The stable isotope composition of organic and inorganic fossils in lake sediment records: current understanding, challenges, and future directions.

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Abstract

This paper provides an overview of stable isotope analysis (H, C, N, O, Si) of the macro- and microscopic remains from aquatic organisms found in lake sediment records and their application in (palaeo)environmental science. Aquatic organisms, including diatoms, macrophytes, invertebrates, and fish, can produce sufficiently robust remains that preserve well as fossils and can be identified in lake sediment records. Stable isotope analyses of these remains can then provide valuable insights into habitat-specific

biogeochemistry, feeding ecology, but also on climatic and hydrological changes in and around lakes. Since these analyses focus on the remains of known and identified organisms, they can provide more specific and detailed information on past ecosystem, food web and environmental changes affecting different compartments of lake ecosystems than analyses on bulk sedimentary organic matter or carbonate samples. We review applications of these types of analyses in palaeoclimatology, palaeohydrology, and palaeoecology. Interpretation of the environmental 'signal' provided by taxon-specific stable isotope analysis requires a thorough understanding of the ecology and phenology of the organism groups involved. Growth, metabolism, diet, feeding strategy, migration, taphonomy and several other processes can lead to isotope fractionation or otherwise influence the stable isotope signatures of the remains from aquatic organisms. This paper includes a review of the (modern) calibration, culturing and modeling studies used to quantify the extent to which these factors influence stable isotope values and provides an outlook for future research and methodological developments for the different examined fossil groups.

Keywords: Stable isotopes; Lake sediment; Organic remains; Inorganic remains; Diatoms; Invertebrates; Ostracods

1. Introduction

Stable isotope analysis provides a versatile tool for investigating lake sediment records based on the link between stable isotope ratios and a range of environmental and biological processes, including climate change, hydrology, biogeochemical cycling, and consumer-diet interactions in food webs (Leng and Henderson 2013). Stable carbon and nitrogen isotope analysis of bulk sedimentary organic matter (SOM) is often used in palaeoenvironmental records as SOM is easy to sample and relatively straightforward to measure (Meyers et al. 2001). Some sediments require chemical pre-treatment to remove carbonates, which in itself can affect the stable isotope composition of SOM (Brodie et al. 2011a, b). The information provided by SOM is always an integrated signal of catchment and in-lake processes, which can make it hard to interpret variations in its isotope composition. For example, an understanding of changes in $\delta^{13}C$ and $\delta^{15}N$ values of SOM

77 requires detailed information from the catchment as they are dependent on, amongst a
78 range of factors, the composition and amount of input from terrestrial vegetation,
79 anthropogenic nutrient input, lake volume, littoral-to-profundal ratio of the lake basin,
80 productivity, groundwater inputs and stratification (e.g., Meyers and Ishiwatari 1993).
81 Stable isotopes of endogenic (bulk) carbonates are also widely used in
82 palaeohydrology and palaeoclimatology. Changes in mineral $\delta^{18}O$ values are interpreted in
83 terms of changes in precipitation and temperature (Leng and Marshall 2004, Leng and
84 Barker 2006). The $\delta^{13}C$ values of endogenic carbonates are a complex sum of processes
85 taking place in the lake and its catchment, including temperature and productivity-
86 dependent fractionation, dissolved inorganic carbon (DIC) inflow, methanogenesis and
87 methane oxidation, CO_2 dissolution and outgassing and lake stratification (Siegenthaler
88 and Eicher 1986; Hollander and Smith 2001; Leng and Marshall 2004; Schwalb et al. 2013).
89 To complement stable isotope data derived from analyses of SOM or carbonates, it has
90 become more common to analyse the stable isotope composition of identifiable fossil
91 remains separated and manually picked from lake sediments (e.g. diatoms, invertebrates,
92 plant macrofossils), or to analyse stable isotopes of specific compounds chemically
93 isolated from the sediments (e.g., lipids, amino acids, pigments). These approaches offer
94 the great benefit of targeting specific organism groups or chemical biomarkers, which can
95 reflect particular habitats or locations in a lake, or provide valuable information about
96 their functional roles in an ecosystem. Targeting specific remains and compounds also
97 means that it is easier to understand and test how the stable isotope composition is
98 affected by biogeochemical and taphonomic processes over time.
99 In this review, we provide an overview of approaches based on stable isotopes
100 measured on taxon-specific samples. We include the isotope systems H, C, N, O, Si,
101 measured on diatoms, calcareous and chitinous invertebrate remains, fish remains, and
102 plant macrofossils. We discuss their palaeolimnological applications and provide an
103 overview of the current understanding of taphonomy and ecology that is required to
104 interpret sedimentary records.
105
106 **2. Diatoms**
107

Diatoms are unicellular, eukaryotic, micro-organisms, which are ubiquitous in nature. As such, diatom silica is an interesting sediment component for isotope measurements in lake (Leng and Barker 2006) and marine (Swann and Leng 2009) environments, where the oxygen ($\delta^{18}O_{diatom}$), silicon ($\delta^{30}Si_{diatom}$), carbon ($\delta^{13}C_{diatom}$) and nitrogen ($\delta^{15}N_{diatom}$) isotope compositions can all be used as proxies for environmental change. $\delta^{18}O_{diatom}$ tends to be used as a measure of temperature/water composition variation (Fig. 1), $\delta^{30}Si_{diatom}$ as a proxy for nutrient availability and utilisation (Fig. 2), and $\delta^{13}C_{diatom}$ and $\delta^{15}N_{diatom}$ for nutrient cycling/source investigation (Leng and Henderson 2013).

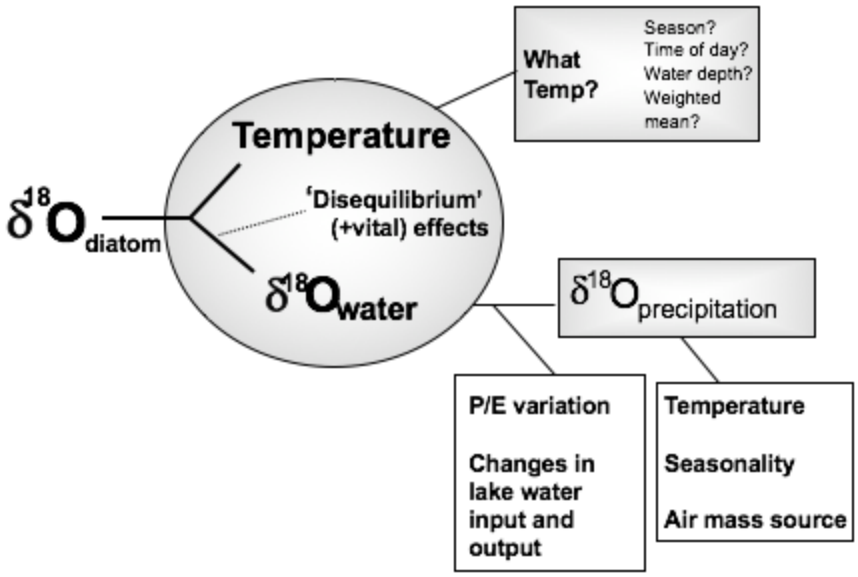

Fig. 1: *Controls on the stable oxygen isotope composition of biogenic silica*

Compared to $\delta^{18}O_{diatom}$ studies, the application of $\delta^{30}Si_{diatom}$, $\delta^{13}C_{diatom}$ and $\delta^{15}N_{diatom}$ techniques are in their relative infancy, with only a few studies being applied in lacustrine systems (Alleman et al. 2005; Street-Perrott et al. 2008; Swann et al. 2010; Hurrell et al. 2011; Hernández et al. 2011, 2013; Opfergelt et al. 2011; Chen et al. 2012; Barker et al. 2013; Cockerton et al. 2015; Webb et al. 2016; Panizzo et al. 2016, 2018a, b). There are several reviews of the use of isotopes in biogenic (including diatom) silica (including Leng and Barker 2006; Swann and Leng 2009; Leng et al. 2009; Leng and Henderson 2013; Sutton et al. 2018), which highlight, in detail, the many issues associated with such analyses. These will be further elucidated upon here and include issues such as equilibrium fractionation, sample purification, post mortem maturation of diatom silica, and

129 standardisation and inter-laboratory calibrations. Given that more recent advances have
130 been made in $\delta^{30}Si_{diatom}$ applications in lacustrine settings, in addition to the body of
131 literature on $\delta^{18}O_{diatom}$, we focus this review on these two proxies.

132 Diatom silica is a structurally complex mineral for $\delta^{18}O_{diatom}$ (see section 2.4 below)
133 measurement due to a hydrous component. O isotope extraction generally adopts
134 fluorination (offline) techniques, with measurement via gas-source isotope ratio mass
135 spectrometry (IRMS), a technique which can also be used to measure $\delta^{30}Si_{diatom}$ (Leng and
136 Sloane 2008). However, the use of multi-collector inductively-coupled-plasma mass
137 spectrometry (MC-ICP-MS) is becoming increasingly more dominant for $\delta^{30}Si_{diatom}$ analyses
138 due to the reduced sample size needed (~ 1 mg), while carbon and nitrogen (for $\delta^{13}C_{diatom}$
139 and $\delta^{15}N_{diatom}$) are measured on very small quantities of organic material hosted
140 (occluded) within the structure (Webb et al. 2016).
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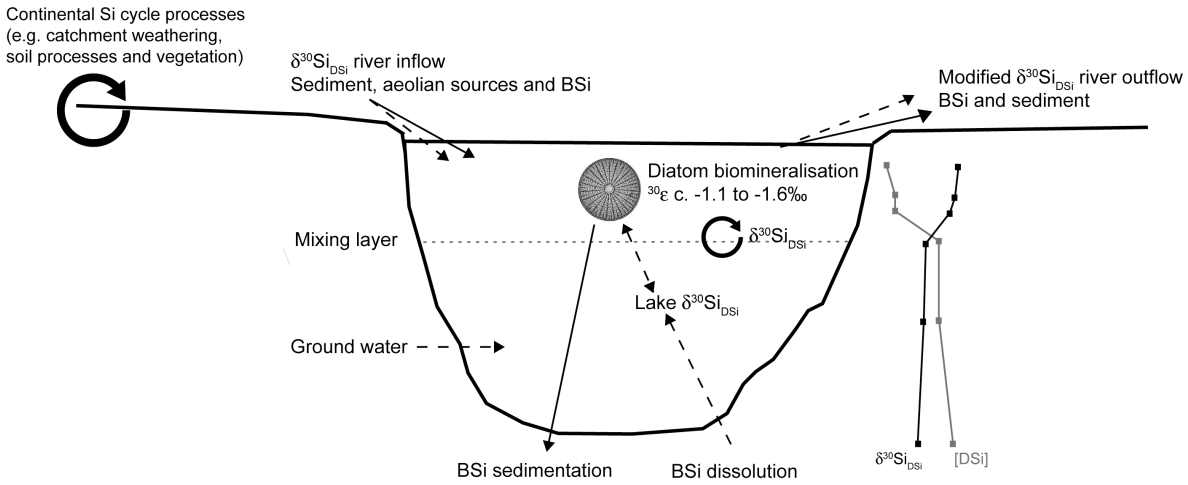

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144 **Fig. 2**: *A schematic drawing of Si cycling in a lake system; solid arrows correspond to*
145 *particulate fluxes and dashed lines to dissolved phases or transformation processes. The*
146 *range in fractionation factors ($^{30}\varepsilon$) associated with the production of biogenic silica (in this*
147 *case, diatoms) from freshwater archives are also provided. Typical water column profiles*
148 *(for both lacustrine and oceanic settings) of DSi concentrations [DSi] and $\delta^{30}Si_{DSi}$ (‰)*
149 *signatures are drawn, highlighting the effects of biological uptake in surface waters (e.g.,*
150 *lower [DSi] and higher $\delta^{30}Si_{DSi}$). Reference to water column mixing is also made, which*
151 *depending on the limnological characteristics of individual sites, can compositionally*
152 *"reset" surface water $\delta^{30}Si_{DSi}$ (e.g., on a seasonal basis).*

153

154 Silicon has three naturally occurring stable isotopes ^{28}Si, ^{29}Si and ^{30}Si with a mean
155 abundance of 92.2%, 4.7% and 3.1% respectively. The isotope composition of any sample
156 (*x*), for ^{29}Si or ^{30}Si (*n*), is expressed in delta notation (δ), compared to the reference
157 standard NBS28, using the following equation:

158 $$\delta^{n}Si_x\ (‰) = ([(^{n}Si/^{28}Si)_x - (^{n}Si/^{28}Si)_{standard}]/[(^{n}Si/^{28}Si)_{standard}]) \times 1000$$

159 An overview of the key processes affecting $\delta^{30}Si$ in lakes are shown in Fig 2. These include
160 processes in a catchment (weathering, soil development, vegetation cover) and in a lake
161 (productivity, water supply, stratification, sedimentation, dissolution) and will be
162 discussed in more detail below.

163

164 *2.1 Interpreting stable oxygen and silicon isotopes of diatom opal*

165

166 $\delta^{18}O_{diatom}$ has been used as a proxy for climate and hydrological change in many studies
167 (Leng and Barker 2006) and the mineral-water fractionation has been estimated
168 previously from analyses of diatoms from freshwater (and marine) sediments, coupled
169 with estimates of the temperatures and isotope compositions of coexisting waters during
170 silica formation (Labeyrie 1974; Juillet-Leclerc and Labeyrie 1987; Matheney and Knauth
171 1989). There are very few calibration studies (e.g., Labeyrie and Juillet 1982; Wang and
172 Yeh 1985; Juillet-Leclerc and Labeyrie 1987; Shemesh et al. 1995), and published estimates
173 of the average temperature dependence for typical ocean temperatures range from –0.2
174 to –0.5 ‰ per ^{o}C (Juillet-Leclerc and Labeyrie 1987; Shemesh et al. 1992; Brandriss et al.
175 1998). However, in lake studies the oxygen isotope composition of diatom silica is often
176 more sensitive to changes in "non-temperature" aspects of climate, such as amount or
177 source of precipitation (Barker et al. 2001; Shemesh et al. 2001) and evaporation
178 (Hernández et al. 2008).

179 In a pioneering study, Schmidt et al. (1997) suggested that there is no regular
180 correlation between temperature and the oxygen isotope fractionation between modern
181 diatoms and the water in which they biomineralise. This led to the hypothesis that the
182 temperature-dependent oxygen isotope fractionation preserved in biogenic opaline
183 sediments may, in some environments, have been established during diagenesis (see

below) rather than acquired during growth, a subject open to recent investigation (Menicucci et al. 2017; Tyler et al. 2017).

$\delta^{30}Si_{diatom}$ is a relatively underused technique in lacustrine systems, with few studies published despite silicon cycling in lakes being a key component of the continental silicon cycle. However, the field is growing, with contemporary studies examining the $\delta^{30}Si$ signature of lake surface waters and diatom opal as a means to validate the technique for palaeolimnological applications (Opfergelt et al. 2011; Panizzo et al. 2016). The method has traditionally been more widely applied in oceanographic settings as a means to reconstruct past biogeochemical cycling, with conventional interpretation of the method as a diatom silica utilisation or water mass/circulation proxy, the latter particularly when coupled with $\delta^{13}C_{diatom}$ and $\delta^{15}N_{diatom}$ or $\delta^{30}Si$ from other silicifying organisms (i.e. sponge spicules and radiolarians) (Abelmann et al. 2015; Beucher et al. 2007; De La Rocha et al. 1998; Hendry and Brzezinski 2014; Hendry et al. 2016; Horn et al. 2011; Maier et al. 2013; Panizzo et al. 2014).

Lake water dissolved silica (DSi) $\delta^{30}Si$ signatures ($\delta^{30}Si_{DSi}$) are essentially a product of upstream catchment processes (e.g., weathering, erosion and soil processes, and vegetation), regulated by regional climate systems, and within-lake biogeochemical processes (e.g., diatom utilisation and dissolution; see section 2.2) (De la Rocha et al. 2000; Frings et al. 2016; Opfergelt and Delmelle 2012; Panizzo et al. 2018b). In instances of more extreme climatic variability, particularly in tropical catchments, lake water budgets can be considerably altered so that there are large opposing (seasonal) variations in overall lake water DSi concentrations and $\delta^{30}Si_{DSi}$ signatures (with increasing compositions of ~0.5 ‰ during dry season periods; Cockerton et al. 2013). This can be further compounded at sites where groundwater inputs are considerable (Street-Perrott et al. 2008). Within-lake biomineralisation can significantly alter the outflowing concentration and composition of DSi and $\delta^{30}Si_{DSi}$ (respectively). As such, lakes have been found to act as a key buffer within the continental Si cycle (in addition to the soil-vegetation system) (Frings et al. 2014).

Studies of $\delta^{30}Si_{DSi}$ and $\delta^{30}Si$ of diatom opal ($\delta^{30}Si_{diatom}$) therefore act as a tracer of biogeochemical processes in nature. Early studies were conducted at lake sites with basalt and volcanic geology, where Si weathering, riverine DSi fluxes and biomineralisation potential are high (e.g., Alleman et al, 2005; Street-Perrott et al. 2008; Opfergelt et al.

2011; Chen et al. 2012; Cockerton et al. 2015). In lacustrine systems these techniques are applied to investigate variations in water column mixing (e.g. DSi supply) and diatom bloom duration (e.g. DSi utilisation), which are all regulated by intrinsic (e.g. water column mixing, stratification, ice-cover duration) and extrinsic processes (e.g. climate and riverine source water changes) (Fig. 2; Alleman et al. 2005; Street-Perrott et al. 2008; Opfergelt et al. 2011; Panizzo et al. 2016, 2017, 2018a, b).

2.2 Fractionation and vital effects of diatom opal

As with other organisms, diatoms are assumed to be precipitated in isotope equilibrium as predicted by thermodynamic fractionation. However, it has been widely shown in carbonates that offsets from oxygen (and carbon) isotope equilibrium may arise in response to variations in kinetic or metabolic processes within and between individual taxa, e.g., changes in growth rates, nutrient availability or rates of calcification/silicification (Duplessy et al. 1970; Wefer and Berger 1991; Spero and Lea 1993, 1996; Spero et al. 1997; Bemis et al. 1998). For biogenic carbonates, such as ostracods (see section 3 below), the impact of vital effects can be overcome by selecting species-specific samples for isotope analysis. This is not feasible for diatoms due to their small size. A number of culture (Binz 1987; Brandriss et al. 1998; Schmidt et al. 2001), sediment trap (Moschen et al. 2005) and down core studies (Sancetta et al. 1985; Juillet-Leclerc and Labeyrie 1987; Shemesh et al. 1995; Bailey et al. 2014) in lacustrine (and marine) systems do not show any oxygen isotope vital effect exists in diatoms. While data in Brandriss et al. (1998) display a 0.6 ‰ difference between two laboratory cultured diatom taxa and Shemesh et al. (1995) found a 0.2 ‰ offset between two different size fractions of diatoms, offsets of this magnitude are within the range of reproducibility routinely achieved when analysing $\delta^{18}O_{diatom}$.

Early studies on the fractionation of diatom opal during valve dissolution suggested a –0.55 ‰ enrichment of $\delta^{30}Si_{diatom}$ (Demarest et al. 2009) although this has since been challenged, to suggest an absence of dissolution fractionation effects, based on analyses of marine (Wetzel et al. 2014) and lacustrine sedimentary diatoms (Panizzo et al. 2016). More complex, and still in dispute to a certain degree, are the isotope fractionations associated with diatom biogenic silica production. When diatoms take up DSi (in the form of silicic acid, $Si(OH)_4$) during biomineralisation they actively discriminate against the

heavier (^{30}Si, ^{29}Si) isotopes in favour of the lighter isotope (^{28}Si), leading to the enrichment of the residual pool ($\delta^{30}Si_{DSi}$) (De La Rocha et al. 1997). Evidence from *in-vitro* and *in-situ* studies from marine diatoms suggests that this per mille enrichment factor ($^{30}\varepsilon_{uptake}$) is independent of temperature, pCO_2 and nutrient availability (De La Rocha et al. 1997; Fripiat et al. 2011; Milligan et al. 2004; Varela et al. 2004). The general consensus for $^{30}\varepsilon_{uptake}$ is −1.1 ± 0.4 ‰ (refer to Frings et al. 2016 for the latest compilation of data) with good agreement with more recent *in-situ,* contemporary studies from freshwater diatoms (published values ranging between −1.1 and −1.6 ‰; Alleman et al. 2005; Opfergelt et al. 2011; Panizzo et al. 2016; Sun et al. 2013) (Fig. 2). However, Sutton et al. (2013) have challenged this consensus reporting species-dependent $^{30}\varepsilon_{uptake}$ ranging from −2.09 to −0.54 ‰ (based on cultured polar and sub-polar marine diatom strains). While such evidence has not (to date) been replicated, these studies highlight the ongoing challenges in applying $\delta^{30}Si$ approaches to down core reconstructions and reinforce the value of modern day, lake site-specific calibrations of the method (as per Panizzo et al. 2016).

2.3 Sample purification

Much effort is placed on diatom purification prior to isotope analysis (e.g., van Bennekom and van der Gaast 1976; Shemesh et al. 1995; Schleser et al. 2001; Morley et al. 2004; Rings et al. 2004; Lamb et al. 2005; Tyler et al. 2007; Brewer et al. 2008; Mackay et al. 2011) as high sample purity is required for $\delta^{18}O_{diatom}$ and $\delta^{30}Si_{diatom}$ analysis. This is because oxygen and silicon are both common elements in many other components found in lake sediments (including clay, silt, tephra and carbonates) and a high proportion of these can introduce significant isotopic offsets. $\delta^{13}C_{diatom}$ and $\delta^{15}N_{diatom}$ are usually measured on occluded (within the frustrule) organic matter and therefore external organic matter, a contaminant, is removed using chemical oxidation (for further information consult Robinson et al. (2004), Singer and Shemesh (1995) and references therein).

In most instances, standard chemical (oxidation) and physical separation approaches (sieving, heavy density liquids) work well for samples with a high proportion of diatom silica (>10 %). However, more complex and time consuming methods are required to clean relatively diatom poor (<10 %) material, where sample sizes are small or where the contaminant is similar in size and density to the diatom silica. These include SPLITT

(gravitational split-flow lateral-transport), micromanipulation, and chemical mass balance modelling. SPLITT is an approach similar to heavy density separation (Giddings 1985), whereby individual particles within a sample are separated under laminar flow of water on the basis of their density, size and shape (Schleser et al. 2001; Rings et al. 2004; Leng and Barker 2006). Micro-manipulation is an alternative approach: where a device is attached to an inverted microscope, with a micro-injector system used to extract individual non-diatom particles from a sample (Snelling et al. 2013). Although time-consuming this technique is routinely used in other fields (e.g., cryptotephra, Lane et al. 2014), and may be the only option, in some cases, to remove contaminants that are chemically and physically similar to diatoms. In instances where all other methods are unsuccessful in removing sample contaminants, mass balance chemical modelling can be applied. Here, whole-rock geochemistry and electron-optical imaging provides a method for the identification, quantification and subsequent removal of (e.g. via an offset correction factor) the effects of different types of contamination (Lamb et al. 2005; Brewer et al. 2008; Mackay et al. 2011). This approach can also work with multiple contaminants so long as they are well characterised (Wilson et al. 2014). Purity is routinely demonstrated in publications either visually (e.g., scanning electron microscopy) or quantitatively, via estimations of sample contamination <1 % (e.g., SiO_2:Al_2O_3 <1).

2.4 The hydrous layer and maturation of oxygen isotopes in biogenic silica

Biogenic silica has an amorphous structure containing Si-O-Si bonds, Si-OH bonds and crystallization water (Knauth and Epstein 1982). These oxygen-bearing compounds (–OH and H_2O) can exchange freely with their environmental lake water (Fig. 3), for example with sedimentary pore water during the burial of diatoms (Mopper and Garlick 1971; Kawabe 1978; Mikkelsen et al. 1978; Schmidt et al. 1997; Brandriss et al. 1998; Moschen et al. 2006) or with laboratory water used in diatom cleaning preparation techniques (Tyler et al. 2017). The hydrous layer must be removed prior to $\delta^{18}O$ measurements due to its ready exchangeability (most notable in modern diatoms), which makes it a complex mineral to analyse (Leng and Sloane 2008). Secondary processes, such as diagenesis, can also alter $\delta^{18}O_{diatom}$ due to the presence of this hydrous layer.

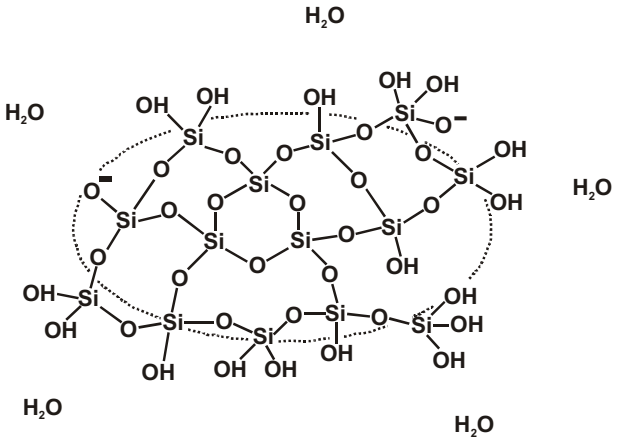


Fig. 3: *Schematic illustration of the nature of amorphous hydrated silica (from Leng and Marshall 2004)*

The influence of silica condensation on the isotope composition of sedimented opal, as a result of isotope exchange, has been described by Schmidt et al. (2001). Diatom silica ^{18}O enrichment is attributed to biogenic silica maturation (dehydroxylation i.e. reduction of Si–OH groups) following the removal of organic coatings (Moschen et al. 2006). Similarly, secondary processes are likely to affect sedimentary diatomaceous silica (especially the hydrous parts), although the vast proportion (c. 90%) of diatom valve oxygen is bound to silicon in SiO_4 tetrahedrons (forming the structurally bound oxygen) which should be more resistant to alteration (refer to reviews of Leng and Henderson, 2013; Swann and Leng, 2009). Furthermore, the absence of trends in $\delta^{18}O$ signatures through time would suggest progressive silica maturation does not occur and it is likely that there is a very slow progression of the maturation process after a fast initial phase of signal alteration. Therefore, some of the diatom $\delta^{18}O$ composition is acquired soon after the formation of biogenic silica, during early diagenesis in the water column and later during early sediment burial (Dodd and Sharp 2010). Interestingly, the conflating effects of temperature on $\delta^{18}O$ recorded by palaeo-diatom silica could be reduced or removed via maturation in deep lacustrine environments with nearly constant temperatures as re-equilibration of diatom silica occurs, thereby providing direct information on the $\delta^{18}O$ of the water (Dodd and Sharp 2010).

2.5 Methods and inter-laboratory calibrations

There are several techniques for the dehydration and release of O_2 from biogenic silica for $\delta^{18}O$ analysis. However, only one silica standard is universally available (NBS28 quartz) distributed by the IAEA, Vienna. Additional standards to calibrate the $\delta^{18}O$ values of biogenic silica were introduced through an inter-laboratory comparison (Chapligin et al. 2011) by eight participating laboratories using their individual bespoke methods and IRMS. The standard materials (diatoms, phytoliths and synthetically-produced hydrous silica) were analysed in accordance to a prescribed protocol. Despite procedural differences at each laboratory (controlled isotopic exchange, stepwise fluorination, inductive high-temperature carbon reduction and inert gas flow dehydration; Chapligin et al. 2011; Leng and Henderson 2013), all methods were in reasonable agreement, with a standard deviation (SD) range for $\delta^{18}O$ between 0.3 ‰ and 0.9 ‰.

There are several methods published for the liberation of $\delta^{30}Si$ from biogenic silica, these include both acid and alkaline dissolution/fusion, Si separation using cation exchange, selective co-precipitation, and gas-source versus plasma-ionization (high and low resolution) mass-spectrometric techniques (Reynolds et al. 2007). Three standards were used for a $\delta^{30}Si$ inter-laboratory comparison exercise using a variety of chemical preparation methods and mass spectrometric techniques. The standard reference materials used were IRMM-018 (a SiO_2 standard), Big-Batch and Diatomite (natural diatomites). All analyses were compared with the international Si standard NBS28 (RM8546) and were in reasonable agreement (within ±0.22 ‰ (1σ) for $\delta^{30}Si$) showing little statistical difference between the mean values obtained by each laboratory, with the notable exception of the IRMM-018. Overall, they concluded that all the methods have similar precision and differences are limited to 0.2 ‰ in mean $\delta^{30}Si$ values for a given sample between laboratories (or differences of 0.13 ‰ in mean $\delta^{29}Si$). On the basis of this study, the reference standard Diatomite, is routinely reported to demonstrate analytical precision (consensus value of +1.26 ‰ ± 0.2 ‰, 2 SD; Reynolds et al. 2007).

While there are no inter-calibration studies on $\delta^{30}Si_{DSi}$ in freshwaters, one was recently undertaken on marine waters, tackling challenges associated with the purification and instrumental precision of waters with low silicic acid concentrations (9 µmol L^{-1}) (Grasse et al. 2017). Overall consensus for high (113 µmol L^{-1}) and low concentration

seawaters was obtained between all laboratories (+1.25 ± 0.06 and +1.66 ± 0.13 ‰ respectively), although some small and significant differences between data were obtained, which the authors attribute to the complex pre-concentration methods (Triethylamine Molybdate; De la Rocha et al. 1996; MAGIC; Georg et al. 2006; or via Mg-induced co-precipitation with purified ammonia; Zhang et al. 2014) and purification steps involved, as well as instrument bias of the different laboratories (e.g., Neptune MC-ICP-MS, Nu Plasma MC-ICP-MS and MAT 252 IRMS) (Grasse et al. 2017).

Diatom silica occluded organic matter is mainly derived of pleuralins, silaffins and long chain polyamines. The $\delta^{13}C$ and $\delta^{15}N$ analysis of these compounds from diatoms is thought to be a better representation of the carbon and nitrogen cycle rather than bulk organic matter (Hecky et al. 1973; Kroger and Poulson 2008; Bridoux et al. 2010). There is no universally accepted method for $\delta^{13}C$ and $\delta^{15}N$ analysis of occluded organic matter in diatom silica (Leng and Swann 2010). These methods are more popular in palaeoceanography with few published studies in (palaeo)limnology (Webb et al. 2016). The advantages are: the C and N isotope composition within the diatom cell walls is not affected by post depositional degradation and therefore potentially preserves an unaltered signal of surface water conditions during diatom growth (Brenner et al. 1999; Ficken et al. 2000) and avoids the generally heterogeneous nature of SOM (Hurrell et al. 2011).

2.6 Future directions

Understanding the isotope composition of diatom silica lags behind work on carbonates by decades, probably because of the ongoing issues of contamination, the hydrous layer and associated maturation of diatom silica. Within the community, research continues in better understanding diatom species-specific fractionation effects for $\delta^{18}O_{diatom}$ and $\delta^{30}Si_{diatom}$. In particular, lacustrine case studies are needed because until now data on fractionation factors are predominantly derived from *in-vitro* and *in-situ* experiments of marine diatom strains. The potential for expanding $\delta^{30}Si_{diatom}$ applications in lacustrine systems has been highlighted here, however a solid understanding of contemporary source water and biogenic opal endmembers (e.g. seasonal variability) is essential in order

to best trace biogeochemical pathways (e.g. changes in DSi supply versus diatom utilisation). In the natural environment, open-system models have been applied to lakes to interpret diatom fractionation processes (Chen et al. 2012; Opfergelt et al. 2011; Panizzo et al. 2016, 2017; 2018b) although closed-system approaches have also been adopted to explain isotope evolution at certain sites (Cockerton et al. 2015). Modern-day calibration studies are needed to best define the most appropriate system and would best constrain any potential for a lake system to periodically shift to a closed-system approach (e.g. during extended periods of ice cover or lake stratification). Furthermore, changes in catchment chemical weathering rates on glacial-interglacial timescales will alter DSi fluxes to lake basins and regulate their isotope composition (e.g., Cockerton et al. 2015; Frings et al. 2016). The coupling of $\delta^{30}Si_{diatom}$ reconstructions with organic-bound $\delta^{15}N_{diatom}$ and $\delta^{13}C_{diatom}$, in addition to stable isotopes of different silicifiers (e.g., sponge spicules), could therefore be further explored as a means to independently constrain the supply of nutrients and their utilisation over such timescales.

3. Biogenic carbonates

The shells of ostracods and aquatic molluscs have often been used in palaeolimnological studies. Ostracods are small (generally microscopic) aquatic crustaceans that are common in lakes. They secrete carapaces made of two low-Mg calcite valves, which are often abundant and well preserved in lake sediments. Ostracods grow by moulting their shells, up to 8 times following hatching from eggs until maturity. Shell formation occurs rapidly, over the course of a few hours to days, and once the shell is formed there is no further addition of calcite (Holmes 1992). Aquatic molluscs, in contrast, are macroscopic and grow incrementally; they thus provide a more time-averaged record of their environment, although carbonate formation may be markedly seasonal (Leng et al. 1999; Leng and Lewis, 2016). Many mollusc species produce aragonitic shells although some may be composed of calcite. Ostracod and mollusc shells are regularly used as sources of carbonate for oxygen and carbon isotope analyses in palaeolimnological reconstructions (Holmes 1996; Holmes and Chivas 2002; Leng and Lewis, 2016). Charophytes are complex algae that are often abundant in shallow, alkaline, fresh to saline lakes (Schneider et al.

2015). Charophyte photosynthetic activity can promote the precipitation of calcium carbonate as encrustations around the thallus and associated with the female reproductive bodies known as gyrogonites, the two components being readily distinguished under a light microscope. Dense beds of charophytes can have significant impacts on the $\delta^{13}C$ of DIC within lakes as a result of bicarbonate utilization for photosynthesis (Pentecost et al. 2006). Whilst the carbonate deposits associated with charophytes are not strictly fossils in the same sense as ostracod or mollusc shells, the encrustations often make up a large component of the carbonate sediments formed in freshwater alkaline lakes (Soulié-Märsche et al. 2010), leading to the formation of so-called *Chara*-marl, and so are considered briefly here. Ostracods shells and *Chara* remains tend to be most abundant in lakes situated on carbonate rocks, or those that are in hydrologically-closed or near-closed basins.

The oxygen isotope composition of lacustrine carbonate is controlled by water temperature and water isotope composition, as for endogenic calcite (Leng and Marshall 2004), together with offsets from isotopic equilibrium, which are discussed further below. The carbon isotope composition of lacustrine carbonate is determined primarily by the carbon isotope composition of DIC: offsets from carbon isotope equilibrium appear to be negligible although, for reasons outlined below, they are difficult to assess.

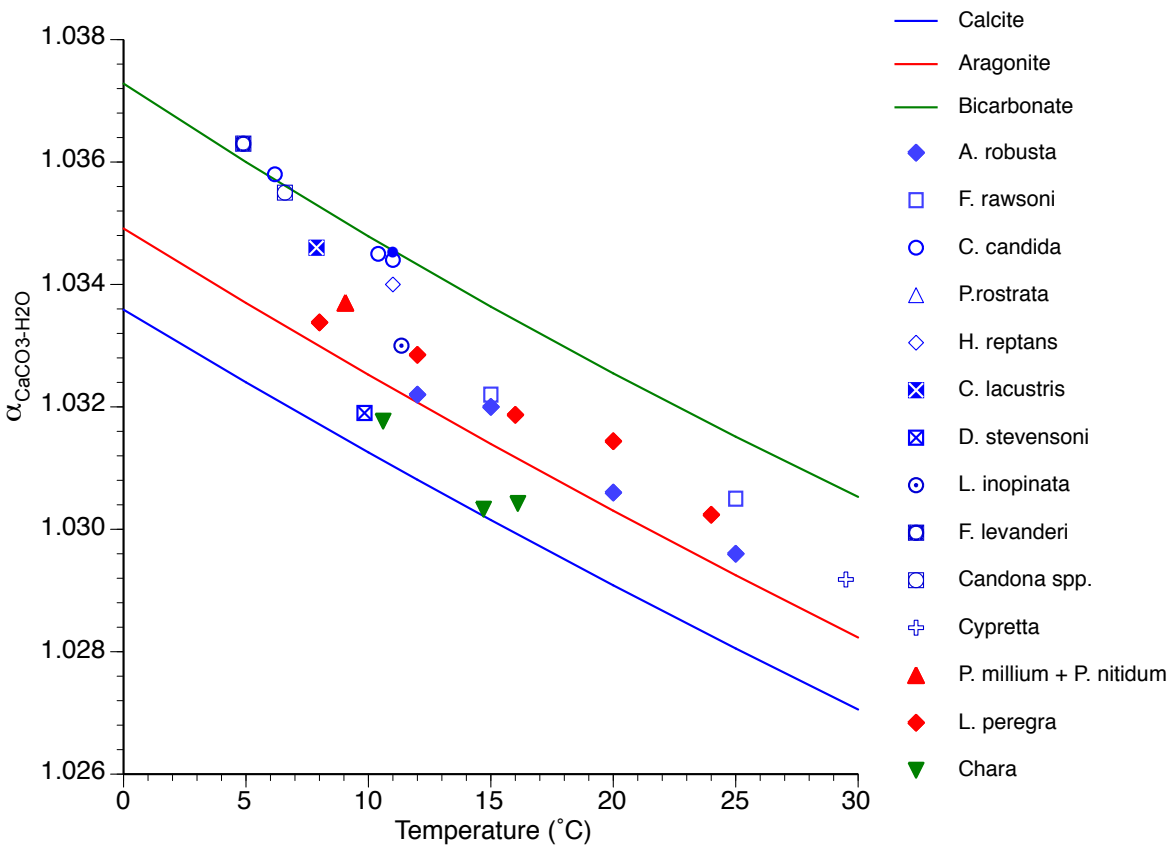
The interpretation of oxygen and carbon isotope signatures derived from lacustrine carbonate depends on the climatic and hydrological characteristics of the lake and its catchment, meaning that an understanding of the modern isotope systematics of the site is beneficial. Species vary in their preferred habitat within a lake and also in their life cycle, with some species calcifying in specific seasons (Decrouy et al. 2011). For molluscs, whereas many taxa are gill-breathing, the fact that some are lung breathing may have an impact on the isotope composition of their shells. Knowledge of an individual species' physiology, ecology and life cycle is therefore also important when interpreting isotope signatures (Shanahan et al. 2005).

There are a number of advantages to using shells as opposed to endogenic carbonate for stable isotope analyses. First, the use of shells assures that the carbonate was formed in water and avoids possible inclusion of detrital material with contrasting isotope composition into the sample analysed. Second, the analysis of shells means that constraints can be placed on the timing and location of carbonate formation within a lake.

462 Ostracods provide a specific temporal and spatial 'snapshot' of water conditions and
463 circumvent the problem of averaging. Moreover, the fact that some species have seasonal
464 preferences and inhabit defined zones (e.g., deep benthic versus littoral) within a lake
465 means that isotopic records derived from species with known life cycles and ecologies may
466 provide seasonal and habitat-specific information (von Grafenstein et al. 1999b). For
467 molluscs, whole-shell analyses provide a more time-averaged signal, since the shells grow
468 incrementally, although non-continuous growth throughout the year may mean that that
469 this signal is still seasonally biased (Leng and Lewis, 2016). Conversely, the isotope analysis
470 of individual growth increments, which is analytically feasible for some larger taxa, may
471 provide information about seasonal or inter-annual variability in environment (e.g., Leng
472 et al. 2009; Dettman et al. 1999), although this may not be possible when the short-term
473 changes in temperature or the isotope composition of water/DIC composition are small
474 (Shanahan et al. 2005). Thirdly, analysis of shells provides some certainty over the
475 mineralogy of the material being analysed. For ostracods, it is low-Mg calcite; for molluscs,
476 often aragonite. Because aragonite is thermodynamically unstable and recrystallization
477 leads to the 'resetting' of the isotopic signature (Leng and Marshall, 2004), it is important
478 to assess the degree of preservation of aragonitic mollusc shells, for example using X-ray
479 diffraction to confirm the presence of aragonite. These advantages also bring some
480 problems, however. The time- and space-specific character of an ostracod isotope
481 signature may not record the 'average' conditions within a lake that are generally required
482 in palaeolimnological reconstructions. Furthermore, because the calcification of ostracod
483 and mollusc shells is under strong biological mediation, isotopic fractionation may not
484 conform to expectations derived from investigations of inorganic carbonate. For
485 charophytes, different isotopic signatures may be recorded depending on where on the
486 plant the carbonate formed (Pentecost et al. 2006). In short, care is required in the
487 interpretation of such signatures.
488
489 *3.1 Oxygen and carbon isotope records from lakes*
490
491 Although the fundamental controls on isotope composition of carbonate are well
492 understood and quantifiable in some instances, the palaeoclimatic and
493 palaeoenvironmental interpretation of isotope records from lake sediments depends

494 strongly on the characteristics of individual lakes including their climatic setting, depth,
495 volume, hydrology, aquatic vegetation and catchment properties (Holmes 1996). Oxygen
496 isotope values derived from ostracod or mollusc shells, or marl, have been used in
497 reconstructions of air temperature and the oxygen isotope composition of rainfall (von
498 Grafenstein 2002; von Grafenstein et al. 1999a), effective moisture (Hodell et al. 1991;
499 Street-Perrott et al. 2000; Holmes et al. 2010; Hodell et al. 1995), meltwater influx
500 (Dettman et al. 1995) and changes in river routing within the lake's catchment (Schwalb et
501 al. 1994). Carbon isotopes are often more difficult to interpret but have been used to
502 reconstruct lake/catchment carbon cycling, productivity, and methanogenesis (Bridgwater
503 et al. 1999; Anadón et al. 2006; Li and Liu 2014; Schwalb et al. 2013), although many
504 studies report only the oxygen isotope results (e.g., Hodell et al. 1991; von Grafenstein et
505 al. 1999a; Holmes et al. 2010; Hodell et al. 1995).
506
507 *3.2 Disequilibrium*
508
509 Despite early suggestions that ostracod calcite is precipitated in isotopic equilibrium with
510 the host water (Durazzi 1977, albeit for marine taxa) it is now well established that
511 significant offsets exist, especially for oxygen. Similar offsets have been shown for some
512 mollusc taxa (e.g., White et al. 1999; Shanahan et al. 2005). Knowledge of such offsets is
513 needed if ostracod or mollusc shell isotope values are to be used in quantitative
514 environmental reconstruction (von Grafenstein et al. 1999a; Decrouy 2012; Devriendt et
515 al. 2017). Moreover, offsets have provided insights into calcification mechanisms (Keatings
516 et al. 2002; Shanahan et al. 2005; Decrouy 2012; Devriendt et al. 2017), although the
517 mechanism behind the observed offsets from oxygen and carbon isotope equilibrium
518 remains incompletely understood. Charophyte isotope records are potentially complicated
519 by differences in isotopic signatures between stem encrustations and calcified remains of
520 gyrogonites, which also vary depending on the strength of water flow (Andrews et al.
521 2004; Pentecost et al. 2006).
522
523 *3.2.1 Oxygen*
524

525 Evidence from field collections of ostracods under closely monitored conditions (e.g., von
526 Grafenstein et al. 1999b; Keatings et al. 2002a; Decrouy et al. 2011) as well as *in vitro*
527 cultures (Xia et al. 1997a; Chivas et al. 2002) have shown that their shells are precipitated
528 out of oxygen isotope equilibrium with the host water (Fig. 4). The offsets from equilibrium
529 are almost invariably positive, up to +3 ‰ or more. Several investigations have shown that
530 the magnitude of the offset varies taxonomically, with members of the same genus, or
531 even sub-family or family, sharing similar offset values (see table 10.1 in Decrouy 2012).
532 Adults and juveniles and males and females of the same species usually show offsets of
533 the same magnitude (Chivas et al. 2002; Decrouy 2012; von Grafenstein et al. 1999b). The
534 two culturing studies cited above suggest that offsets are greater at higher temperature.
535 Decrouy et al. (2011) have demonstrated that there are some differences in the magnitude
536 of the vital offset *within* taxa from different localities, suggesting that water chemistry, in
537 addition to taxonomy, may also play a role. Devriendt et al. (2017) have confirmed these
538 observations based on a comprehensive meta-analysis of studies undertaken over a very
539 large range of water types, and have argued that they can be explained by a carbonate ion
540 effect. At high pH, a greater proportion of the DIC is present as the CO_3^{2-} ion rather than as
541 HCO_3^-, which dominates in lower pH waters: the degree of fractionation between water
542 and the CO_3^{2-} ion is less than that with HCO_3^-, and since ostracod shells are formed from
543 DIC, they will have lower $\delta^{18}O$ values in higher pH waters (Devriendt et al. 2017). Positive
544 offsets from oxygen isotope equilibrium have been observed in some mollusc taxa (White
545 et al. 1999; Shanahan et al. 2005) although some species appear to precipitate their shells
546 in isotopic equilibrium (Leng et al. 1999).
547



548
549

550 **Fig. 4**. Fractionation factors between selected ostracod and mollusc species and water.
551 Fractionation between bicarbonate (Beck et al. 2005), synthetic calcite (Kim and O'Neil
552 1997) and synthetic aragonite (Kim et al. 2007) is also shown. Key to taxa and sources: *A.*
553 *robusta – Australocypris robusta* (Chivas et al. 2002): *F. rawsoni – Fabaeformiscandona*
554 *rawsoni* (Xia et al. 1997a): *C. candida – Candona candida* (Keatings, 1999; von Grafenstein
555 et al. 1999b): *P. rostrata – Pseudocandona rostrata: H reptans – Herpetocypris reptans*
556 (Keatings et al. 2002): *C. lacustris – Cytherissa lacustris: D. stevensoni – Darwinula*
557 *stevensoni: L. inopinata – Limnocythere inopinata: F. levanderia – Fabaeformiscandona*
558 *levanderi: Candona* spp. (von Grafenstein et al. 1999b): *C. brevisaepta - Cypretta*
559 *brevisaepta* (J. A. Holmes, unpublished): *P. millium + P. nitidum - Pisidium millium +*
560 *Pisidium nitidum* (Keatings 1999): *L. peregra - Lymnaea peregra* (White et al. 1999): *Chara*
561 – charophyte stem encrustations (Andrews et al. 2004).
562
563
564 The offsets from isotopic equilibrium must be corrected for if calculations of past water
565 temperature or past water isotope composition are to be derived from the oxygen isotope

values of ostracod shells, otherwise significant errors may arise (von Grafenstein 2002; Devriendt et al. 2017).

3.2.2 Carbon

Assessing offsets from carbon isotope equilibrium in shells is more difficult because the $\delta^{13}C$ composition of DIC, the source of carbon for calcification, varies significantly at the micro scale meaning that there may be a weak relationship between the $\delta^{13}C$ at the site of calcification and that within the main lake, which is the value that is typically measured. This is much more so than for oxygen isotopes, which tend to be relatively more homogenous within a lake. For example, the DIC within sediment pores may be ^{13}C-depleted as a result of mineralisation of SOM. In methane-producing lakes, however, the formation of ^{13}C-enriched co-genetic CO_2 may have the opposite effect (Durand et al. 1984), which may lead to very high $\delta^{13}C$ values in the shells of infaunal ostracods (Bridgwater et al. 1999). Interestingly, the opposite (very low ostracod $\delta^{13}C$ values) can be observed when methane is oxidized close to the sediment-water interface and ^{13}C-depleted carbon is added to pore water DIC that is then available for incorporation into ostracod shells (Schwalb et al. 2013). Close to the leaves of submerged macrophytes that utilise HCO_3^-, DIC may also be ^{13}C-enriched as a result of preferential uptake of ^{12}C by plants for photosynthesis (Kelts and Talbot 1990). Hence, organisms co-existing in different micro-environments within a lake, or calcifying at different seasons, may have markedly contrasting $\delta^{13}C$ values (Bridgwater et al. 1999). For molluscs, contrasts can be seen between gill-breathing and lung-breathing species, which may relate to differences in physiology or life history (Shanahan et al. 2005). Variations in $\delta^{13}C$ values between different taxa are therefore often regarded as habitat effects rather than offsets from equilibrium *sensu stricto* (Heaton et al. 1995).

3.3 Sample preparation and shell cleaning

Shells destined for isotope analysis need to be well preserved (cf. section 3.4, below) and free from detrital contamination. Various methods have been used to remove

597 contamination from ostracod shells, especially organic material, which may interfere with
598 isotope determinations. However, the potential of these techniques to modify the primary
599 isotope composition of the shell as well as to remove any contaminant has not been fully
600 assessed. Five techniques have been used to remove contaminants, namely simple manual
601 cleaning with a fine paint brush and (usually) methanol, roasting *in vacuo*, heating in an
602 oxygen plasma and chemical oxidation with sodium hypochlorite (clorox) or hydrogen
603 peroxide (Keatings et al. 2006). For each technique, a variety of conditions (treatment
604 times, temperatures, reagents strengths) has been used. Keatings et al. (2006) compared
605 treated and untreated (manually cleaned) valves of the same carapace of late Pleistocene
606 lacustrine ostracods and showed that in most instances, the mean impact of the cleaning
607 techniques over manual cleaning was small, although treatment other than simple
608 mechanical cleaning typically increased within-sample variability. Roberts et al. (2018)
609 confirmed that treatment can cause changes in both oxygen and carbon isotope values,
610 but concluded that hydrogen peroxide treatment would be preferable if treatment were
611 required, since this reagent does not appear to have a significant impact on the isotope
612 composition. Despite the relatively small changes imparted by each of the treatment
613 methods, the increase in variability that may result suggests that anything other than
614 simple mechanical cleaning should only be undertaken if absolutely necessary (i.e. to
615 remove detrital contamination that could not otherwise be eliminated). The potential
616 impact on variability needs to be borne in mind if single-shell samples are analysed
617 (section 3.5) and, moreover, implications of cleaning for trace elements must be taken into
618 account in cases where 'tandem' isotope and trace-element determinations are
619 undertaken on the same shells, as in Chivas et al. (1993) and Xia et al. (1997b). Similar
620 methods have been employed for the removal of organic material from mollusc shells
621 destined for isotope analyses: modifications to techniques involving heating and or
622 grinding are required in order to prevent inversion of aragonite to calcite (White et al.
623 1999; Dettman et al. 1999). Marl samples, including those dominated by charophyte
624 carbonate, are commonly treated by heating or chemical oxidation to remove organic
625 matter (e.g., Apolinarska and Hammarlund 2009).
626
627 *3.4 Signal preservation and diagenetic alteration*
628

An advantage of analysing shell calcite over endogenic calcite is that diagenetic alteration can usually be detected. For ostracods, early diagenetic alteration can usually be detected by the presence of etching, opaque rather than transparent or translucent appearance, and obscured or altered surface ornament (Keatings et al. 2002b). Ideally, shells showing signs of such alteration should be excluded from analyses. However, in some cases pristine material may not be preserved in sediment sequences, meaning that ostracod specimens that have undergone some degree of alteration need to be analysed. Limited attention has been paid to the impacts of early diagenesis on isotope signatures. Keatings et al. (2002b) found no clear evidence of an impact of preservation on the oxygen isotope signature of late Pleistocene lacustrine ostracods from a Jamaican hardwater lake: evidence for the absence of an impact on carbon isotopes was less conclusive. The potential for alteration increases for older material. Bennett et al. (2011) showed that isotope values, especially for oxygen, of Carboniferous non-marine ostracods from Scotland primarily reflected the degree of diagenetic alteration rather than a palaeoenvironmental signature and warned of the potential for cryptic diagenesis to alter the shells and their isotope values. However, Bajpai et al. (2013) derived plausible palaeoenvironmental signatures from both the oxygen and carbon isotope values from Late Cretaceous non-marine shells from peninsular India, some pristine and others showing signs of alteration. For aragonitic mollusc shells, recrystallization to calcite can effectively 'reset' the isotopic signature: mineralogical assessment of shells destined for isotope analyses is therefore often undertaken (e.g., Leng et al. 1999).

3.5 Measurement of multiple versus single-shell samples

Adult ostracod shells weigh anything from a few micrograms for a single shell of small or weakly calcified taxa, such as species belonging to the genera *Limnocythere, Darwinula* and *Cypria,* to several hundred micrograms for large and well-calcified taxa belonging to genera such as *Sclerocypris*, although many taxa have shells in the tens of micrograms range (J. A. Holmes, unpublished). Juveniles are not only smaller than adults of the same species but also less well calcified, meaning that they weigh substantially less. Duel-inlet mass spectrometers are therefore able to measure isotope ratios for single shells of adults of many ostracod species (Chivas et al. 1993). Most stratigraphic studies of ostracods have

661 used single, multiple-shell, monospecific samples, where the number of shells analysed is
662 determined not by the material requirements of the instrument, but by the need to obtain
663 a sample that is representative of 'average' conditions within the lake and over the time
664 interval represented by the increment of sediment from which the shells are recovered.
665 However, there is increasing interest in undertaking analyses of multiple single shells at
666 individual stratigraphic intervals in a lake-sediment sequence. Such an approach has the
667 advantage of providing information about short-term variability within the lake as well as
668 allowing any analytical outliers to be identified, although it does of course increase
669 analytical costs. Pilot studies of large numbers of shells from a few intervals can be used to
670 determine optimum sample size (Escobar et al. 2010). Such an approach has been used to
671 assess short-term changes in effective moisture in NW India (Dixit et al. 2015) and in
672 Mayan lakes from Central Mexico (Escobar et al. 2010), and variations in meltwater input
673 in Lake Huron (Dettman et al. 1995). Despite the fact that a single mollusc shell typically
674 integrates a longer time interval than a single ostracod shell, and would typically provide
675 sufficient material for an isotope determination, similar issues have arisen with the isotope
676 analysis of single mollusc shells, especially for species that have short life spans
677 (Apolinarska et al. 2015).
678
679 *3.6 Other isotopes and future developments*
680
681 Further work is needed to compare oxygen-isotope signatures in different minerals or
682 biomolecules. Theoretically, comparison of signatures in calcite or biogenic silica, in which
683 oxygen-isotope fractionation is temperature dependent (section 3.2.1), with those in
684 materials such as cellulose or chitin, which show temperature-independent fractionation
685 (section 4.2.2), should lead to quantitative reconstructions of water temperature.
686 However, existing studies show that this does not always work in practice. For example,
687 Rozanski et al. (2010) attributed unrealistically-high estimates of temperature from
688 coupled calcite and cellulose oxygen isotope analyses for an eastern European lake to
689 kinetic effects during rapid carbonate formation. Comparison of $\delta^{18}O$ values of diatom
690 silica and endogenic calcite in Lake Pinarbasi, Turkey, show contrasting trends in spite of
691 their mutual dependence on the water $\delta^{18}O$ and lake-water temperature. The most likely
692 explanation for this divergence is difference in seasonality of biological productivity

mediated by the strongly continental climate of the Anatolian plateau. The endogenic calcite $\delta^{18}O$ is thought to be temporally limited to a few summer months and the diatom silica $\delta^{18}O$ provides seasonally-specific water isotope composition in the spring and autumn and, at least in the record in question, captures periods of heavy snow through the spring thaw (Leng et al. 2001). Further investigations are needed to indicate the circumstances in which lake water temperature can be reconstructed from $\delta^{18}O$ offsets between calcareous/siliceous and organic remains.

Ostracod shells have been used in analyses of other isotopes in a limited number of studies, including $^{87}Sr/^{86}Sr$ (Janz and Vennemann 2005; Holmes et al. 2007) and U-series (for dating) (Bischoff et al. 1998). The chitinous shell linings, which remain after dissolving the calcium carbonate with a 5 % HCl solution, can be used for measuring $\delta^{13}C$ and $\delta^{15}N$ to provide insights in the position of ostracods in the aquatic food web (Fig. 6). Future developments require additional calibration work to improve understanding of the mechanisms behind vital offsets and their quantification for additional species, especially for oxygen isotopes. Future work may also lead to the use of 'novel' isotope systems like Ca (Oehlerich et al. 2015) and clumped isotope analysis (Mering 2015) on biogenic carbonates.

4. Organic remains of aquatic plants and animals

Many organisms living in lakes produce organic structures that are remarkably robust to degradation once buried in lake sediments. These remains are composed (partly) of flexible polymers that are difficult to break down including chitin, keratin, lignin, collagen, and cellulose. These polymers are bonded or otherwise associated with proteins, calcium, carbonate, or other compounds to provide strength (Leschine 1995; Nation 2002; Kornilłowicz-Kowalska and Bohacz 2011; Jex et al. 2014). We will focus on identifiable organic remains of some of these organisms, including invertebrates, aquatic macrophytes and fish. We will review how taxon-specific analysis of stable isotopes on these remains are used in palaeolimnological studies to understand changes in carbon cycling, food web structure, eutrophication, hydrology and climate.

724 *4.1 Chitinous invertebrate remains*

725

726 Invertebrates are ubiquitous in lakes and their exoskeleton fragments and resting eggs are
727 preserved in lake sediments for tens of thousands of years (e.g., Engels et al. 2010).
728 Remains of a wide range of invertebrates can be identified using microscopy and the
729 composition of fossil invertebrate assemblages can be indicative of ecological and
730 environmental conditions (Frey 1964). The exoskeleton fragments from various insect
731 orders (e.g., Coleoptera, Diptera, Ephemeroptera, Trichoptera), crustaceans (e.g.,
732 Cladocera, Ostracoda), and mites (Oribatida) are commonly found in lake sediments, as
733 are the resting stages of Cladocera and moss animals (Bryozoa).

734 Robust organic remains of many invertebrate groups consist predominantly of
735 chitin cross-linked with protein (Stankiewicz et al. 1996; 1998). In suitable conditions they
736 can remain relatively unchanged over thousands of years (Miller 1991; Verbruggen et al.
737 2010a). Isotope analyses of these remains can therefore be used to reconstruct past
738 changes in the isotope composition of the formerly living organisms (Wooller et al. 2004;
739 Verbruggen et al. 2011). The chemical composition of these remains means that they
740 contain relatively large amounts of hydrogen, carbon, oxygen, some nitrogen, and to a
741 lesser extent sulphur. Isolating the small remains from sediments and preparing them for
742 stable isotope analysis can be time consuming, but efficient protocols are available (Wang
743 et al. 2008), which can be adapted depending on the chemical pre-treatment steps
744 required (e.g., Heiri et al. 2012). Using a 200-micrometre mesh (instead of the commonly
745 used 90 to 100-micrometre mesh) can decrease processing time by 30 to 58 % because of
746 the disproportionally large gain in sample mass from larger fragments (van Hardenbroek
747 et al. 2010a). Using large mesh sizes may cause the loss of smaller fragments, which could
748 lead to systematic bias against particular taxa and body parts – and subsequently against
749 certain habitats, or feeding habits.

750 The amount of sample required for stable isotope analysis depends on the
751 chemical element being analysed, the relative abundance of the element in the
752 invertebrate remains as well as on the analytical equipment used. The carbon content of
753 chitinous invertebrate remains is relatively high, followed by oxygen, nitrogen, and
754 hydrogen (Table 1). The elemental composition (% by weight) of invertebrate remains is
755 generally 40-50 %C, 25-30 %O, 7-10 %N, and 5-6 %H. Interestingly, the nitrogen content of

Bryozoa remains (12.7 ± 1.7 %N) is consistently higher than that of other invertebrates. A lower carbon and nitrogen content has been observed in the carapaces of Cladocera (18.3 ± 2.7 %C and 2.8 ± 0.7 %N, respectively). At present it is unclear what causes the differences between taxa and different types of remains; further analysis of the chemical composition of the remains is required.

Table 1: *Elemental composition (% by weight) with 1 standard deviation (SD) of invertebrate remains in surface and down core sediments (n = number of data points). Data from van Hardenbroek et al. (2010b, 2012, 2013b, 2014, 2018, van Hardenbroek, Heiri and Wooller unpublished data, Perga unpublished data).*

	%C	SD	n	%N	SD	n
Chironomidae head capsules	46.4	5.4	247	8.6	1.3	247
Cladocera ephippia	44.4	5.4	380	8.8	1.8	379
Cladocera carapaces	18.3	2.7	28	2.8	0.7	28
Bryozoa statoblasts	45.7	3.9	168	12.7	1.7	167
Ephemeroptera mandibles	39.0	10.0	19	7.5	3.0	19
Ostracoda shell lining	41.7	7.6	11	10.4	2.1	11
Trichoptera frontoclypeus/mandible)	42.5	9.5	20	8.1	1.7	20
Sialis (frontoclypeus/mandible)	47.0	3.7	13	10.0	1.2	13
Chaoborus (mandible)	45.3	7.4	22	8.8	1.4	22
Coleoptera (elytron)	43.1	2.8	14	8.2	1.0	14

	%O	SD	n	%H	SD	n
Chironomidae head capsules	29.6	1.8	27	5.6	0.3	27
Cladocera ephippia	28.6	2.6	77	5.5	0.5	77
Bryozoa statoblasts	25.5	3.9	48	5.2	1.1	48

For $\delta^{13}C$ and $\delta^{15}N$ analyses an Elemental Analyser IRMS (EA-IRMS) setup is commonly used, whereas $\delta^{18}O$ and δD analysis often rely on a Thermal Conversion / Elemental Analyzer (TC/EA)-IRMS setup. With this equipment, analyses are typically based on tens to hundreds of invertebrate remains. A reduction of the sample size is possible using Laser Ablation nano Combustion Gas Chromatography (LA/nC/GC)-IRMS (Schilder et al. 2018) or Spooling-Wire Microcombustion (SWiM)-IRMS (Zhao et al. 2017), allowing $\delta^{13}C$ analysis of individual invertebrate remains. The Supplementary Table provides an overview of mean weight of individual remains, to allow better estimates of the minimum number of remains required for stable isotope measurements. The mean weight of

777 remains in down core samples is seemingly smaller than that of remains in surface
778 sediments (Supplementary Table). Material from surface and down core samples
779 originates from different sites and includes different species, making it difficult to make
780 conclusive statements about differences between remains in surface and down core
781 samples in the Supplementary Table without more work on the taphonomy of
782 invertebrate remains.
783
784 *4.2 Dietary and environmental isotopes reflected by chitinous remains*
785
786 Analyses of the isotopic offsets between aquatic invertebrates, their food and their
787 chitinous remains focussed on different organism groups such as marine crustaceans,
788 freshwater crustaceans and aquatic insects. Much of this work started in the 1980s mainly
789 with marine decapods (Schimmelmann and DeNiro 1986a, b; Schimmelmann 2011).
790 However, results based on marine crustaceans may not be representative for chitinous
791 microfossils of many freshwater invertebrate taxa. This is because calcium carbonate
792 forms an important component of the exoskeleton of many crustaceans (Greenaway
793 1985; Willis 1999), but calcium may be less relevant in some planktonic freshwater
794 crustaceans (Jeziorski and Yan, 2006) and the cuticles of other freshwater invertebrates
795 groups (e.g., aquatic insects, Willis 1999) do not contain calcium carbonate. Many
796 experimental studies assessing the relationships between isotope sources and isotopic
797 contents of invertebrates have selectively isolated and analysed chitin or chitin-derived
798 compounds (Schimmelmann and DeNiro 1986a, b; Schimmelmann 2011) and this needs to
799 be considered when using this information for interpreting stable isotope studies based on
800 whole chitinous remains (including proteins, lipids, etc.) of aquatic invertebrates.
801
802 *4.2.1 Carbon and nitrogen isotopes*
803
804 Carbon and nitrogen in chitin of heterotrophic organisms originates from their diet
805 (DeNiro and Epstein 1978, 1981; Schimmelmann 2011). Metabolic activities and life stage
806 may also influence the isotope composition for some elements and organism groups
807 (Schimmelmann 2011). Chitinous remains of aquatic invertebrates in lake sediments do
808 not only consist of chitin but include other organic components such as proteins or lipids

809 (e.g. Verbruggen et al. 2010a). The exact composition of fossilising exoskeleton parts may
810 differ between organism groups but also within the same organism, between different
811 types of structures (e.g., Jeziorski et al. 2008). Ideally, the relationship between the
812 isotope composition of diet, bulk tissue and fossilizing structures is therefore established
813 for each organism group and isotope pair of interest before developing down core isotope
814 records. Experiments and environmental measurements constraining these relationships
815 for C and N are presently available for planktonic cladocerans, chironomid larvae and
816 bryozoans. Laboratory experiments with *Chironomus* (Chironomidae) larvae showed that
817 there are only very minor offsets (reported here as Δ values) between the $\delta^{13}C$ values of
818 the food and chironomid biomass ($\Delta^{13}C$ of ca. –1.5 to +1.5 ‰ in most cases; Goedkoop et
819 al. 2006; Wang et al. 2009; Heiri et al. 2012; Frossard et al. 2013). In contrast, observed
820 offsets between foods and body tissue for $\delta^{15}N$ were more pronounced ($\Delta^{15}N$ –1.5 to +3.4
821 ‰; Goedkoop et al. 2006; Wang et al. 2009; Heiri et al. 2012). For fourth instar larvae,
822 head capsule $\delta^{13}C$ and $\delta^{15}N$ values were very similar to but on average ca. 1 ‰ lower than
823 the isotope composition of the remaining larval tissue (Heiri et al. 2012; Frossard et al.
824 2013).
825 The $\delta^{13}C$ values of the exoskeleton of the cladocerans *Daphnia* and *Bosmina* were
826 shown to be very similar to the values for the bodies: offsets are –0.8 ± 0.2 ‰ and 1.4 ±
827 0.7 ‰ for *Daphnia* and *Bosmina*, respectively (Perga 2010). Lower $\delta^{15}N$ values for *Daphnia*
828 exoskeletons were observed compared with the whole bodies (by as much as 7.9 ± 0.5 ‰;
829 Perga (2010). This offset was very consistent along a gradient of *Daphnia* $\delta^{15}N$ values,
830 however, and a strong relationship between $\delta^{15}N$ values of *Daphnia* exoskeletons and the
831 entire organisms was found. The fossilizing sheaths of resting eggs (ephippia) of *Daphnia*
832 *pulicaria* are very similar in their isotope composition to the *Daphnia* they originated from,
833 with mean offsets of +0.2 and –1.5 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively (Schilder et al.
834 2015a). Experimental results indicate that the $\delta^{13}C$ values of *Daphnia* are also close to the
835 isotope composition of the available food, with offsets between food and *Daphnia* of 0.5 ±
836 0.3 ‰ ($\Delta^{13}C$) and +3.4 ± 0.3 ‰ ($\Delta^{15}N$) and between food and ephippia of 0.7 ± 0.2 ‰
837 ($\Delta^{13}C$) and 1.8 ± 0.4 ‰ ($\Delta^{15}N$) (Schilder et al. 2015a).
838 Experimental results indicate that $\delta^{13}C$ values of chitinous resting stages
839 (statoblasts) of the bryozoans *Plumatella* and *Lophopus* are similar as those of both their

food and soft tissue parts of these organisms: statoblasts have between 0 to 1.7 ‰ lower $\delta^{13}C$ values than the living colonies (van Hardenbroek et al. 2016). In a survey of lakes in Northwest and Central Europe, median $\delta^{13}C$ values of bryozoan colonies of *Cristatella mucedo*, *Pectinatella magnifica* and *Plumatella* were observed to be closely related with the isotope composition of the statoblasts for most sites (offsets generally between –3 to +4.5 ‰; van Hardenbroek et al. 2016). The seemingly large variability of offsets recorded in the above study suggests that, in field situations, food sources can change between the formation of fossilizing structures and the formation of new soft tissue. Chitinous fossilising invertebrate structures are formed within a period of 1-2 weeks (Candy and Kilby 1962; Nation 2002) and their stable isotope values will largely represent food sources in this period and shortly before. In the limited studies comparing living invertebrates and their fossilising structures, the $\delta^{13}C$ values of fossilising structures are within one standard deviation of the mean $\delta^{13}C$ values of living chironomids and cladocerans (Morlock et al. 2017; Schilder et al. 2017). This strongly suggests that $\delta^{13}C$ values of samples of fossilising structures are representative for mean $\delta^{13}C$ values of the invertebrates at longer (annual to decadal) time scales. The large number of sedimentary remains required for regular EA-IRMS measurements will not normally allow detection of short-term (weekly to seasonal) variability in stable isotope values found in living invertebrates (but see Zhao et al. 2017 and Schilder et al. 2018). Future studies should quantify the impact of tissue turnover time and the sources of material incorporated during the formation of chitinous structures on the stable isotope composition of these remains. This would be similar to stable isotope studies on turnover time of different tissues in other aquatic organisms such as fish (e.g., Pinnegar and Polunin 1999; Hanisch et al. 2010).

When quantifying offsets between soft tissues and fossilising structures, controlled laboratory experiments give more consistent results than field studies. Culturing experiments have demonstrated that variability in these offsets is generally low: 0.2–0.9 ‰ for $\delta^{13}C$ and 0.3–0.5 ‰ for $\delta^{15}N$ (Perga 2010; Heiri et al. 2012; Frossard et al. 2013; Schilder et al. 2015a). Information from such controlled experiments is crucial as it demonstrates that variability > 0.9 ‰ ($\delta^{13}C$) and > 0.5 ‰ ($\delta^{15}N$) is most likely related to environmental processes rather than natural variability in offsets between soft tissues and fossilising structures.

871

872 *4.2.2 Oxygen and hydrogen isotopes*

873

874 Less information, compared with C and N, is available that documents the relationship
875 between the stable O and H isotope composition of aquatic invertebrates, their fossilizing
876 remains, and their diet and source water. Available evidence from lakes indicates that
877 most of the O in aquatic invertebrates originates from lake water (56 to 84 %; Wang et al.
878 2009; Nielson and Bowen 2010; Soto et al. 2013; Schilder 2015a) with smaller
879 contributions from dietary O. The exact proportions vary between the available
880 experiments and therefore may also vary between different organism groups, tissue types
881 and chemical compounds. Schilder et al. (2015a) demonstrated that changing the oxygen
882 isotope composition of the water influenced $\delta^{18}O$ values of *Daphnia* ephippia, which were
883 on average 0.8 ± 0.4 ‰ lower than for the entire *Daphnia*. Limited evidence from this
884 culturing experiment also suggested no significant temperature-dependent fractionation
885 between $\delta^{18}O$ values of water and $\delta^{18}O$ values of *Daphnia* tissues (Schilder et al. 2015a). A
886 close relationship between $\delta^{18}O$ of aquatic invertebrate remains and lake water has also
887 been confirmed by environmental surveys demonstrating a high correlation between $\delta^{18}O$
888 values of chitinous invertebrate remains in lake sediments and lake water $\delta^{18}O$ (Fig. 5 and
889 Wooller et al. 2004; Verbruggen et al. 2011; Lombino 2014; Mayr et al. 2015; Chang et al.
890 2016, 2017; Lasher et al. 2017).

891 The greatest proportion of H in freshwater invertebrate biomass and chitinous
892 remains apparently originates from the diet of these organisms (Solomon et al. 2009;
893 Wang et al. 2009; Soto et al. 2013; Belle et al. 2015b). Based on controlled experiments,
894 estimates of the amount of H in freshwater invertebrate tissue originating from water
895 range from 20 to 47 % (Solomon et al. 2009; Wang et al. 2009; Soto et al. 2013). Belle et al.
896 (2015b) reported offsets between chironomid larval bodies and fossilizing head capsules of
897 –24 ± 7 ‰. In a field study of bryozoan colonies in 23 lakes, median δD values of the
898 fossilizing statoblasts of the colonies were relatively close to the median δD values of their
899 soft tissues for *Plumatella* (observed offsets –34 to +16 ‰) but distinctly more negative (–
900 75 to –16 ‰) for *Cristatella* (van Hardenbroek et al. 2016). This finding might be related to
901 differences in food type, mobility and seasonality of resting stage production between the

902 examined bryozoan groups. The H-isotope composition of many food types available in
903 lakes (e.g., algae and organisms feeding on these) will be related to the isotope
904 composition of the lake water. Therefore, δD values of aquatic invertebrate remains will in
905 many cases be related to lake water δD values, even if food sources play a major role in
906 determining aquatic invertebrate δD values (see, e.g., van Hardenbroek et al. 2016).
907 The observed variability in offsets between soft tissues and fossilising structures can
908 be as large as 50‰ for δD in field studies due to natural variability. Controlled laboratory
909 experiments are required to evaluate what part of this variability is due to changes in the
910 environment (food supply, temperature, isotope composition of lake water) and what part
911 is the inherent natural variability in the offset between soft tissues and fossilising remains.
912 Limited data from controlled studies suggests that natural variability in this offset may be
913 as small as ca. 0.4‰ for δ^{18}O (Schilder et al. 2015a) and ca. 7% for δD (Belle et al. 2015b).
914 This implies that variations > 0.4 ‰ for δ^{18}O and > 7 ‰ for δD will in many instances be
915 related to environmental processes and could be interpreted in palaeoenvironmental
916 records.
917
918 *4.3 Effects of chemical treatment and taphonomy*
919
920 Chitinous invertebrate remains for isotope analysis are isolated from sediments by a
921 combination of mechanical and chemical treatments (van Hardenbroek et al. 2010a).
922 Sediments are usually first chemically treated by exposing the samples in a 5 to 10 % KOH
923 solution to facilitate sieving and eliminate easily degradable organic material. For C and O
924 isotopic analysis it is essential that carbonates are eliminated prior to isotope analysis by
925 exposing the samples to acids (e.g., low concentration HCl solution or fumigation
926 Verbruggen et al. 2010a; Heiri et al. 2012; Belle et al. 2014) or a buffered NH_4Cl solution
927 (Verbruggen et al. 2010b). Commonly used chemical pre-treatment methods apparently
928 do not strongly affect the δ^{13}C values of chitinous and fossilizing structures (head capsules)
929 of chironomid larvae. In contrast, the oxygen isotope composition of chironomid cuticles
930 can be strongly affected by strong alkali or acid treatments (Verbruggen et al. 2010a;
931 Lombino 2014) and such treatments can lead to selective removal of protein or chitin from
932 the cuticles. Furthermore, acid solutions can promote oxygen exchange between the

933 solution and the cuticles (Verbruggen et al. 2010b). Less information is available on the
934 effects of chemical pre-treatments on the fossilizing structures of other chitinous
935 invertebrates and on the N- and H-isotope composition of remains. Treatment with 10 %
936 KOH solution has no apparent effect on the C, O and N isotope composition of the
937 ephippia sheaths of *Daphnia pulicaria* (Schilder et al. 2015a).
938 Taphonomic changes in chitinous arthropod cuticles have mainly been studied for
939 marine crustaceans, whereas much less information is available for lacustrine
940 invertebrates. Major losses of chitin and protein have been observed during the first
941 weeks of biodegradation of shrimp cuticle and other arthropod remains (Stankiewicz et al.
942 1998; Schimmelmann et al. 1986). The C, N, O and H isotope composition was found to be
943 preserved during partial degradation of chitin leading Schimmelmann et al. (1986) to
944 conclude that chitin in ancient archaeological deposits can still be expected to faithfully
945 carry the original isotopic signature. However, as mentioned above, experimental results
946 based on decapod cuticles may not be representative for chitinous cuticles of other
947 aquatic invertebrate groups. Perga (2011) studied the effects of degradation of cladoceran
948 exoskeletons in in lake water and anoxic sediments. In degradation experiments with
949 cladoceran exoskeletons changes in $\delta^{13}C$ were very small (<1 ‰) and happened within the
950 first three months (Perga 2011). No major changes in $\delta^{15}N$ were observed if exoskeletons
951 were incubated in sediments, but effects were larger in oxic or anoxic lake water (+2 to +5
952 ‰). The chemical structure of chironomid head capsules isolated from 15,000-year old
953 sediments was shown to be very similar to modern head capsules, suggesting that no
954 extensive chitin or protein decomposition occurred in these structures after initial
955 decomposition (Verbruggen et al. 2010a). Down core analyses demonstrated that
956 chironomid head capsules from sediments 11,000-15,000 years old were still
957 characterized by the centennial- to millennial-scale changes in $\delta^{18}O$ values that
958 characterized precipitation and lake water compositions in Europe, during the end of the
959 last ice age (Verbruggen et al. 2010b; Lombino 2014).
960 In summary, available evidence indicates that pre-treatment and initial decomposition
961 affects the isotope composition of different elements differently. Only very small sample
962 pre-treatment effects are reported for $\delta^{13}C$ values of invertebrate remains. More
963 significant effects have been described for $\delta^{18}O$ values, whereas little information is

964 available for $\delta^{15}N$ and δD values. Similarly, some evidence indicates that effects of
965 degradation may be more relevant for $\delta^{15}N$ than $\delta^{13}C$ values of invertebrate remains,
966 whereas studies describing these effects for $\delta^{18}O$ or δD values are lacking. Nevertheless, it
967 appears that chitinous remains preserve well and (as far as evidence is available) retain
968 their isotopic signature in lake sediments once initial stages of decomposition have been
969 completed, which is also confirmed by the interpretable shifts in isotope composition
970 reported for invertebrate remains in late Quaternary sediment records (see sections 4.4
971 and 4.5 below).
972
973 ## 4.4 *Stable carbon and nitrogen isotope records based on chitinous invertebrate remains*
974
975 ### *4.4.1 Impacts of land use, eutrophication, and food sources*
976
977 Section 4.2.1 discussed how $\delta^{13}C$ and $\delta^{15}N$ values of invertebrates and their remains
978 closely reflect dietary composition, usually with a systematic offset. This makes it possible
979 to provide information about the diet of invertebrates using the carbon and nitrogen
980 isotope composition of their remains. Diets usually consist of a mixture of sources, each
981 with its own isotope composition, and more nutritious or more easily digestible sources
982 may therefore be assimilated preferentially (Kamjunke et al. 1999; Goedkoop et al. 2006).
983 The food sources available to invertebrates also strongly depend on invertebrate feeding
984 ecology and will differ for detritivores, grazers, filterer-feeders, or predators (Merritt et al.
985 2008; Thorp and Rogers 2015), so it is crucial to understand the parent organism's modern
986 habitat and ecology when interpreting stable isotope values (more detailed examples in
987 van Hardenbroek et al. (2012, 2014)). Overall, $\delta^{13}C$ and $\delta^{15}N$ values of invertebrate
988 remains and bulk sediment tend to follow the same trends in many lakes (Struck et al.
989 1998; Wooller et al. 2008; Griffiths et al. 2010; van Hardenbroek et al. 2014; Kattel et al.
990 2015), but significantly different $\delta^{13}C$ profiles have also been reported (Perga et al. 2010;
991 Schilder et al. 2017). Recent palaeolimnological studies that have measured taxon-specific
992 $\delta^{13}C$ values of chironomid head capsules indicate that benthic invertebrates are sensitive
993 to changes in available carbon sources (van Hardenbroek et al. 2014; Belle et al. 2017a,b;
994 Schilder et al. 2017), including methane-derived carbon (see section 4.4.2).

995 Planktonic cladocerans are typically filter-feeding on particulate organic matter
996 (POM) in the water column, predominantly consisting of algae and bacteria (Kamjunke et
997 al. 1999) and their stable isotope composition follows that of their food sources during the
998 annual cycle (Perga and Gerdeaux 2006; Morlock et al. 2017). As primary consumers,
999 Cladocera are very sensitive to changes in primary productivity and the stable isotope
1000 composition of their remains can be used to trace the impact of anthropogenic nutrient
1001 enrichment of lakes and recovery from it (Perga et al. 2010; Frossard et al. 2014; van
1002 Hardenbroek et al. 2014). In large lakes, there can be a strong relationship between
1003 Cladocera $\delta^{13}C$ values and the CO_2 concentration in the lake water (Smyntek et al. 2012).
1004 This is due to the strong relationship between algal carbon fractionation and dissolved CO_2
1005 availability (e.g., Hollander and McKenzie 1991; Laws et al. 1995). Based on this
1006 mechanism, Perga et al. (2016) have shown that in three large, temperate lakes the $\delta^{13}C$
1007 values of Cladocera remains can be used as an indicator of past summer surface water CO_2
1008 concentrations. In smaller lakes, however, Cladocera are more likely to also incorporate
1009 allochthonous or methanogenic (see section 4.4.2) carbon, which can compromise the
1010 relationship between Cladocera $\delta^{13}C$ values and CO_2 concentrations.
1011 Bryozoa are another invertebrate group that produces resting stages (statoblasts),
1012 which have been used for stable isotope analyses (van Hardenbroek et al. 2016). Bryozoa
1013 are mostly sessile organisms, filter-feeding on suspended POM, which consists to a
1014 considerable extent of algae and bacteria (Kaminski 1984). Bryozoan $\delta^{13}C$ values generally
1015 follow the trend in $\delta^{13}C$ values of POM or, in down core studies, SOM (Turney 1999; van
1016 Hardenbroek et al. 2014, 2018; Morlock et al. 2017; Rinta et al. 2016).
1017 The impact of urban and agricultural pollution to lakes can result in the increase of
1018 $\delta^{15}N$ values of SOM of aquatic origin (Cabana and Rasmussen 1996; Laevitt et al. 2006). A
1019 number of studies have found that $\delta^{15}N$ of Cladocera remains and SOM are related (Struck
1020 et al. 1998; Wooller et al. 2008), but the addition of nutrients can disturb this relationship.
1021 In Lake Annecy, Perga et al. (2010) observed increasing $\delta^{15}N$ values of SOM and *Bosmina*
1022 carapaces during the 1960s and 1970s eutrophication. During re-oligotrophication $\delta^{15}N$
1023 values of SOM decreased again, but $\delta^{15}N$ values of *Bosmina* remained high, possibly as a
1024 result of a shift in their diet from algae to flagellates: changing the trophic position of
1025 *Bosmina*. Griffiths et al. (2010) investigated the effect of marine-derived nutrients from

1026 sea-bird colonies on arctic lakes and showed that this input led to an increase in SOM $\delta^{15}N$
1027 values. Chironomid remains showed the same trend, albeit with greater variability,
1028 whereas *Daphnia* remains did not show any change at all, indicating that both groups of
1029 organisms can assimilate different sources of nitrogen, at least in the periods when the
1030 fossilizing structures are formed.
1031 Aquatic food webs can be highly complex, with large spatial and temporal variations in
1032 the quantity of carbon and nitrogen sources as well as the isotope composition of these
1033 sources. Attempts to interpret sedimentary stable isotope records of invertebrate remains
1034 to estimate the relative contribution of different dietary components (algae,
1035 allochthonous OM, bacterial biomass) requires reliable estimates of the stable isotope
1036 composition of these dietary components. This is challenging in modern food web studies,
1037 and even more so in palaeoenvironmental applications. Emerging compound-specific
1038 stable isotope analysis of microbial and algal compounds (e.g., Castañeda and Schouten
1039 2011; Middelburg 2014; Taipale et al. 2015), combined with stable isotope analysis of
1040 specific organic remains may hold the key to a substantial increase in understanding
1041 trophic interactions over long timescales.
1042
1043 *4.4.2 Methane-fuelled food webs*
1044
1045 Notably low $\delta^{13}C$ values of invertebrates compared with $\delta^{13}C$ values of algae and SOM
1046 occur where ^{13}C-depleted methane and methane oxidizing bacteria (MOB) provide carbon
1047 sources for lacustrine food webs (e.g., Gebruk 1993; Bunn and Boon 1993; Jones et al.
1048 2008; Devlin et al. 2015; Grey 2016). Filter-feeding and deposit-feeding invertebrates can
1049 partially feed on MOB biomass (or on ciliates feeding on MOB) and their $\delta^{13}C$ values
1050 become lower with increasing contributions of methane-derived carbon.
1051 The $\delta^{13}C$ values of some invertebrate groups and their remains in surface
1052 sediments are systematically related to methane concentrations or fluxes in lakes, at least
1053 in some regions and lake types. For example, $\delta^{13}C$ values of Chironomini larval head
1054 capsules and of *Daphnia* ephippia were negatively correlated to diffusive methane fluxes
1055 in 17 lakes studied in Sweden and arctic Siberia (van Hardenbroek et al. 2013b). Also, $\delta^{13}C$
1056 values of *Daphnia* ephippia in 15 European lakes were found to correlate to lake methane

concentrations (Schilder et al. 2015b) and $\delta^{13}C$ values of Chironomini larvae in 6 lakes in arctic Alaska were correlated to methane oxidation rates (Hershey et al. 2015). These findings agree with the observation that, in small European lakes, the abundance of ^{13}C-depleted fatty acids in surface sediments, originating at least partially from MOB, also increases with increasing CH_4 concentrations (Stötter et al. 2018). This suggests that MOB concentrations in these lakes also increase with CH_4 concentrations. Sediment core studies indicated higher uptake of methane-derived carbon in certain invertebrate groups (mainly belonging to the chironomids and cladocerans) with increased primary productivity in lakes. This was either driven by anthropogenic nutrient addition (Frossard et al. 2015; Belle et al. 2016a, b; Rinta et al. 2016; Schilder et al. 2017), or triggered by warmer/wetter climatic conditions (Wooller et al. 2012; van Hardenbroek et al. 2013b). Quantifying the uptake of methane-derived carbon using isotope mixing models is possible, using the $\delta^{13}C$ values of the two end member food sources, MOB and algae. POM/SOM has been used as approximation for algae (Wooller et al. 2012; Belle et al. 2014, 2015b, 2016b; Schilder et al. 2017), although this approach is associated with high levels of uncertainty due to variations in $\delta^{13}C$ values of methane and algal material between lakes.

Measurements of δD values of invertebrates can also potentially be used to assess the contribution of MOB to their diet, since methanogens also strongly discriminate against the heavier D, and the resulting low δD values are transferred to certain taxa of chironomid larvae (Deines et al. 2009; Belle et al. 2015b). In addition, in lakes with significant contributions of methane to the lacustrine food web, ^{14}C analyses of invertebrate remains may in some situations be used to constrain the amount of old carbon entering the food web: e.g., via methane from decomposing Pleistocene deposits (Wooller al. 2012; Elvert et al. 2016). However, in hard water lakes this approach is further complicated by other old carbon sources such as carbonates derived from local bedrock.

The stable isotope composition of invertebrates can vary seasonally in relation to MOB availability, notably during autumn turnover when methane stored in the hypolimnion is mixed (e.g., Grey et al. 2004; Kankaala et al. 2010; Yasuno et al. 2012). The $\delta^{13}C$ values of invertebrate remains, however, are more likely to represent average values of these seasonal variations (Morlock et al. 2017; Schilder et al. 2017, 2018; van Hardenbroek et al. 2018). Likewise, spatial variation of methane production in lakes can

lead to spatial variability of $\delta^{13}C$ values of invertebrates (Deines and Grey 2006; Agasild et al. 2013), which can also be observed in the remains (van Hardenbroek et al. 2012; Belle et al. 2015a). Interesting examples of spatial variability of methane-derived carbon are provided by Frossard et al. (2014, 2015), who observed an expansion (to shallower depths) of the anoxic hypolimnion with ongoing eutrophication. As a result, chironomid head capsule $\delta^{13}C$ values became progressively lower, a process that was first observed in the deepest cores, followed a few decades later in shallower cores.

4.5 Stable oxygen and hydrogen isotope records based on chitinous invertebrate remains

The $\delta^{18}O$ values of organic remains can provide information on the $\delta^{18}O$ values of lake water in which the organic remains were produced. Wooller et al. (2004) were the first to measure $\delta^{18}O$ values of chironomid head capsules in lake sediment records and demonstrated that, within analytical error, chironomid $\delta^{18}O$ values agree with the expected $\delta^{18}O$ values of modern regional precipitation. The relationship between $\delta^{18}O$ values of lake water and chironomid remains has since been further validated and explored via laboratory experiments (section 4.2.2) and field surveys. The field relationship has been reproduced and further explored over larger transects and multiple regions (Fig. 5), including Europe (Verbruggen et al. 2011; Lombino 2014), South America (Mayr et al. 2015), Greenland (Lasher et al. 2017), and Australia (Chang et al. 2016, 2017). In lakes with short residence times (≤ 1 year), i.e. open basins, the relationship between chironomid head capsules and lake water $\delta^{18}O$ values is strong (Fig. 5). Here, chironomid $\delta^{18}O$ values are enriched relative to lake water by ~ 22.5 ‰. This relationship appears to fail in closed basin lakes with long residence times. Sampled lake water from such lakes may not reflect $\delta^{18}O$ values of waters during the growing season due to evaporation effects. In lakes where lake water – chironomid enrichment factors differ greatly from the mean relationship demonstrated in open basins, an assessment of lake hydrology is necessary to clarify what chironomid $\delta^{18}O$ values are recording.

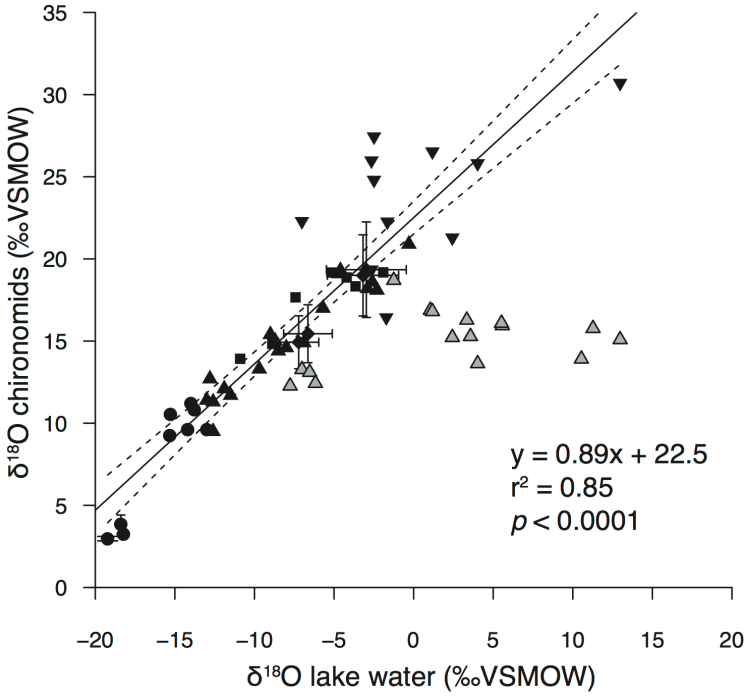


Fig. 5: *Relationship between $\delta^{18}O$ values of (lake) water and $\delta^{18}O$ values of chironomid head capsules from surface sediment samples. Plotted data points are from Verbruggen et al. 2011 (closed triangles), Lombino 2014 (closed diamonds), Mayr et al. 2015 (closed squares), Chang et al. 2016 (shaded triangles), Chang et al. 2017 (closed inverted triangles), and Lasher et al. 2017 (closed circles). Error bars indicate 1 standard deviation variability in replicate data from individual sites, where available. The linear regression excludes data points from Chang et al. 2016, as these contain closed systems with high evaporation, where the $\delta^{18}O$ of sampled lake water may not be indicative of $\delta^{18}O$ value of water during chironomid growth.*

Although chironomid larvae are often abundant in lake sediments, recalcitrant organic structures of other aquatic organisms have also been used to measure $\delta^{18}O$ values, including cladoceran ephippia (Verbruggen et al. 2011; Schilder et al. 2015a) and elytra of aquatic beetle genera *Helophorus* and *Hydroporus* (van Hardenbroek et al. 2013a).

Based on these demonstrated positive relationships between the $\delta^{18}O$ values of organic invertebrate remains and those of lake water (Fig. 5), a growing number of palaeoecological and palaeoclimatic studies have used $\delta^{18}O$ analyses of organic

1138 invertebrate remains as a palaeoclimatic proxy. Records have largely been developed from
1139 sites from higher latitudes including Greenland (Wooller et al. 2004; Lasher et al. 2017),
1140 Iceland (Wooller et al. 2008), Alaska (Wooller et al. 2012; Graham et al. 2016) and
1141 Svalbard (Arppe et al. 2017; Luoto et al. 2018), but also from mid-latitudes (Verbruggen et
1142 al. 2010b; Lombino 2014). These $\delta^{18}O$ records do not only reflect temperature changes,
1143 but a combination of drivers including (1) seasonality and source region of precipitation,
1144 (2) the balance between inputs (precipitation, snow melt, inflow) and evaporation, and (3)
1145 composition of invertebrate assemblages, their habitat and ecology. As a result, the $\delta^{18}O$
1146 records may in some situations be more variable than independent temperature
1147 reconstructions (e.g., Wooller et al. 2004, 2008, 2012). In some records, however,
1148 chironomid $\delta^{18}O$ values closely follow minor climate oscillations (Verbruggen et al. 2011b;
1149 Lombino 2014; Arppe et al. 2017). Chironomid $\delta^{18}O$ has also been used to show the
1150 reduction in freshwater availability attributed to the local extinction of woolly mammoth
1151 on St. Paul Island (Graham et al. 2016).

1152 In contrast to the $\delta^{18}O$ values from chironomids and other aquatic invertebrates, the
1153 δD values of these organisms have been found to be primarily controlled by the δD values
1154 of diet rather than source water (section 4.2.2). Given this finding, δD values from
1155 chironomids have found to be a valuable dietary biomarker, and used to trace the use of
1156 terrestrial and methanogenic carbon by aquatic organisms (Deines et al. 2009; Karlsson et
1157 al. 2012; Belle et al. 2015b; Mariash et al. in press, and see section 4.4.2).

1158

1159 ## 4.6 *Other organic remains*

1160

1161 ### *4.6.1 Aquatic plant macrofossils*

1162

1163 Records of $\delta^{13}C$ and $\delta^{18}O$ of aquatic cellulose in lake sediments are widely available (e.g.,
1164 Wolfe et al. 2007; Mayr et al. 2015; Street-Perrot et al. 2018) but are not discussed here. In
1165 contrast, continuous, high-resolution sediment records of aquatic plant remains have only
1166 in rare cases been analysed for their stable isotope composition. For example, Turney
1167 (1999) found little variation in the $\delta^{13}C$ values of terrestrial plant remains compared with
1168 $\delta^{13}C$ of SOM in two kettle-hole lakes in the UK, while $\delta^{13}C$ values of aquatic plant remains

(*Potamogeton*) was more variable, possibly in response to changes in productivity. A systematic study of the difference between $\delta^{13}C$ values of *Potamogeton* and $\delta^{13}C$ values of DIC in lakes on the Tibetan Plateau and Yakutia (Herzschuh et al. 2010a), demonstrated that this difference is dependent on the growth rate, which can be interpreted in terms of productivity and applied to sediment records. Another study linked variations in $\delta^{15}N$ values of aquatic macrophyte remains with N-limitation and productivity (Herzschuh et al. 2010b). When the organic matter produced within lakes by aquatic macrophytes and algae is an important component of SOM, the stable isotope composition of SOM and algae (e.g. *Botryococcus*) are strongly coupled, even on millennial time scales (Heyng et al. 2012). In a study of 40 lakes on the Tibetan Plateau $\delta^{13}C$ values of aquatic macrophytes correlated positively with $\delta^{13}C$ values of SOM, although much stronger correlations existed between $\delta^{13}C$ values of *Potamogeton* and $\delta^{13}C$ values of mid-chain n-alkanes, which are largely produced by the macrophytes (Aichner et al. 2010). This study also demonstrated the effect of terrestrial organic matter on $\delta^{13}C$ of SOM and the importance of measuring $\delta^{13}C$ values of different carbon sources to understand palaeolimnological records of SOM $\delta^{13}C$ values (e.g., Gu et al. 2006; Das et al. 2008; Drew et al. 2008). The use of spooling-wire microcombustion IRMS makes is possible to analyse very small samples of carbonised terrestrial plant material (Urban et al. 2010, 2013). This approach could also be applied to aquatic plant remains to disentangle the relative importance of terrestrial and aquatic carbon sources in SOM.

Stable oxygen isotopes of aquatic plant macrofossils have been studied in a similar way as aquatic invertebrate remains (section 4.5), indicating a strong relationship between $\delta^{18}O$ values of lake water and aquatic moss fragments (Zhu et al. 2014; Lasher et al. 2017). Furthermore, stable oxygen isotope values of aquatic moss macrofossils and those of purified cellulose are strongly correlated in some lakes, with a reported mean $\delta^{18}O$ offset from cellulose of ~2.7 ‰ for aquatic vascular plants and ~1.3 ‰ for aquatic mosses (Zhu et al. 2014).

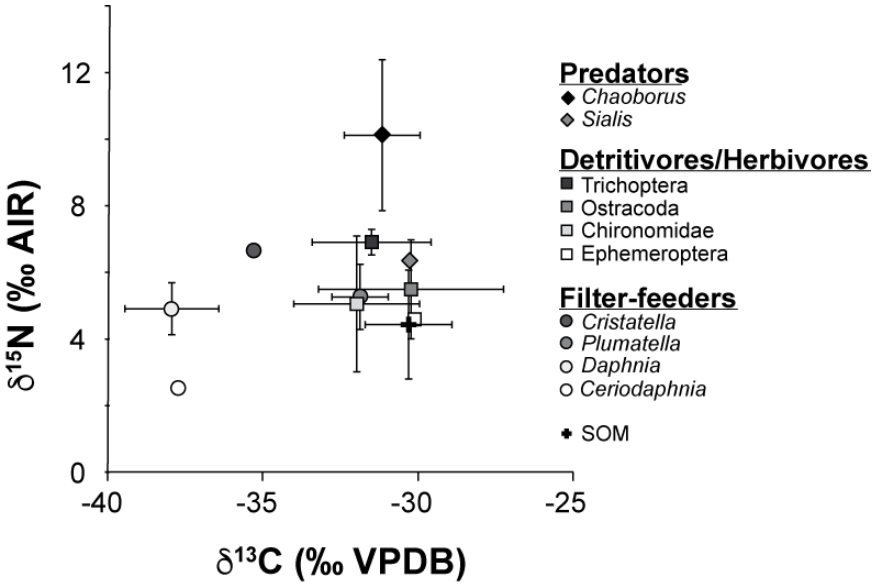
4.6.2 Fish remains

Fish have crucial top-down impact on the structure of lake ecosystems, generally taking up

1200 the highest trophic positions in lacustrine food webs. Fish remains (e.g., scales, teeth,
1201 bones and otoliths) can be preserved in lake sediments (e.g., Wooller et al. 2015) and the
1202 assemblages in surface sediments broadly reflect the species in living fish communities
1203 (Davidson et al. 2003). Fish remains contain organic and calcified moieties. The organic
1204 fraction, mostly collagen, is derived from diet, whereas the calcified fraction can come
1205 from both diet and DIC in lake water (Hoie et al. 2003). A good relationship between the
1206 $\delta^{13}C$ values of scales and muscle tissue was found, with systematic offsets of ca. 2.5 to 4 ‰
1207 and –1.5 to 0 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively (Perga and Gerdeaux 2003; Kelly et al.
1208 2006). Decalcifying scales can improve this relationship (Perga and Gerdeaux 2003), but
1209 has a minimal effect on $\delta^{13}C$ and $\delta^{15}N$ values (Ventura and Jeppesen 2010). Stable isotope
1210 analysis of fish remains from lake sediments (Patterson et al. 1993; Wooller et al. 2015),
1211 archived fish scale collections (Gerdeaux and Perga 2006), and fish remains from
1212 archaeological settings (Häberle et al. 2016) hold great potential for investigating changes
1213 in food chain length and trophic interactions in response to, for example, eutrophication,
1214 non-native species introductions, fisheries and aquaculture.
1215
1216 *4.7 Future directions*
1217
1218 An increasing number of studies use stable isotope analyses on organic remains in
1219 sediment records from arctic, boreal, and temperate regions. In contrast, subtropical and
1220 tropical sediment records are almost completely absent, although the same pattern
1221 applies to modern limnological studies (e.g., Iglesias et al. 2017; Sanseverino et al. 2012).
1222 Other aspects that deserve further study are understanding drivers of spatial variability
1223 within lake basins, for example by comparing shallow and deep-water cores (Frossard et
1224 al. 2015), and by measuring the stable isotopes on components of the modern lake
1225 (eco)system and catchment to better understand taphonomic processes and constrain
1226 palaeolimnological interpretations (Morlock et al. 2017; Arppe et al. 2017). Related to this
1227 is the continued need for controlled growth and feeding experiments, especially those that
1228 investigate temperature-dependent fractionation (Schilder et al. 2015a).
1229 A novel direction is the use of dual $\delta^{13}C$ and $\delta^{15}N$ values from organic remains of
1230 organisms, at different trophic positions throughout a sediment record, which allows the

1231 study of changes in food web structure over time. Initial work on invertebrate remains
1232 from surface sediments indicates that ranges of ~10 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values can
1233 be observed (Fig. 6), similar to the ranges found in soft tissues of these organisms (e.g.,
1234 Jones and Grey 2011). Related to this is the potential for using $\delta^{34}S$ values (e.g., Grey and
1235 Deines 2005) to understand trophic relationships as preserved in fossil assemblages.

1236



1237
1238

1239 **Fig. 6**: *$\delta^{13}C$ and $\delta^{15}N$ values of invertebrate remains and SOM from surface sediments in*
1240 *Lake De Waay, The Netherlands (van Hardenbroek, Heiri, Schilder, Wooller unpublished*
1241 *data)*.

1242

1243 A further approach to understanding food web structure is by analysing stable
1244 carbon isotopes of essential amino acids in tissues (Larsen et al. 2009). These amino acids
1245 have specific stable isotope values, or isotope "fingerprints", depending on the primary
1246 producer type that synthesised the essential amino acids: aquatic photosynthetic (derived
1247 from dissolved organic carbon), terrestrial photosynthetic (derived from atmospheric
1248 carbon dioxide) and microbial (Fig. 7). These fingerprints are independent of the isotope
1249 composition of the carbon source that was originally used to synthesise the essential
1250 amino acids and they are retained in consumer tissues (Larsen et al. 2009). These
1251 fingerprints can also be used in mixing model approaches to determine the proportional
1252 contribution of different primary production sources (e.g. terrestrial vs. aquatic vs.

microbial) to consumers (Larsen et al. 2009; Larsen et al. 2013). This approach has been tested in some marine ecosystems (Larsen et al. 2013; Arthur et al. 2014) and initial palaeolimnological work is promising (Carstens et al. 2013).

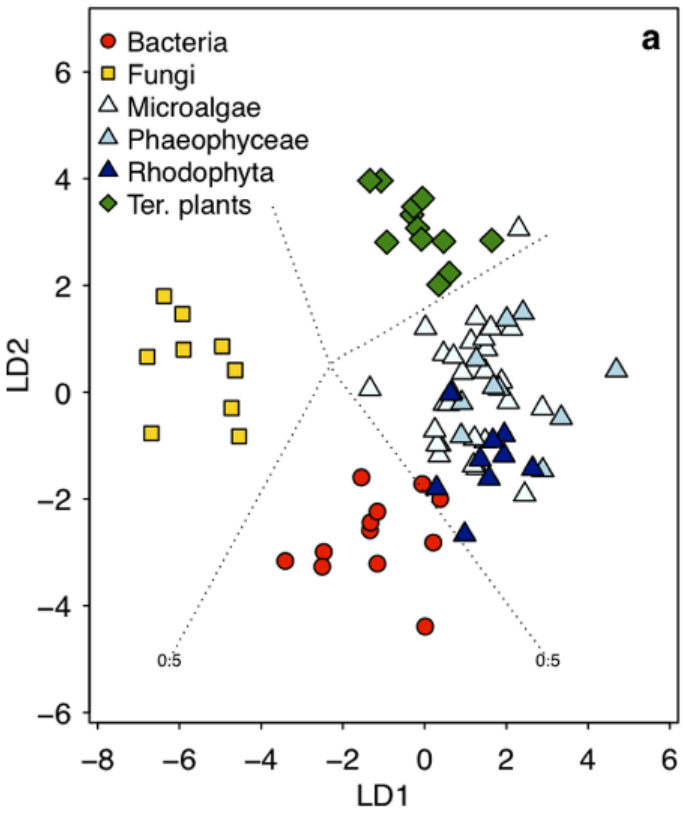


Fig. 7: *Linear discriminant (LD) function analysis of the stable carbon isotope composition of essential amino acids demonstrates the "fingerprinting" of a range of primary production sources (from Larsen* et al*. 2013).*

5. Stable isotope modelling in palaeolimnology

Stable isotopes, for all their attractive qualities as environmental, ecological and behavioural tracers, are at times rather blunt instruments: tissue isotope compositions are influenced by a wide range of potential drivers, so that interpreting the cause(s) contributing to any measured variation in isotope compositions can be challenging.

Modern ecology and hydrology have greatly profited from explicit modelling of variations in stable isotope composition due to known stressors, impacts, and processes, which can help to separate realistic from unrealistic scenarios even in complicated situations with overlapping variabilities due to seasonal changes, different drivers, and site- or taxon-specific isotopic offsets. This type of modelling is mostly lacking when interpretations are made in palaeolimnological studies, and some examples are outlined here that show how approaches developed in modern modelling studies could be applied to palaeorecords.

This section will focus on the potential and pitfalls of modelling carbon and nitrogen isotopes in modern and palaeo food webs and how this can contribute to the interpretation of stable isotope records based on fossil remains in lake sediments. Recent advances in the use of stable isotopes of oxygen and hydrogen in (palaeo)hydrological modelling are only briefly highlighted, as the topic was reviewed recently (Jones et al. 2016a).

5.1 Carbon and Nitrogen

In a review of the use of stable isotopes in ecology, Boecklen et al. (2011) identify 46 separate sources of variation in stable isotope compositions (predominantly $\delta^{13}C$ and $\delta^{15}N$ values) of organisms. In their review, the isotope composition of an organism is viewed as an emergent property of that organism, influenced by the factors that are frequently the subject of palaeontological investigation such as diet, trophic level, and environmental variability (e.g., habitat and seasonality), but also by a large range of additional physiological, ecological and biochemical factors that are often difficult to assess in palaeoecological and palaeoenvironmental studies such as temporal and spatial variations in stable isotope compositions of nutrients and food sources and physiological influences on isotopic fractionation. Interpretations of stable isotope data are therefore vulnerable to making overly simplified assumptions, and it is helpful to consider how sources of uncertainty and variance can impact the validity of isotope-based ecological or environmental interpretation before identifying possible solutions to deal with uncertainty.

In the simplest ideal case, the isotope composition of carbon and nitrogen in consumers reflects the isotope composition of their prey. Where a consumer derives

resources from two or more isotopically distinct prey sources, the relative importance of those sources can be determined from a mass-balance based isotope mixing model (for example, in the case of carbon derived from phytoplankton and methane, see section 4.4.2). Measuring stable isotopes on 'pure' algal or bacterial biomass is challenging in modern food webs and culturing experiments (Templeton et al. 2006; Vuorio et al. 2006). This hinders defining the isotope values of sources at the base of lake food webs and leads to assumptions and uncertainties in mass-balance models. Compound-specific stable isotope analysis of biomarkers typical for particular algal (n-alkanes, pigments, fatty acids, amino acids) and bacterial groups (fatty acids, hopanols, amino acids), are now emerging as palaeolimnological tools (Huang et al. 1999; Aichner et al. 2010; Castañeda and Schouten 2011; Larsen et al. 2013; Middelburg 2014; Taipale et al. 2015; Elvert et al. 2016) and will potentially address this issue.

Mass-balance type models also assume the isotopic offset between diet and tissue is known (see, e.g., McCutchan et al. 2003 and sections 4.2). More advanced Bayesian mixing models such as SIAR (Parnell et al. 2013), mixSIR (Semmens et al. 2009) and FRUITS (Fernandes et al. 2014) have been developed to incorporate uncertainties in diet-tissue isotopic fractionation and to consider isotopic distributions of potential food sources. However, mixing models are still simplifications of food webs with relatively limited consideration of temporal or spatial variation in either isotope compositions or trophic interactions. Model outputs should be interpreted with care to avoid oversimplification, as highlighted by authors of isotopic mixing models and isotope metrics themselves (Layman et al. 2012; Phillips et al. 2014), but can nevertheless provide estimates of the likelihood of different scenarios, although it remains difficult to include temporal and spatial variability.

Seasonality has a marked effect on biogeochemical cycles, associated nutrient isotope compositions, food availability and food web structure (e.g., Woodland et al. 2012; Junker and Cross 2014; Visconti et al. 2014). Most isotope-based ecological analyses either explicitly compare the isotope composition of the animal in question to a reference baseline animal, assuming a constant isotopic baseline or infer that temporal change in consumer isotope values reflects changes in the baseline isotope compositions. In all these cases, short-term (e.g., seasonal) variability in isotope baselines can complicate interpretations of consumer isotope data. This will be particularly pertinent for consumers with high growth or metabolic rates such as chironomids, cladocerans, and

ephemeropterans, where dynamic changes in baseline isotope values will be rapidly incorporated into consumer tissues, although this is mediated to some extent because many fossil specimens are needed for one stable isotope measurement, and therefore represent a larger period of time. Woodland et al. (2012) built a time dynamic isotope mixing model by coupling combined time-dependent functions of temporal baseline $\delta^{13}C$ variation and functions predicting consumer isotope composition as a function of growth. Relaxing assumptions of isotopic equilibrium, both in terms of consumer growth and isotopic baselines, resulted in different and more realistic reconstructions of the contribution of benthic resources to diets. Studies that quantify and model the isotope variability in aquatic organisms are essential for understanding stable isotope records from lake sediment cores, although few modern surveys are designed with palaeoenvironmental applications in mind (e.g., von Grafenstein et al. 1999b; Dixit et al. 2015; Morlock et al. 2017; Schilder et al. 2017; section 4.5).

When different locations and palaeolimnological studies are considered, potentially confounding aspects of spatial/geographical variability, diagenesis and time averaging are also added. Very quickly the number of potential variables that could be drawn on to explain an observed temporal change either in means or distributions of organism stable isotope compositions increases, leading to a range of possible scenarios that could explain a measured isotopic response. One approach to quantitatively choosing between alternative mechanisms responsible for measured isotopic variability is through mechanistic modelling. For example, Magozzi et al. (2017) estimated spatial variations in inter-annual ranges of $\delta^{13}C$ values for phytoplankton, and show that these vary systematically with latitude, at less than 1.5 ‰ in equatorial regions to >9 ‰ in highly seasonal arctic systems. Estimates of spatial and temporal variability in isotopic baselines can form the basis for more complex simulations of isotopic variability in food webs under differing scenarios, and with differing sampling protocols.

As a simple conceptual example, a time series of $\delta^{13}C$ and $\delta^{15}N$ values from benthic insect remains from a sediment record within a small temperate lake can be assumed. Changes in isotope composition and distributions through time are observed and the question is what contributed to the observed change. A change in external conditions is suspected (e.g., a change in the supply of terrestrial organic matter into the lake), but uncertainty remains whether any other potential driving mechanisms (e.g., Boecklen et al.

2011) or systematic changes in sampling associated with sedimentation could have contributed to the observed pattern. To set up a basic conceptual model of the isotopic systematics in the lake it is necessary to establish variables associated with isotope composition of nutrient inputs to the lake, pelagic production and benthic production. Each of these variables will have defined seasonal, inter-annual and longer-term variability, which can be varied in the model.

Table 2: *Example of variables needed to derive a basic simulation of time series of lake sediment isotopic records.*

Nutrient input	Primary production	Benthic production	Taphonomy
The number of isotopically distinct sources of carbon and nitrogen entering the lake. Proportional contribution of each nutrient source to primary production. The isotope composition of each source. **Temporal variation in the above**	Isotopic fractionation associated with primary production (with uncertainty) – can be linked to temperature and/or cell growth rate. Proportion of primary production formed in each season. **Temporal variation in the above**	Proportional contributions of detrital and pelagic organic matter to sediment. Contribution of methane to sediment organic matter. **Temporal variation in the above**	Time of year represented in the sample. Number of years assimilated into a single sample. Variability in sample time averaging. Differential loss of isotopically distinct fractions of production.

Simple conceptual model systems building on variables suggested in Table 2 allow investigators to explore sensitivity of the measured output (the expected means or isotopic distributions of a population of invertebrates) to a range of variables, including seasonality, benthic vs. pelagic production, temperature-related differences in isotopic fractionation in phytoplankton, proportional methane contributions and terrestrial detrital matter input. In addition, the sensitivity of the system to naturally uneven sampling can be explored. Using this type of modelled time series in tandem with palaeoenvironmental records from lake sediments make it possible to test the likelihood of alternative scenarios

and interpretations. This is not unlike studies that test models of fossil assemblage data from sediment record to understand sensitivity or response of lake ecosystems to critical transitions (e.g., Seddon et al. 2014; Doncaster et al. 2016).

5.2 Oxygen and hydrogen

An increasing number of palaeoenvironmental studies uses the systematic relationship between climatic variables and δD and δ^{18}O values measured from environmental water (Dansgaard 1964; Gat 1996) to interpret proxies of palaeo lake water recorded in sediment archives (Darling et al. 2006, Jones et al. 2016a; Swann et al. 2018). Mass-balance models based on the linear resistance model of Craig and Gordon (1965) for the isotope composition of evaporating water have been used to explain large proportions of the variability in lake water isotopes, although the many steps between the isotope composition of precipitation and the isotopic signal preserved in proxy records require a thorough understanding of the 'proxy systems' and how they filter and adapt this variability (Feng et al. 2016; Gibson et al. 2016; Jones et al. 2016b). Jones et al. (2016a) suggest that such proxy system models (PSMs) could potentially be used to predict 'forward' modelled proxy time series. These predictions could then be tested against output from General Circulation Models (GCMs) that have stable water isotope physics included, although downscaling GCM output to make it compatible with local palaeo records is challenging (e.g., Sturm et al. 2010). The inclusion of stable water isotopes in GCMs now also allows quantitative validation of GCMs using water isotope data from palaeoenvironmental records (especially when based on large, amalgamated data sets that go beyond the local scale, as described by Horton et al. 2016). The water isotope component of GCMs also creates opportunities for hypothesis-driven exploration of palaeo climate data based on GCM output (e.g., Holmes et al. 2016). Further effort is needed from the modelling, monitoring, and palaeo communities to quantifying the physical processes in the atmosphere that drive spatial and temporal heterogeneity in water isotopes. It might be challenging, however, to find 'well-behaved' lake systems due to large variations and complexity of local hydrology (both spatially and temporally) that affect the stable isotope composition of lake systems compared to marine systems.

6. Concluding remarks

This review shows the extraordinary potential for using stable isotope systems of H, C, N, O, and Si from the macro- and microscopic remains of a wide range of organisms commonly preserved in lake sediments. The stable isotope composition of these siliceous, carbonate and organic components have been related to a range of ecological and environmental variables and processes including evaporation, climate, nutrient cycling, productivity, and methane cycling. Stable isotope measurements on fossil remains have been used to reconstruct past changes in these variables and processes. Furthermore, new approaches for reconstructing past environmental and ecosystem change (e.g., analysing changes in primary productivity or the structure of palaeo food webs) are continuously being developed, expanding the range of applications for isotope analyses based on macro- and microscopic remains in lake sediments. Our review has provided an overview of the level of understanding of the driving variables for environmental proxies based on isotope measurements. Complicating factors such as the effects of seasonality, transport, sample preparation, offsets between different tissues, and of confounding environmental variables, have also been highlighted. These factors remain a major challenge for emerging but also established isotope based approaches analysing biotic remains in lake sediments. An important aim of the review is therefore to also highlight the importance of calibration studies, be it controlled experiments or in field surveys. Such calibrations are essential to better understand the relationships between stable isotope composition and ecological/environmental variables, and are crucial to interpret variability measured in down core applications in terms of an ecological/environmental 'signal' or stochastic 'noise'. Modelling can be a valuable tool to increase our ability to distinguish likely scenarios from unlikely ones. We have also touched on recent methodological advances that have led to expanding the use of Si isotope measurements on diatoms, or reducing the sample size needed for analysis of C isotopes on organic samples, making it clear that technical developments are necessary to continue to increase the stable isotope toolkit available to palaeolimnological studies.

Author contributions

MvH initiated the workshop on stable isotopes in fossils and organic compounds in lake sediments, coordinated the review and manuscript, and drafted sections 1, 4.5, 4.6, 4.7 and 5.2; ML and VP drafted section 2; JAH drafted section 3; OH drafted section 4.2 and 4.3; MJW drafted section 4.4; CT drafted section 5.1. All co-authors participated in workshop discussion groups, provided input for the manuscript, and suggested improvements.

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Supplementary Table: *Mean weight and standard deviation of individual invertebrate remains. Data are derived from surface and down core sediments after chemical processing with 10 % HCl and 10 % KOH (van Hardenbroek et al. 2010b, 2012, 2013; van Hardenbroek, Heiri and Wooller unpublished). Note that values are means for samples containing different sizes of remains, including head capsules from different larval instars.*

	Surface samples weight (µg)	SD	n	Down core samples weight (µg)	SD	n
Chironomid head capsules						
Chironomus anthracinus-type	2.5	3.4	18			
Chironomus plumosus-type	2.8	2.7	14			
Chironomus spp.	2.1	2.1	81	1.2	0.4	30
Dicrotendipes	1.2	0.7	6			
Endochironomus	1.0	0.5	6			
Glyptotendipes	2.0	2.3	6			
Microtendipes	1.0	0.4	5			
Polypedilum	0.6	0.2	5			
Sergentia	0.9	0.3	1			
Chironomini (including all taxa)	1.6	2.2	241	1.0	0.4	76
Corynocera ambigua				1.3	0.1	7
Tanytarsini	0.6	0.5	122	0.5	0.2	39
Orthocladiinae	0.7	0.5	105	0.9	0.5	42
Diamesinae	1.9	0.9	3			
Tanypodinae	1.0	0.7	89	1.0	0.3	17
Cladoceran ephippia						
Daphnia spp.	2.8	2.9	148	2.8	2.7	51
Simocephalus vetulus	2.8	1.4	11			
Ceriodaphnia spp.	0.8	0.7	45			
Leydigia	1.1	0.8	5			
Chydorid	0.9	0.5	26			
Bryozoan statoblasts						
Plumatella spp.	1.2	0.7	116	0.6	0.3	23
Cristatella mucedo	30.3	28.1	66	21.0	8.1	15
Lophopus crystallina	4.6		1			
Pectinatella magnifica	18.1		2			
Other remains						
Ephemeroptera (mandible)	0.6	0.3	32			
Turbularia (coccoon)				1.2	0.2	18
Ostracoda (shell lining)	1.8	1.5	56			
Trichoptera (frontoclypeus/mandible)	2.2	3.7	32			
Sialis (frontoclypeus/mandible)	2.5	1.6	23			
Chaoborus (mandible)	1.5	1.0	37			
Chaoborus (thoracic horn)	0.9	0.2	7			