_2$  and  $\text{HNO}_3$  in the latter stages of the experiment. It is encouraging to note that following  $\text{HNO}_3$  treatment  $\delta^{13}\text{C}_{\text{diatom}}$  values reached a plateau of 31.9‰ with a standard deviation of 0.4‰ (Figure 2) indicating that cleaning of external sample carbon had been effective. Both the species and broken valve experiments, produced values that lay within one standard deviation, suggesting that species considerations played a small role in comparison with the total analytical error for these small samples (Figure 4).

#### *Methodology recommended for carbon isotope analysis of diatoms*

Based on the above discussion, we propose the following methodology as appropriate for diatomaceous sediments such as those from Challa. An initial treatment in 10% HCl for 2h, 30%  $\text{H}_2\text{O}_2$  for 7.5h and concentrated  $\text{HNO}_3$  for 1h is sufficient for preparing samples for  $\delta^{13}\text{C}_{\text{diatom}}$  analysis (Stage 1-3). More extensive treatment may damage the diatom silica and result in leaching of the internal organic material. The percentage organic carbon should be measured and values greater than 1% should be suspected of containing non-diatom C. If samples are obtained from carbonate lakes, the HCl stage may need to be prolonged to ensure that all carbonates are removed

especially if carbonate is of the less soluble types (e.g., dolomite/siderite). Similarly, samples from lakes with abundant, or resistant, initial organic matter in their sediments might need longer oxidation.

In Lake Challa the effect of silicate mineral contamination on  $\delta^{13}\text{C}_{\text{diatom}}$  is thought to be minimal, because minerals in these sediments are unlikely to have large quantities of carbon relative to that in the diatoms. More minerogenic sediments from Mt. Kenya were also investigated, but artificial mixtures of diatoms and silicate minerals had no effect on the isotope values [23]. Other biogenic silica materials such as phytoliths and sponge spicules sometimes contain significant quantities of organic matter and samples rich in these forms should also be treated with caution [32].

### *Prospects for the application of $\delta^{13}\text{C}_{\text{diatom}}$ in lakes and oceans*

A broad range of  $\delta^{13}\text{C}_{\text{diatom}}$  values have been recorded by the few studies which have utilized this technique (Figure 6), highlighting that it is not possible to classify all diatom frustule organic matter into narrow isotope ranges. Encouragingly, these data suggest that environmental conditions play a large role in influencing the isotopic signature although the processes leading to these values are likely to be diverse. The highest  $\delta^{13}\text{C}_{\text{diatom}}$  values, observed in the Southern Ocean, show a similar range to other carbon isotope studies of marine algae that have reported values of  $-16$  to  $-25\text{‰}$  [33]. High isotopic values in the marine environment may result from diatoms having an ability to use  $\text{HCO}_3^-$  as a carbon source [34]. As the input of terrestrial organic matter to the open oceans is low, productivity plays a large role in determining the isotopic signature of oceanic DIC which then contributes to  $\delta^{13}\text{C}_{\text{diatom}}$ . This supports the interpretation of  $\delta^{13}\text{C}_{\text{diatom}}$  values from Southern Ocean cores as representing changes in productivity, where low  $\delta^{13}\text{C}_{\text{diatom}}$  during the Last Glacial Maximum is thought to suggest an increase in the availability of  $^{12}\text{C}$  due to low phytoplankton productivity [21, 35].

In acidic and circumneutral lakes, mineralisation of terrestrial material can take place directly in the lake as a result of inputs from the surrounding catchment, resulting in lake DIC and Dissolved Organic Carbon (DOC) being isotopically depleted and therefore explaining the lower  $\delta^{13}\text{C}_{\text{diatom}}$  values. In Small Hall Tarn and Simba Tarn, two high altitude lakes on Mt. Kenya, the limited Afroalpine vegetation and thin soils today and for most of the Holocene means that the lake catchment carbon pool has always been relatively small and high aquatic productivity enriches lake DIC and DOC through preferential use of  $^{12}\text{C}$ . In this scenario, the  $\delta^{13}\text{C}_{\text{diatom}}$  signature reflects the balance between carbon inputs from the catchment and lake productivity [23]. The sedimentary

record of  $\delta^{13}\text{C}_{\text{diatom}}$  from Small Hall Tarn and Simba Tarn suggests that when carbon inputs increased, lake productivity also increased, either because carbon was a limiting nutrient, or because carbon and nutrient influxes were coeval highlighting the strong relationship between catchment and lake productivity<sup>[36]</sup>. Modern  $\delta^{13}\text{C}_{\text{diatom}}$  samples from the Lake District, UK tell a similar story, except that the more highly productive lakes also have large carbon pools due to their location in productive catchments. In this Lake District study, values of  $\delta^{13}\text{C}_{\text{diatom}}$  are similar to the carbon isotope values that might be expected from surrounding  $\text{C}_3$  vegetation (Barker, unpublished data).

Finally, the  $\delta^{13}\text{C}_{\text{diatom}}$  values observed in oligotrophic Lake Challa are the lowest yet measured ( $-28\%$  to  $-37\%$ ) and the  $\delta^{13}\text{C}$  of the diatomaceous bulk sedimentary organic matter was  $-24$  to  $-35\%$ . This is surprising since the dominant  $\text{C}_4$  grasses surrounding the lake have much higher  $\delta^{13}\text{C}$  values and the  $\delta^{13}\text{C}$  of DIC in the water column today are  $-3$  to  $-7\%$  (Keppens Personal Communication). These data imply that the diatoms were largely using biogenic carbon leached from catchment soils and respired from sediments<sup>[37]</sup>. Therefore, changes in carbon source and in authigenic carbon cycling have heavily influenced  $\delta^{13}\text{C}_{\text{diatom}}$  of this lake.

## CONCLUSION

We have shown that it is possible to analyse diatom  $\delta^{13}\text{C}_{\text{diatom}}$  from lake sediments and that this could become a useful tool to limnologists interested in reconstructing aquatic carbon cycling. The method we propose is robust and effective in the removal of carbon external to the diatom frustules. We note that the interpretation of  $\delta^{13}\text{C}_{\text{diatom}}$  from lakes will differ from that in the oceans, and is likely to be an indicator of changes in the nature and concentration of lake carbon, either through changes in productivity or carbon source, or the balance between the two. The  $\delta^{13}\text{C}$  signal is therefore site specific and a function of the characteristics of the lake catchment influencing C supply and in-lake processing. Nevertheless, this proxy is able to trace lake carbon cycling and/or lake productivity on various ecological to geological timescales and will add to the limited methods available to address key questions relevant to the global carbon cycle.

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**Table 1.** Selected samples from Lake Challa were sieved to examine the effect of broken diatom frustules on  $\delta^{13}\text{C}_{\text{diatom}}$ . The  $>20\mu\text{m}$  fraction contained a greater proportion of intact diatom cells than the  $<20\mu\text{m}$  fraction. Each fraction was measured independently for  $\%C_{\text{diatom}}$  and  $\delta^{13}\text{C}_{\text{diatom}}$ .

| Sample (depth cm) | %Intact          | %Broken | $\delta^{13}\text{C}_{\text{diatom}}$ |        |
|-------------------|------------------|---------|---------------------------------------|--------|
| <b>1216</b>       | $>20\mu\text{m}$ | 65%     | 35%                                   | -31.3‰ |
|                   | $<20\mu\text{m}$ | 30%     | 70%                                   | -30.9‰ |
| <b>1392</b>       | $>20\mu\text{m}$ | 62%     | 38%                                   | -30.9‰ |
|                   | $<20\mu\text{m}$ | 16%     | 84%                                   | -32.4‰ |
| <b>1600</b>       | $>20\mu\text{m}$ | 88%     | 11%                                   | -28.8‰ |
|                   | $<20\mu\text{m}$ | 33%     | 67%                                   | -30.8‰ |

## Figure Captions

**Figure 1(a)** SEM image of *Gomphocymbella* spp. from Lake Challa showing both forms of this taxon encountered. Scale bar 10 $\mu$ m. **(b and c)** Sample after combustion indicating formation of Sn-silica crystals and diatom fragments. Scale bar 10 $\mu$ m. **(d)** Samples were separated using a gravitational Split Thin Flow Fractionation (SPLITT) system. This created two distinct end fractions: Fraction a' was comprised of *Nitzschia paleacea* and Fraction b' was comprised of *Gomphocymbella* spp.

**Figure 2** Staged cleaning experiment showing %C content (bars) and carbon isotope signature (triangles). All chemical treatments were made at 80°C.

**Figure 3** Organic geochemical analysis of samples taken from different stages of the cleaning experiment. Loss of *n*-alkane compounds occurred after heating in HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>. The star notes the location of a peak in Phthalic acid, a ubiquitous plasticiser that was likely introduced during the preparation of samples for GC-MS.

**Figure 4** Comparison of the ratio of *Nitzschia* to *Gomphocymbella* (by volume) and  $\delta^{13}\text{C}_{\text{diatom}}$  values. The error shows the standard deviation from three replicates.

**Figure 5** Samples from Lake Challa sediments were prepared for  $\delta^{13}\text{C}_{\text{diatom}}$  using the method described here<sup>[23]</sup>. A correlation between  $\delta^{13}\text{C}_{\text{diatom}}$  and percentage carbon was found for %C>1%.

**Figure 6** Comparison of  $\delta^{13}\text{C}_{\text{diatom}}$  values measured in sediments from Lake Challa, Simba Tarn and Small Hall Tarn<sup>[23]</sup> with studies from ocean sediments<sup>[20-22]</sup> and contemporary diatoms from the English Lake District (Barker, unpublished data).