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Is small beautiful? A review of the advantages and limitations of using small mammal teeth and the direct laser fluorination analysis technique in the isotope reconstruction of past continental climate change

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ABSTRACT

Oxygen isotopes in mammalian tooth enamel have become a widely used proxy for reconstructing past continental climate change. However, owing to sample size constraints in the chemical separation and precipitation of the phosphate prior to oxygen isotope analysis, this technique has been, until recently, limited to relatively large mammal teeth. As the result of recent developments in chemical and analytical techniques, including the development of a novel pre-treatment technique and a direct laser fluorination methodology of analysis, small mammal teeth (rodents), which are relatively more abundant in the fossil record than large mammal teeth, can now be used. Published examples from the recent literature are provided with the aim of examining the advantages and limitations of using small mammal teeth in palaeoclimate reconstruction studies. Issues that are addressed include 1) the rationale behind the calculation of the mean oxygen isotope composition of local water and the problems associated with the intake of ^{18}O enriched water from plant food and from small water bodies fractionated as a result of evaporation; 2) the methodology, justification and limitations in determining mean annual temperatures (MATs) from calculated $\delta^{18}O$ local water values and also combining the $\delta^{18}O$ local water values with $\delta^{18}O$ values from freshwater biota to calculate, using established thermometry equations, multiple palaeotemperatures of differing climatic significance; 3) specific published examples of how the phosphate oxygen isotope composition of small mammal teeth are being used in studies of continental climate change.

1. Introduction

With climate change becoming an increasing governmental and societal issue, a detailed understanding of non-anthropogenic climate cycles in the geological past and their effect upon both marine and terrestrial biota is required if society is to plan for the future. The investigation of past global climate change and its impact upon ecosystems has demonstrated that there is a wide range of biotic responses to changes in environmental conditions (see Culver and Rawson, 2000, for a comprehensive review of biotic responses to global change over the last 145 million years). The isotope study of climate change has extensively exploited the marine sedimentary record. Studies have involved the collection of isotope data from the tests of benthic and planktonic foraminifera (for a recent comprehensive review see Zachos et al., 2001 and references therein) and to a lesser extent from other marine palaeoproxies such as the otoliths (ear stones) (Ivany et al., 2000) and teeth (Lécuyer et al., 2003) of marine fish and also the shells of molluscs (Kobashi et al., 2001). However, because of the difficulties in correlating the marine record to that in the continental realm, the value of using marine palaeoproxies to interpret biotic changes in the continental realm has been called into question (Alroy et al., 2000). Therefore, over time independent palaeoproxies have been developed for the study of continental climate change. Examples of such studies, beyond that of the late Neogene (Quaternary), include fossil leaf analysis (e.g., Wing et al., 2005), mammal species richness analysis (e.g., Legendre et al., 2005) and the oxygen isotope analysis of freshwater biota (e.g., gastropods by Schmitz and Andreasson, 2001). However, following the seminal work of Longinelli (1984) on oxygen isotope fractionation between ingested water and precipitated biogenic apatite (bone and teeth), there has been a rapid expansion of isotope studies using vertebrate skeletal remains. For example, Kolodny and Luz (1991) combined the marine and freshwater realms in their phosphate oxygen isotope study of fossil fish from the Devonian to the Recent. However, even though this was a wide-ranging study it had low palaeoclimatic and temporal resolution. Bryant et al. (1996), Fricke et al. (1998), Kohn et al. (2004), Grimes et al. (2005) and Tütken et al. (2006) have, to

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mention a few, all conducted studies purely in the continental realm using the oxygen isotope composition of mammalian phosphate in tooth enamel as a proxy for changes in precipitated meteoric water and climate. Building upon the pioneering work of Longinelli (1984) these papers have further highlighted the fact that the body water of mammals is related to ingested water (either local water or precipitated meteoric water) via metabolic isotope fractionations that are species dependent. Thus, for a given species, the $\delta^{18}O$ variation in tooth enamel phosphate proxies $\delta^{18}O$ variability of local precipitated meteoric water. As a consequence of this relationship the phosphate oxygen isotope analysis of tooth enamel from relatively large mammals has become a routine tool for the reconstruction of past continental palaeoclimates during the Cenozoic (e.g., Late Paleocene to Early Eocene, Koch et al. (1995), Fricke et al. (1998); Late Eocene to Early Oligocene, Bryant et al. (1996), Kohn et al. (2004); Miocene, Fox and Fisher (2001), Tütken et al. (2006); Pleistocene, Delgado-Huertas et al. (1997) and Holocene, Shahack-Gross et al. (1999), Stephan (2000)). Lindars et al. (2001) and Grimes et al. (2003) developed a direct laser fluorination technique, with a new pre-treatment procedure, to analyse the phosphate oxygen from the enamel of small mammal teeth (for example, rodents) for use in continental palaeoclimate reconstruction. One of the advantages of using small mammal teeth in such studies is that they are relatively more abundant in the fossil record than large mammal teeth (e.g., Hooker, 1994), thus potentially allowing higher temporal resolution studies to be conducted. However, there are limitations in using small mammal teeth. Therefore, this paper will review the advantages and limitations of using phosphate oxygen isotopes from small mammal teeth as a proxy for reconstructing continental climate change.

2. Key underlying principles and theoretical considerations

Mammal teeth have two main anatomical parts, the crown and the root. The crown is that part of the tooth which is covered with enamel and protrudes from the gum line. The root is the part embedded in the jaw and which anchors the tooth. Enamel is the hard outer layer of the crown and contains on average ~96 wt.% calcium phosphate, ~3 wt.% water, and ~1 wt.% organic matter (Driessens and Verbeeck, 1990; Hillson, 1986). Structurally enamel is extremely compact with small pore spaces, large phosphate crystals (<100 nm long), and a fibrous texture. It is one of the hardest substances in the human body. Dentine on the other hand is not as hard as enamel, forms the bulk of the tooth, and contains on average 70–75 wt.% calcium phosphate, ~20 wt.% organic matter, and 5–10 wt.% water (Driessens and Verbeeck, 1990; Hillson, 1986). Dentine is also more porous then enamel with 1 μm diameter tubules, and smaller crystals ((<100 nm long) (Hillson, 1986). Two other components of mammal teeth are pulp and cementum. Pulp is a soft tissue containing the blood and nerve supply to the tooth and is therefore rarely fossilised. The pulp extends from within the crown to the tip of the root. Cementum is the layer of bone-like tissue covering the root and often extending to envelope the crown.

Primary biogenic apatite, in the form of either bone, dentine or enamel, can be represented by the chemical formula $Ca_{4.5}[(PO_4)_{2.7}(HPO_4)_{0.2}(CO_3)_{0.3}](OH)_{0.5}$ which corresponds to a calcium hydroxyl apatite mineralogy (Driessens and Verbeeck, 1990). According to the stoichiometry of Elliott (1997), 91.5% of all oxygen in enamel occurs as phosphate, 6.5% as carbonate and 2.9% as hydroxyl. In modern teeth, enamel and dentine phosphate oxygen are precipitated in equilibrium with each other and their corresponding carbonate oxygen values are, at equilibrium, 8.6–9.1‰ higher (Iacumin et al., 1996). This 'apparent' fractionation between phosphate and carbonate oxygen in modern biogenic apatite assumes that the oxygen isotope fractionation between acid-liberated CO_2 and mineral apatite carbonate oxygen is the same as that observed for calcite. The isotope relationship between hydroxyl oxygen and phosphate oxygen, on the other hand, has not yet been demonstrated analytically (Passey and Cerling 2006). However, owing to the reported similarity in the total oxygen isotope composition of enamel and that reported for separated phosphate oxygen in the same teeth, it is assumed that because both the carbonate and hydroxyl bound oxygen appear to compensate for each other when mixed with the phosphate oxygen, then the hydroxyl oxygen must have an isotope value lower than that of phosphate oxygen (e.g., Jones et al., 1999 estimate it to be −16.6‰ relative to phosphate oxygen). This hypothesis, however, still remains to be proven quantitatively.

As enamel is more crystalline and less porous than dentine and bone, it has been demonstrated in a number of studies to be more resistant to diagenesis (e.g., see Kohn and Cerling, 2002 and references therein). Furthermore, because the phosphate-oxygen (P–O) bond has a greater bond strength than the carbon–oxygen (C–O) bond then in the majority of palaeoclimate studies the phosphate oxygen (PO_4^{3-}) has been the preferred oxygen phase to use (e.g., see Grimes et al., 2003 and references therein).

Grimes et al.'s (2003) use of enamel PO_4^{3-} in their continental palaeoclimate studies relied upon Longinelli and Nuti's (1973) phosphate-water thermometer, which was re-calibrated by Kolodny et al. (1983) (1) such that:

$$T°C = 113.3 - 4.38\left(\delta^{18}O_{PO4} - \delta^{18}O_{water}\right) \quad (1)$$

Assuming $\delta^{18}O_{water}$ represents the $\delta^{18}O$ of a mammal's body water ($\delta^{18}O_{bw}$), from which biogenic apatite is precipitated, Eq. (1) can predict $\delta^{18}O_{bw}$ because biogenic apatite is precipitated in equilibrium with a mammal's body water at a near constant body temperature. For example, if we assume a typical mean body temperature for modern rodents of 37 °C (Hart, 1973), this leads to the reduction of Eq. (1) to:

$$\delta^{18}O_{bw} = \delta^{18}O_{PO4} - 17.42 \quad (2)$$

Furthermore, as illustrated in Fig. 1, $\delta^{18}O_{bw}$ is controlled by oxygen influxes (of which local meteoric water predominates), metabolic rate (a function of mammal size), and oxygen outfluxes (Longinelli, 1984; Luz et al., 1984; Ayliffe et al., 1992; Bryant, 1995; Kohn et al., 1996). However, numerous studies on present-day mammals have demonstrated that animals drinking plentifully, generally those eating semi-dry food, have a linear relationship between their $\delta^{18}O_{bw}$ and the $\delta^{18}O$ of the local water ($\delta^{18}O_{lw}$) from which they drank (e.g., Longinelli and Peretti Padalino, 1980; Luz et al., 1984; D'Angela and Longinelli, 1990; Ayliffe et al., 1992). For captive lab rodents (rats) eating semi-dry food in a constant relative humidity of 50% this linear relationship has been expressed by Luz et al. (1984) as

$$\delta^{18}O_{lw} = \left(\delta^{18}O_{bw} - 0.24\right)/0.59 \quad (3)$$

Combining Eqs. (2) and (3) leads to:

$$\delta^{18}O_{lw} = \left(\delta^{18}O_{PO4} - 17.66\right)/0.59 \quad (4)$$

Though this equation is a valid expression of captive lab rodents eating semi-dry food under controlled conditions, Lindars et al. (2001), pointed out that it may not be representative of a wild population. An alternative equation derived from a wild population of rodents (*Apodemus sylvaticus*) was proposed by D'Angela and Longinelli (1990) Eq. (5).

$$\delta^{18}O_{mw} = \left(\delta^{18}O_{PO4} - 21.61\right)/0.79 \quad (5)$$

However, little was recorded about the *Apodemus sylvaticus* habitat and diet in the D'Angela and Longinelli (1990) study, especially the location of a significant local water body from which the rodents could

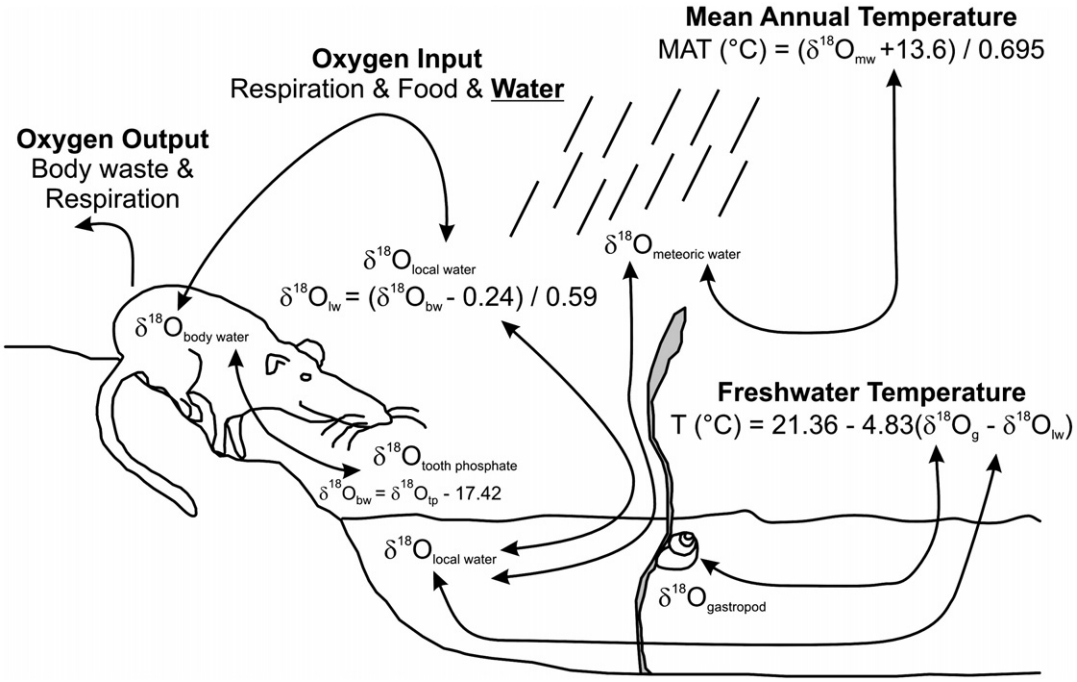


Fig. 1. A schematic diagram outlining how the $\delta^{18}O$ values of tooth enamel phosphate can be used to calculate $\delta^{18}O$ local water ($\delta^{18}O_{lw}$) values, and how this can be combined with another palaeoproxy, in this case *Lymnaea* shell carbonate, to calculate a freshwater mean palaeotemperature. The references for the biogenic fractionation equations and the thermometry equations (for freshwater and mean annual temperature) can be found in the text (Section 2) and in Fig. 2.

have regularly drunk. As a consequence Eq. (5) is calibrated to regional records of precipitated meteoric water ($\delta^{18}O_{mw}$), rainwater, and not a local water source. Because of this drawback and because Lindars et al. (2001) provided clear evidence for a single drinking source, they argued that their study was more in line with the captive rodent study of Luz et al. (1984), and therefore they used Eq. (4) in their palaeoclimate studies (e.g., Lindars et al., 2001; Grimes et al., 2003, 2005).

A third rodent palaeothermometry equation has recently been proposed by Navarro et al. (2004). Based upon the study of present-day European arvicoline teeth and their correlation to recorded regional precipitated $\delta^{18}O_{mw}$ values they proposed the following equation.

$$\delta^{18}O_{mw} = \left(\delta^{18}O_{PO4} - 20.98\right)/0.572 \quad (6)$$

Navarro et al. (2004) noted that their equation had a similar intercept to that of the other wild population study conducted by D'Angela and Longinelli (1990), but the slope of the latter was significantly different. This they argued could be due to their use of a different genus of rodent and therefore be a consequence of different metabolic activities. However, they also noted that it could be due to uncertainties related to the composition of ingested water derived from food and not from free standing water sources.

Eqs. (4–6) are the three published palaeothermometry equations for rodents. These and other published biogenic fractionation equations for different mammal taxa, which can all be used in the reconstruction of the oxygen isotope composition of local water (drinking water), are listed in Table 1. The left hand part of Fig. 1 also displays a schematic representation of the biogenic fractionation processes proposed by Luz et al. (1984) that occur during the ingestion of local water by rodents.

Either by directly calculating the local precipitated $\delta^{18}O_{mw}$ value, or by assuming that the calculated $\delta^{18}O_{lw}$ value is comparable to that of the local precipitated $\delta^{18}O_{mw}$ value, then changes in the isotope ratio of either can be used to track changes in past continental climate regimes. This is because the isotope composition of rainwater depends on temperature and/or precipitation amount, as well as the source and movement of moisture (e.g., Dansgaard, 1964; Rozanski et al., 1993). Therefore, if it can be demonstrated that there are no significant changes in either the amount of precipitation, or the source and

Table 1
A list of some of the published mammalian biogenic fractionation equations that can be used in the reconstruction of the $\delta^{18}O$ local water ($\delta^{18}O_{lw}$) value from which the mammal drank

Equation: $\delta^{18}O_{lw}=(\delta^{18}O_{PO4}-K1)/K2$					
Animal	Life mode of animal	Material analysed	*K*1	*K*2	Reference
Human	N/A	Blood & bone	22.37	0.64	Longinelli (1984)
Pig	Domesticated	Blood & bone	22.71	0.86	Longinelli (1984)
Rodent (rat)	Captive	Bone	17.66	0.59	Luz et al. (1984)
Rodent (wood mouse)	Wild	Bone	21.61	0.79	D'Angela and Longinelli (1990)
Deer	Wild	Bone	25.55	1.13	D'Angela and Longinelli (1990)
Sheep	Domesticated	Bone	27.21	1.48	D'Angela and Longinelli (1990)
Cattle	Domesticated	Bone	24.90	1.01	D'Angela and Longinelli (1990)
Elephant	Wild	Teeth & bone	23.30	0.94	Ayliffe et al. (1992)
Horse	Wild	Teeth & bone	22.60	0.71	Huertas et al. (1995)
Goat and roe-buck	Domesticated & wild	Bone	24.39	0.91	Huertas et al. (1995)
Reindeer	Wild	Teeth & bone	15.96	0.39	Iacumin and Longinelli (2002)
Fox	Wild	Teeth & bone	25.49	1.34	Iacumin and Longinelli (2002)
Rodent (vole, lemming)	Wild	Teeth	20.98	0.57	Navarro et al. (2004)
Bison	Wild	Teeth	21.23	0.70	Hoppe (2006)

Depending on the study the $\delta^{18}O_{lw}$ value either is representative of regional precipitated meteoric water or is that of a known free standing water body.

movement of moisture in a specific area, then any changes in $\delta^{18}O_{mw}$ values over time must be related to changes in temperature. Such an approach was used by among others Kohn et al. (2004) to demonstrate possible climate stability across the Eocene–Oligocene transition.

Other studies, including that of Navarro et al. (2004), have proposed that the precipitated $\delta^{18}O_{mw}$ value can be used to calculate a mean annual temperature (MAT). For example, for mid to high latitudes experiencing the same meteorological conditions as those operating today, this can be achieved using for example Dansgaard's (1964) equation such that:

$$MAT(°C) = \left(\delta^{18}O_{mw} + 13.6\right)/0.695 \quad (7)$$

However, it should be noted that in some cases Dansgaard's (1964) equation may not be the most suitable equation to use in calculating a MAT. For example, Tütken et al. (2006), in a multi-proxy palaeoenvironmental reconstruction of the Middle Miocene Steinheim Basin of SW Germany, used a regional $\delta^{18}O_{H20}$-MAT equation. Furthermore, caution must be exercised when applying a present-day derived $\delta^{18}O_{H20}$-MAT relationship to certain ancient environments as there is theoretical and empirical evidence that the same meteorological and environmental parameters that govern the current relationship may not always have applied in the past (e.g., see Boyle, 1997; Fricke and O'Neil, 1999; Fricke and Wing, 2004).

The calculation of a MAT is fortunately not the only way to quantifiably reconstruct the palaeoclimate of past continental environments using biogenic apatite. As will be detailed later in Section 4.2 of this paper, Grimes et al. (2003, 2005) have extended the use of mammal teeth in climate studies by combining the $\delta^{18}O_{lw}$ value determined from mammalian enamel, with oxygen isotope values from co-existing freshwater biota to obtain freshwater summer season palaeotemperatures of varying climatic significance.

3. Methodologies for phosphate oxygen isotope determination

3.1. Chemical separation techniques and the analysis of silver phosphate

Oxygen is present at three different sites in the biogenic apatite structure. However, because phosphate oxygen (PO_4^{3-}) is considered the most resistant to post-depositional diagenetic alteration (e.g., see Kohn and Cerling, 2002 and references therein), it is PO_4^{3-} that is typically separated from the apatite structure, analysed and used in the majority of palaeoclimate reconstructions involving biogenic apatite. The PO_4^{3-} ion was first separated from biogenic apatite by Tudge (1960) using a rather time consuming and complicated methodology that involved acid digestion and re-precipitation as bismuth phosphate ($BiPO_4$). Firsching (1961) proposed an alternative and simpler methodology that involved the re-precipitation of PO_4^{3-} as silver phosphate (Ag_3PO_4). However, despite the lengthy chemical procedure and the hygroscopic nature of $BiPO_4$, this was the standard methodology until Crowson et al. (1991) resurrected and refined the Ag_3PO_4 methodology. Because of the non hygroscopic nature of Ag_3PO_4 this methodology is now the standard chemical separation technique, though minor modifications to the Crowson et al. (1991) methodology have been proposed by among others Lécuyer et al. (1993) and Dettman et al. (2001).

The oxygen isotope analyses of Ag_3PO_4 can be conducted using a variety of methodologies. These include resistance heating of Ag_3PO_4–graphite mixtures in isolated silica tubes (O'Neil et al., 1994); reaction with bromine (Stuart-Williams and Schwarcz, 1997); laser heating Ag_3PO_4–graphite mixtures (Wenzel et al., 2000); and fluorination (with either BrF_5, ClF_2 or F_2) which has been specifically refined for phosphate analysis by Crowson et al. (1991), Lécuyer et al. (1996) and Grimes et al. (2003). All of these reaction methodologies generate molecular oxygen (O_2) which, with the exception of the Grimes et al. (2003) methodology, is converted to CO_2 for isotope analysis. Another methodology, which generates and measures O_2, was proposed by Holmden et al. (1997). The methodology involves filament loaded Ag_3PO_4 which is analyzed by thermal ionization in a mass spectrometer configured for negative ion analysis. This technique, however, is considered time consuming, relatively expensive and its accuracy and precision are not considered comparable to other techniques (Vennemann et al., 2002). A more recent technique, which was specifically developed for the analysis of small amounts of Ag_3PO_4, involves the quantitative on-line high-temperature reduction of Ag_3PO_4 using graphite in a glassy carbon reactor to produce carbon monoxide (CO), which is then analysed by a continuous flow mass spectrometer (see Kornexl et al., 1999, for details). Vennemann et al. (2002) reviewed, in more detail than can be presented here, each of the aforementioned analytical techniques. In particular they highlighted the fact that because of the lack of an international biogenic phosphate oxygen isotope standard (NIST120c, though used by the majority of laboratories is not an international recognized isotope standard, nor is it biogenic in origin, or composed of 100% carbonate fluoro-apatite) it is important that the different techniques used by different laboratories are calibrated to allow comparison of different data sets.

One of the early perceived limitations of the PO_4^{3-} chemical separation technique was the requirement that a starting mass of 2–5 mg of sample was needed to ensure that, after the digestion and re-precipitation of Ag_3PO_4, which does not always produce 100% yields, there was enough material retrieved for the isotope analysis. Partially as a result of this early limitation the use of small mammals in continental palaeoclimate studies was restricted. This in part led to the development of numerous in-situ laser techniques (see Section 3.2 for a summary), including the direct laser fluorination technique of Lindars et al. (2001), which was designed specifically for the analysis of enamel samples of around 1 mg in weight (see Section 3.3 for a summary). However, around this same time refinements were being made to the chemical separation technique. Therefore, there are now numerous studies that have shown that it is possible to run samples of ~1 mg through the chemical separation technique and, assuming a yield of around 100% is obtained, still generate enough Ag_3PO_4 to allow reliable $\delta^{18}O$ values to be determined (e.g., see Dettman et al., 2001; Straight et al., 2004; Zazzo et al., 2006).

3.2. Laser techniques employed in the analysis of untreated teeth

A number of different laser techniques have been published which vary considerably in their mode of analysis and, more significantly, in terms of which oxygen species are targeted for isotope analysis. The first technique to be published used an infrared laser to locally heat untreated tooth enamel to liberate CO_2 by thermal decomposition of the carbonate component (Cerling and Sharp, 1996). Although a clear linear relationship was found between results from laser and conventional H_3PO_4 techniques (which liberate the carbonate component of biogenic apatite by acid digestion) the $\delta^{18}O$ laser results were offset by on average −7.0±1.4‰. Similar results were also obtained by Passey and Cerling (2006) using a refined CO_2 laser technique, which was specifically designed for the analysis of small mammal teeth. They also recorded a clear linear relationship between results from laser and conventional H_3PO_4 techniques, though the laser results on fossil teeth were offset by on average only −5.1±1.2‰. In contrast to the more recent study of Passey and Cerling (2006), the original work by Cerling and Sharp (1996) also went one stage further. They argued that when their $\delta^{18}O$ laser results were compared to Ag_3PO_4 isotope results from splits of the same teeth, the offset between the two data sets was reduced to around 1.2‰. Cerling and Sharp (1996) suggested that this was the result of their laser technique mixing oxygen from all the sites in the biogenic apatite structure (i.e., the phosphate, carbonate and hydroxyl sites). This resulted in a bulk $\delta^{18}O$ value, which they argued was within error (i.e., sample

heterogeneity) of the phosphate oxygen value due to the carbonate and hydroxyl oxygen compensating for each other (see Section 2 for a background discussion). Finally, Kohn et al. (1996) and Jones et al. (1999) both developed laser fluorination techniques, one infrared and the other ultraviolet, to specifically obtain bulk $\delta^{18}O$ values on untreated biogenic apatite samples. In addition to noting low oxygen yields they also both reported that their $\delta^{18}O$ values were subject to a uniform offset of ~1.7‰ relative to Ag_3PO_4 isotope results. This offset is similar in magnitude to that reported by Cerling and Sharp (1996), at 1.2‰, which they attributed in part to sample heterogeneity in relatively large teeth where seasonal changes in temperature will cause variations in $\delta^{18}O_{PO4}$ values. Therefore, Cerling and Sharp (1996), Kohn et al. (1996) and Jones et al. (1999) have claimed, with cited limitations, to have developed laser analytical techniques that can be exploited in palaeoclimate reconstruction studies.

More recently, Passey and Cerling (2006) have developed a revised in-situ thermal laser ablation technique specifically for the high resolution analysis of intra-tooth carbon and oxygen isotope profiles in small mammal teeth with enamel as thin as ~100 μm. Though the authors clearly state that the accuracy of oxygen isotope analyses obtained using their new technique is lower than that of more conventional chemical separation, large-scale oxygen isotope, and detailed carbon isotope, patterns are resolvable. This they demonstrated by measuring intra-tooth profiles on marmot incisors from Utah and Mongolia. Their isotope results show quasi-sinusoidal variations with amplitudes of 7.1‰ and 8.2‰ for oxygen, and 3.4‰ and 3.8‰ for carbon. Furthermore, the carbon and oxygen isotope minima in both incisors correspond spatially with disruptions in enamel morphology similar to 'hibernation marks' described in squirrels and marmots. Therefore, even though there are limitations with using any laser ablation technique this revised in-situ thermal laser ablation method does offer the possibility of conducting, for the first time, high resolution intra-tooth reconnaissance analyses on small mammal teeth.

All of the aforementioned laser techniques are time efficient, as they do not require a chemical separation technique, and they offer the opportunity of conducting very high resolution studies; they are, however, not widely exploited for a number of reasons. Firstly, they require specialist laser equipment which is not available in many stable isotope laboratories. Secondly, and more importantly, they either directly or indirectly target all forms of available oxygen (phosphate, carbonate and hydroxyl) in the biogenic apatite structure and if they are to be used in quantifiable palaeoclimate studies they rely upon the notion that hydroxyl and carbonate oxygen equally compensate for each other such that the bulk $\delta^{18}O$ would equal the phosphate oxygen $\delta^{18}O$ value. Though this may be a feasible notion in modern calibration studies, when using fossil samples this may not be the case as the carbonate and hydroxyl groups are prone to diagenetic alteration (Kohn et al., 1999), which may not be of an equal magnitude.

3.3. A direct laser fluorination technique for phosphate oxygen isotope analysis

Lindars et al. (2001) reported a direct laser fluorination (DLF) technique for the analysis of small mammal teeth, which only required a sample mass of ~1 mg, and, as a result of a new pre-treatment methodology, is still believed to be the only laser fluorination technique to target just the phosphate oxygen component of biogenic apatite. Based upon a series of step heating experiments, they demonstrated that the first stage of the pre-treatment, which involved heating at 400 °C for 1 h, removed water bound oxygen and oxidised and removed organically bound oxygen. Secondly, after the samples were loaded into an online sample chamber and fused using a 25 W CO_2 laser under a continuously pumped high vacuum, carbonate oxygen was removed, leaving a calcium phosphate residue, which could be analysed by laser fluorination (Lindars et al., 2001). Grimes et al. (2003) further tested the validity of the DLF technique using both untreated enamel and also enamel derived Ag_3PO_4 samples with known $\delta^{18}O$ values from the inter-lab calibration study of Vennemann et al. (2002). Furthermore, they conducted Fourier Transform Infrared (FT-IR) spectral analyses on fused beads of enamel and dentine to ascertain the nature of the oxygen present prior to DLF. Their results showed that the pre-treatment technique and the DLF analysis generated $\delta^{18}O$ values for enamel, which were within error (±0.4‰) of those obtained from the silica tube analysis of Ag_3PO_4 precipitates from the same homogenised sample. However, Grimes et al. (2003) also noted that their new technique generated $\delta^{18}O$ values for dentine rich samples (whole teeth) which were on average 6‰ below that obtained from the silica tube analysis of Ag_3PO_4 precipitates from the same homogenised sample. A possible explanation for this was provided by the FT-IR spectral results, which demonstrated that the dentine samples appeared to have retained hydroxyl oxygen after fusing, which Grimes et al. (2003) argued could account for the negative offset. The reason why hydroxyl oxygen was retained in the fused dentine and not in the fused enamel is not fully understood. Nevertheless, as a result of these combined experimental observations Grimes et al. (2003) argued that the pre-treatment and DLF technique was a reliable methodology for the phosphate oxygen isotope analysis of enamel samples, but not for dentine or, by association, bone.

More recently, Grimes et al. (2004a) reported a minor modification to the original analytical protocol that governs the DLF technique. The Lindars et al. (2001) protocol originally stated that a single DLF run should only consist of one biogenic apatite (enamel) sample with two non-apatite standards for daily offset correction. The reason for this original protocol was the perceived potential for premature fluorination of multiple apatite samples leading to a mixing of phosphate oxygen from a number of sources (e.g., different enamel samples). Evidence for this was seen in early analytical runs which showed a progressive reduction in the oxygen yield of individual samples as the run progressed. The requirement that only one biogenic apatite sample be analysed per run seriously reduced the efficiency of the DLF technique. However, further experimentation, with a slightly modified sample tray and new fusing protocol, now means that up to 8 enamel samples, with 4 standards (quartz and apatite) can be included in a single DLF run. Other important results reported by Grimes et al. (2004a) included evidence that teeth, belonging to the extinct rodent *Thalerimys*, etched by digestion (Vasileiadou et al., 2006), had not experienced any isotope fractionation. It was also demonstrated that moulding of *Thalerimys* teeth to produce a 3D cast prior to sample preparation and analysis did not affect enamel phosphate oxygen isotope values. Both of these findings are important because they enable the use of small mammal teeth that would have previously been considered either unsuitable or too rare for destructive isotope analysis.

4. Small mammal teeth versus large mammal teeth

4.1. Oxygen intake (food versus drinking water) and diagenesis

Though large mammal teeth have routinely been used in the reconstruction of the oxygen isotope composition of local water, this is not the case for small mammal teeth. Previously, relatively large mammals have been preferred to small mammals because drinking water has been clearly shown in either modern relatives, or in mammals of a similar body mass, to form a large proportion of their oxygen intake (Bryant and Froelich, 1995). However, numerous studies (Grimes et al., 2003, 2005; Navarro et al., 2004; Tütken et al., 2006) have all argued that with relatively large mammals, the potential of long distance migrations means that the $\delta^{18}O_{PO4}$ values in their cheek tooth enamel (which unlike bone mineralizes early in the life of the animal and is not renewed) is representative of their site of weaning rather than their site of death, which is the one being reconstructed. In

contrast, the home ranges of small mammals (including rodents) and their predators, which can be responsible for localised rich accumulation deposits of prey remains, are typically small, thus favouring their use in such isotope studies. However, even though a small home range is a benefit when reconstructing the palaeoclimate of a region there are nevertheless potential drawbacks with using small mammals. A number of these issues were addressed in a test study by Grimes et al. (2003). They argued that before small mammals could be routinely used in the determination of $\delta^{18}O_{lw}$ values two key requirements had to be met.

First, it is necessary to demonstrate that the majority of a small mammal's water intake came from the consumption of water, rather than from their food. Grimes et al. (2003) used a variety of evidence to demonstrate that the extinct theridomyid rodents *Thalerimys* and *Isoptychus* obtained their water intake principally from groundwater rather than food. Firstly, ecological diversity analysis of the mammal fauna from the fossil localities independently suggested that the habitat ranged from forest to more open areas with woodland patches (Hooker, 1992). These were mesic environments, the kind where modern rodents typically obtain their water by drinking rather than from their food (Schröpfer, 1974; Wright, 1976). Furthermore, it has been demonstrated that *Thalerimys* and *Isoptychus*, from the anatomy of attributed ankle bones (JJH Pers. Obs. 2000) and articulated skeletons of near relatives (Schmidt-Kittler and Storch, 1985), were essentially ground dwelling animals. Grimes et al. (2004b) in a palaeodietary study concluded that these genera foraged amongst wetland vegetation in close association with local water bodies. The ready availability of these local water bodies to the rodents for drinking is evidenced by the occurrence of their remains in sediments that accumulated in freshwater ponds and lakes. Finally, the combined dominance of *Thalerimys* and *Isoptychus* through almost all of a four million year UK Paleogene succession, regardless of lithofacies, and evidence for predator–prey interaction with no evidence for long distance transport of remains (Vasileiadou et al., 2007), indicates that they lived as part of local mammal communities and died close to the edges of the water bodies in which they became entombed.

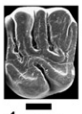
The second requirement for suitability of using small mammal tooth enamel in the determination of a mean $\delta^{18}O_{lw}$ value is evidence that the enamel $\delta^{18}O_{PO4}$ values used in its calculation have not been diagenetically altered. In previous climate related studies using mammal teeth, the enamel layers were thick simply because the animal's body size was large and partly because of this enamel was shown to be resistant to diagenesis (among others, horse, Bryant et al., 1996 and Paleogene *Coryphodon*, Fricke et al., 1998). The enamel of small mammals, like rodents, on the other hand, is necessarily much thinner. Therefore, it is important to show that phosphate oxygen in this thin enamel is as robust to diagenetic alteration as that in thick enamel. Grimes et al. (2003) demonstrated that this was indeed the case by comparing the equilibrium fractionation offset of carbonate (CO_3^{2-}) and phosphate (PO_4^{3-}) oxygen isotope values of enamel from co-existing teeth and also by Electron Microprobe Wave Dispersive Spectral (EM-WDS) analysis of the Ca/P and the F/P molar ratios in enamel and dentine. Their EM-WDS results provided clear evidence that diagenetic alteration had converted the original hydroxyl apatite mineralogy of the *Thalerimys* and *Isoptychus* dentine to that of carbonate fluoro-apatite, thus suggesting that their CO_3^{2-} and PO_4^{3-} oxygen isotope values would be diagenetically altered (Bryant, 1995). In contrast, the enamel of *Thalerimys* and *Isoptychus* appeared to have retained its original hydroxyl apatite mineralogy. However, even though there was still an equilibrium offset between the PO_4^{3-} and the CO_3^{2-} components of the enamel (average 4.5‰), this was significantly reduced compared to that which was expected (between 8.6 and 9.1‰). This, in connection with the stronger P–O bond strength, suggested that the CO_3^{2-} component of the enamel had suffered diagenetic alteration, while the PO_4^{3-} component had remained largely unaltered. Therefore, as for larger mammals, (e.g., Longinelli 1984; Luz et al., 1984, 1990; Ayliffe and Chivas, 1990; Fricke et al., 1998; Stuart-Williams and Schwarz, 1997; Kohn et al., 1998) the enamel PO_4^{3-} oxygen isotope composition of small mammal teeth (in this case theridomyid rodents) can also be used reliably in continental palaeoclimate reconstructions. Navarro et al. (2004) came to a similar conclusion for arvicoline teeth, though based upon slightly different criteria (e.g., preservation of the original apatite stoichiometry).

A critical point discussed by Grimes et al. (2005), is the constancy of the body temperature of mammals. This value forms a key component of the biogenic fractionation equations from Luz et al. (1984) given in Section 2, which enable the $\delta^{18}O_{PO4}$ in rodent tooth enamel to be used to calculate $\delta^{18}O_{lw}$ values. Hart (1973) recorded the typical mean body temperature for modern rodents as 37±2 °C. A 1 °C change in body temperature would generate a 2 °C difference in calculated freshwater palaeotemperatures (Grimes et al., 2005; see Section 4.2). This potential error factor should be considered in all palaeoclimate change studies using biogenic apatite palaeoproxies, regardless of the body mass of the animal being used.

4.2. Calculation and significance of palaeotemperatures

Because Grimes et al. (2003) demonstrated that the small mammals they used in their palaeoclimate studies had a close association with a single water body, and that the PO_4^{3-} in their tooth enamel was not diagenetically altered, they argued that the $\delta^{18}O_{lw}$ values they calculated could in theory be used to calculate a MAT, though there are theoretical and practical limitations in doing so (see Section 5.1 for details). They also demonstrated that $\delta^{18}O_{lw}$ values could be combined with $\delta^{18}O$ values from freshwater biota to calculate, using established thermometry equations, multiple palaeotemperatures of differing climatic significance (see Fig. 1 for a schematic example and Fig. 2 for the palaeoproxies and thermometry equations used by Grimes et al., 2003, 2005). To interpret the climatic significance of these palaeotemperatures it is necessary to first understand the timing of enamel mineralization. Relatively low crowned molars, like those of *Thalerimys* and *Isoptychus* mineralize and erupt a short time (weeks) after birth. However, the exact time interval in the year when the theridomyid enamel, which is used to calculate the $\delta^{18}O_{lw}$ values, mineralized is dependent upon the breeding season of the rodents (Grimes et al., 2003; Navarro et al., 2004). Modern studies suggest that the smaller the mammal the less seasonal their breeding, probably because of a positively correlated short gestation period (Kiltie, 1988). Relatively small rodents like theridomyids can, therefore, be treated as low seasonality breeders. The mineralization of their cheek teeth, and therefore the calculated $\delta^{18}O_{lw}$ value, should, as a result, encompass many, but perhaps not all, of the temperature fluctuations throughout a year (Grimes et al., 2003; Navarro et al., 2004). Therefore, any MAT calculated from $\delta^{18}O_{lw}$ values determined on small mammal teeth are likely to be over-estimated, as they may not incorporate the coldest months of the year. Furthermore, before calculating any MAT it is necessary first to take account of potential localised evaporation affecting $\delta^{18}O_{lw}$ values, such that they no longer proxy meteoric water (Grimes et al., 2003, 2005; Tütken et al., 2006), and secondly to ascertain if present-day $\delta^{18}O_{H20}$–MAT relationships can be applied to ancient environments (see Section 5 for more details).

In order to understand the significance of any palaeotemperatures calculated by combining a $\delta^{18}O_{lw}$ value with that obtained from a freshwater biotic proxy, it is important to take account of the mineralization period of that proxy. For example, Grimes et al. (2003, 2005) noted that fish scales and gastropod shells are known to mineralize throughout the growing season but that charophyte gyrogonites mineralize over a one month period towards the end of the growing season (Jones et al., 1996). Therefore, the mean palaeotemperatures calculated by combining the $\delta^{18}O_{lw}$ value with the $\delta^{18}O$ values of separated ganoine from whole fish scales and whole gastropod shells, using published thermometry



A After Luz *et al.*'s (1984) equation for captive lab rodents with a body temp. of 37°C.
$\delta^{18}O_{lw} = (\delta^{18}O_{phos.\ (VSMOW)} - 17.66) / 0.59$
Dansgaard's (1964) **Mean Annual Temperature** equation; $MAT(°C) = (\delta^{18}O_{lw} + 13.6) / 0.695$

1mm

B White *et al.*'s (1999) *Lymnaea* genus-specific carbonate thermometer.
$Temp.\ (°C) = 21.36 - 4.83(\delta^{18}O_{carb.\ (VPDB)} - \delta^{18}O_{lw})$
Mean Growing Season Temperature

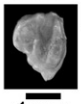
5mm

C Kolodny *et al.*'s (1983) re-calibration of Longinelli and Nuti's (1973) phosphate thermometer.
$Temp.\ (°C) = 113.3 - 4.38(\delta^{18}O_{phos.\ (VSMOW)} - \delta^{18}O_{lw})$
Mean Growing Season Temperature

5mm

D Hays and Grossman's (1991) freshwater carbonate thermometer.
$Temp.\ (°C) = 15.7 - 4.36(\delta^{18}O_{carb.\ (VPDB)} - \delta^{18}O_{lw}) + 0.12\ (\delta^{18}O_{carb.\ (VPDB)} - \delta^{18}O_{lw})^2$
Mean Temperature of a Single Month in the latter part of the Growing Season

1mm



E Patterson *et al.*'s (1993) freshwater otolith specific thermometer.
$Temp\ (°C) = (18564 / ((Ln\ (((10^3 + (\delta^{18}O_{carb.\ (VPDB)} \cdot 1.03091 + 30.91)) / (10^3 + \delta^{18}O_{lw}))) . 10^3) + 33.491)) - 273.15$
Mean Temperature of Warmest Months of the Growing Season

1mm

Fig. 2. Representative images of the five palaeoproxies from the Late Eocene Headon Hill Formation, Hampshire Basin, UK and which were used by Grimes et al. (2003, 2005) in their studies of continental palaeoclimate reconstruction. A) an upper left first or second molar tooth of the rodent *Thalerimys fordi*; B) a shell of the pulmonate gastropod *Lymnaea longiscata*; C) a scale of the fish *Lepisosteus*; D) a gyrogonite of the charophyte *Nitellopsis*; E) an otolith (sagitta) of the fish *Umbra valida*. Explanations for the use of the thermometry equations and justifications for the climatic significance of each mean palaeotemperature is reported in the text (Section 4.2).

equations, should be concordant and represent the mean growing season temperature (Fig. 2). The mean palaeotemperature calculated using whole charophyte gyrogonites and the thermometry equation of Hays and Grossman (1991), on the other hand, should represent the mean temperature of a single month in the later part of the growing season. In the case of freshwater fish otolith growth, temperature tolerance, and thus the length of the growing season, can vary widely, but, even in eurythermic species, growth is most rapid during the warmest part of the year (Patterson et al., 1993). Therefore, Grimes et al. (2003, 2005) concluded that palaeotemperatures calculated using whole fish otoliths will be biased towards the mean temperature of the warmest months of the growing season (Fig. 2).

The derivation of thermometry equations is a factor that should also be considered. Grimes et al. (2003, 2005) used a range of thermometry equations, some of which were genus specific, some only composition specific. For example, these authors used otoliths of the freshwater fish species *Umbra valida*, which is extinct. The genus *Umbra* (family Umbridae, mud minnows) is, however extant, but neither it, nor its closest family relative Esocidae (pike), were represented in the calibration study by Patterson et al. (1993). Therefore, even though Patterson et al. (1993) used nine different genera of freshwater fishes in their determination of a freshwater otolith thermometry equation, it may not be valid for all freshwater fish families. It could be argued that a family or genus specific thermometry equation would be preferable, like those of the marine fish *Micropogonias undulatus* (Thorrold et al., 1997) and the freshwater gastropod *Lymnaea* (White et al., 1999). However, it should also be noted that the thermometry equation used by Grimes et al. (2003) in the calculation of the mean growing season temperature from the ganoine of fish scales was not taxon specific but composition specific (i.e., phosphate) and it still generated a mean growing season temperature similar to that of the genus specific *Lymnaea* equation. Therefore, although genus (or at least family) specific thermometry equations are preferable, they may not be essential for all palaeotemperature reconstructions.

4.3. Calculation of errors, standard deviations and seasonality

It is important, whenever considering any analytical data set, to be aware of the error surrounding any individual results. This particularly applies to the summer season palaeotemperatures determined by Grimes et al. (2003, 2005). In their study, two independent analytical results (unknown variables) combined to determine an outcome. For example, the *Lymnaea* genus specific carbonate thermometer of White et al. (1999) (see Fig. 2) can be written as

$$Y = K_1 - K_2(A - B) \tag{8}$$

Where Y is temperature, K_1 and K_2 are constants and A and B unknown variables (in this case $\delta^{18}O_{lw}$ and the $\delta^{18}O$ of the *Lymnaea* shell). From this simplified equation the 1(error ((∇) on the calculated temperature propagates as

$$\nabla^2 Y = \nabla^2 A(dY/dA)^2 + \nabla^2 B(dY/dB)^2 \tag{9}$$

Similar error propagations were applied by Grimes et al. (2003, 2005) to all the thermometry equations shown in Fig. 2. Furthermore, Grimes et al. (2003) argued that as the propagated standard error represents the accuracy to which the mean of a data set has been calculated then it should be used to compare the level of agreement between calculated mean palaeotemperatures of the same climatic significance (e.g., mean growing season temperatures). The propagated standard deviation, on the other hand, represents the fluctuation of data around its mean value. This, according to Grimes et al. (2003), contains four principle components; 1) analytical error; 2) variable water source intake, such as water sourced from food; 3) variable non-equilibrium metabolic fractionation during carbonate and phosphate oxygen isotope precipitation; 4) variable water temperatures due to seasonal fluctuations. Only that contributed by analytical error can be directly determined and this is relatively small in the majority of

phosphate oxygen isotope studies (e.g., ±0.2‰ using the DLF technique of Grimes et al., 2003) with an average contribution of ~ 1 °C to the standard deviation on any calculated palaeotemperature. Furthermore, Grimes et al. (2003, 2005) also argued that the variability contributed by theridomyids sourcing their water intake from food is likely to be minor (see Section 4). Models derived by Bryant and Froelich, (1995) suggest that the larger the mammal's body size, the greater its proportion of oxygen intake from drinking water and therefore the lower its body-mass fractionation and potential error on the $\delta^{18}O_{lw}$ determination. However, Lindars et al. (2001) showed, in a study of modern rodents, that there was a good correlation between the calculated $\delta^{18}O$ of local water using phosphate oxygen tooth enamel of the small rodent *Apodemus sylvaticus* and the measured $\delta^{18}O$ of the local water source from which they drank, which suggests that body-mass related fractionation in small mammals is not a major source of variation. Navarro et al. (2004) came to a similar conclusion when they demonstrated that there was a good correlation between the $\delta^{18}O$ of meteoric water and the $\delta^{18}O$ of phosphate precipitated in arvicoline rodent teeth. Grimes et al. (2005) therefore used this combined evidence to argue that a significant proportion of the standard deviation in their multi-proxy derived summer season palaeotemperatures is likely to be the result of variations in water temperature attributable to seasonal fluctuations.

5. Specific examples of the use of small mammal teeth in continental palaeoclimate reconstruction studies

5.1. Evaluating isotope data sets: considering evaporative and ice volume effects

Grimes et al. (2003) used the new analytical techniques described by Lindars et al. (2001), i.e., small mammal teeth with the pre-treatment and the DLF technique of measuring phosphate oxygen isotope values in separated enamel. These new techniques were applied to the study of a bed in the Late Eocene Headon Hill Formation of the Hampshire Basin, UK. Five different palaeoproxies were used; enamel from rodent cheek teeth, gastropod shells, charophyte gyrogonites, fish otoliths, and fish scale ganoine (Fig. 2), all of which co-occurred in the same fossil horizon. Firstly, evidence was provided of at most only minimal or insignificant diagenetic alteration of both the carbonate (gastropod shells, charophyte gyrogonites and fish otoliths) and phosphate proxies (rodent tooth enamel and fish scale ganoine). Secondly it was demonstrated that there were no significant differences in the enamel $\delta^{18}O_{PO4}$ values between different types of permanent cheek teeth (i.e., between first or second molars [M1/2], third molars [M3] and fourth premolars [P4]) or between the two genera (*Thalerimys* and *Isoptychus*). Deciduous teeth were avoided as other studies had shown that oxygen (e.g., Wright and Schwarcz, 1998) and carbon isotope (e.g., Balaisse et al., 2001) results are likely to be influenced by intake of the mother's milk in a pre-weaning individual. Later work (Grimes et al., 2004b) showed this caveat to be justified for theridomyids with respect to $\delta^{13}C$ values (on average a 1.5‰ offset) and therefore by association also with respect to $\delta^{18}O$ values. Grimes et al. (2003) reported that the DLF analysis of separated enamel from 74 individual teeth gave a calculated $\delta^{18}O_{lw}$ value of 0.0 ± 3.4‰. However, they also discovered that the frequency of the measured $\delta^{18}O_{lw}$ values clearly showed a skewed distribution towards more positive isotope values.

This skewed distribution in the $\delta^{18}O_{lw}$ values (Grimes et al., 2003) was not caused by a single taxon type or tooth type, as all contributed at least one result to the skew. The skew was also unlikely to be due to diagenetic alteration or mixing of carbonate and phosphate oxygen sources during DLF, as analysis of the carbonate oxygen component of the enamel indicated that both processes, if significant, would result in a lowering of $\delta^{18}O_{lw}$ values. There was also no evidence to indicate that the few teeth with the highest $\delta^{18}O_{PO4}$ values could have been reworked from older horizons. However, evidence, in the form of mud cracks and palaeosols, in the overbank deposits of the sedimentary unit involved, did suggest that short-lived periodic evaporation had affected water bodies on the floodplain where the animals lived. Grimes et al. (2003) argued that this would have led to isotope fractionation of oxygen in the water body from which the mammals

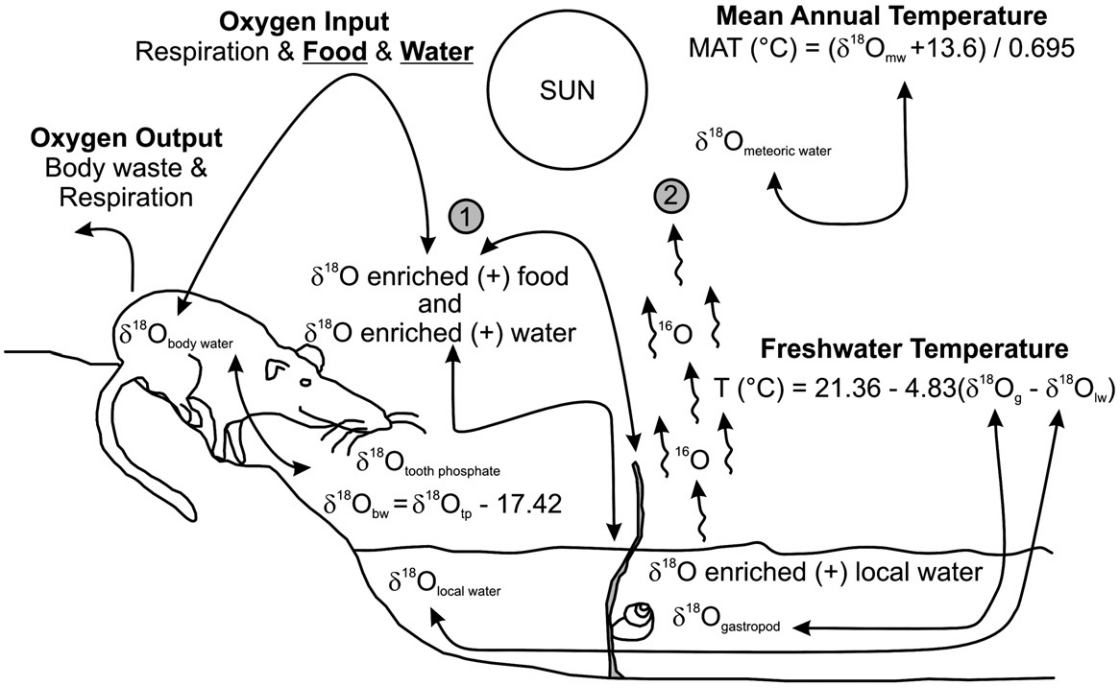


Fig. 3. A schematic diagram outlining how the $\delta^{18}O$ of tooth enamel phosphate can be affected by 1) an increased intake proportion of dietary (plant) ^{18}O enriched water versus local water in some animals and 2) evaporative fractionation of oxygen in the local water source drunk by some of the rodents. The references for the biogenic fractionation equations and the thermometry equations can be found in the text (Section 2) and in Fig. 2.

drank (and in which they became entombed) so that ingestion of this fractionated water source during a drier interval could account for the skewed distribution in the $\delta^{18}O_{lw}$ values (Fig. 3).

Furthermore, Grimes et al. (2005) found this same skewed distribution in data sets compiled from five other Eocene–Oligocene horizons and also attributed it to evaporative fractionation of oxygen in the local water source from which the rodents drank (e.g., in isolated embayments of shallow lakes amongst marginal vegetation). However, they also accepted that an increased intake proportion of dietary (plant) ^{18}O enriched water versus meteoric water in some animals might also contribute to the higher $\delta^{18}O_{lw}$ values (Fig. 3) and thus generate a skewed distribution. In order to account for the skewed distribution more objectively than previously (e.g., Grimes et al., 2003), Grimes et al. (2005) used the log-normal mean (rather than the mean) of the individual $\delta^{18}O_{lw}$ values at each horizon to calculate a representative $\delta^{18}O_{lw}$ value. However, even with this new approach, the log-normal mean $\delta^{18}O_{lw}$ value for each fossil assemblage was more positive than ice volume-corrected seawater $\delta^{18}O$ values for this time (Lear et al., 2000) (Fig. 4). Tütken et al. (2006) obtained a similar result, albeit from a smaller data set of small mammal teeth, in a study of a Middle Miocene lake deposit in SW Germany. As $\delta^{18}O_{mw}$ (precipitation) values cannot be more positive than seawater values both Grimes et al. (2005) and Tütken et al. (2006) stated that their small mammal derived $\delta^{18}O_{lw}$ values, even if they were representative of $\delta^{18}O_{mw}$ (precipitation) values, could not be used to calculate reliable MATs as they had probably been affected by evaporation. Furthermore, even if MATs had been calculated by Grimes et al. (2005), using a present-day $\delta^{18}O_{H20}$-MAT correlation, they would need to be treated with caution. This is because Fricke and Wing (2004), in a study of the Early Eocene of North America, clearly showed that the reconstructed relationship between the oxygen isotope ratios of precipitation and MAT during the whole of the Eocene was significantly different from the present-day relationship. Therefore, a present-day $\delta^{18}O_{H20}$-MAT relationship should not be applied to fossil proxy data from this time period to calculate MAT.

The problems with MAT determinations, however, do not prevent the calculation of multi-proxy derived summer season palaeotemperatures from the Eocene. Therefore, even with evidence of post-precipitation evaporation processes affecting the $\delta^{18}O_{lw}$ values, Grimes et al. (2005) argued that they could be combined with carbonate or phosphate proxy $\delta^{18}O$ values to calculate summer season palaeotemperatures. Furthermore, as long as the ^{18}O enrichment was dominated by evaporative effects then with large enough data sets, summer season palaeotemperatures would become increasingly independent of this post-precipitation process. This is because the palaeotemperatures are dependent upon the difference between the calculated $\delta^{18}O_{lw}$ values and the carbonate or phosphate proxy $\delta^{18}O$

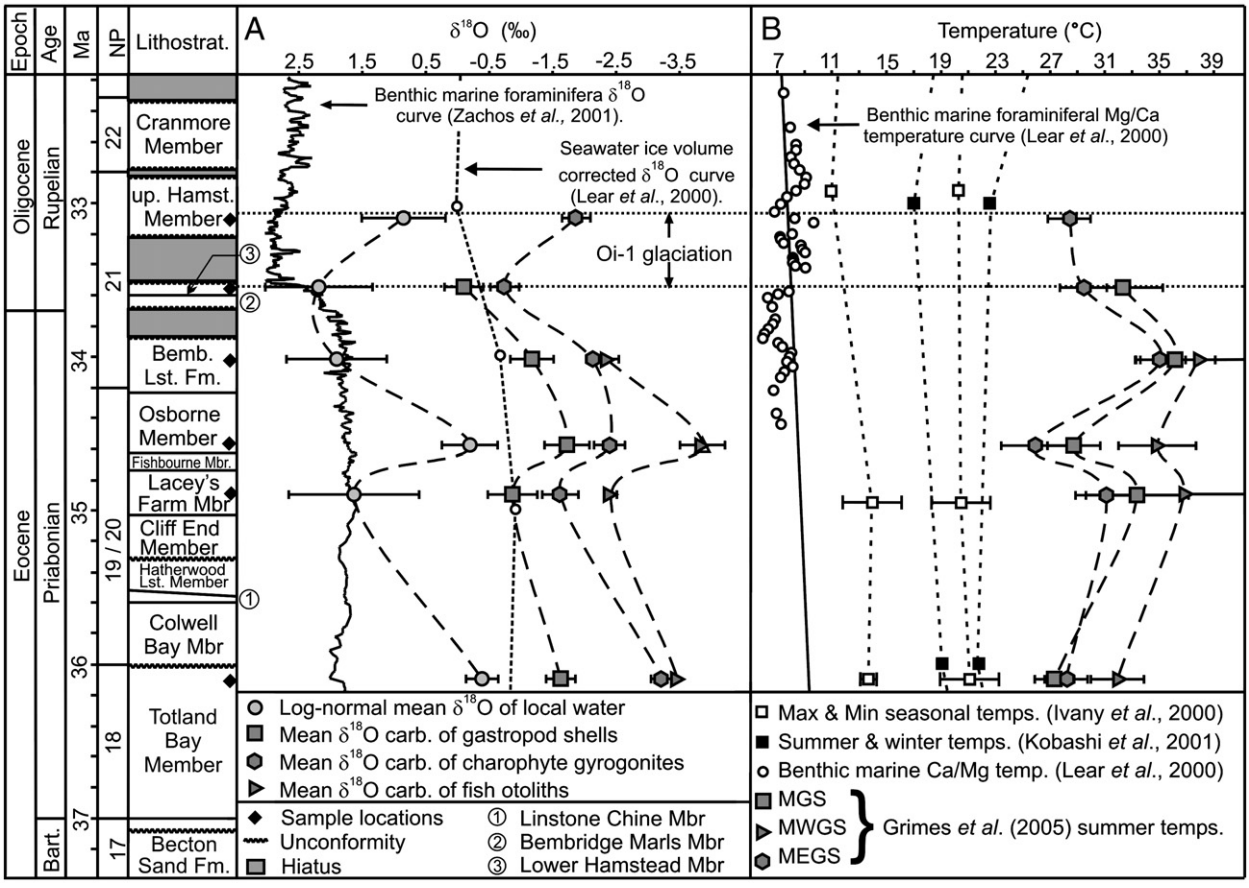


Fig. 4. A compilation of mean $\delta^{18}O$ results and calculated summer season paleotemperatures reported in Grimes et al. (2005) and which were obtained from six fossil assemblages from Late Eocene (Priabonian) to Early Oligocene (Rupelian) Solent Group, Hampshire Basin (Isle of Wight, UK). A: Plot of log-normal mean local-freshwater $\delta^{18}O$ values (in per mil relative to VSMOW [Vienna standard mean ocean water]). Also plotted are marine $\delta^{18}O$ data (Lear et al., 2000 {in per mil relative to VSMOW}; Zachos et al., 2001 {in per mil relative to VPDB [Peedee belemnite]}) correlated to Hampshire Basin stratigraphy. B: Calculated mean palaeotemperatures. MGS-Mean growing season temperature. MWGS-mean temperature of warmest months of growing season. MEGS-mean temperature of a single month in later part of growing season. Also plotted are surface marine palaeotemperatures (Ivany et al., 2000; Kobashi et al., 2001) and deep sea palaeotemperatures (Lear et al., 2000) correlated to Hampshire Basin stratigraphy. All error bars are individual or propagated 2σ standard errors. Dashed trend lines between our measured data points are smoothed interpolated lines. Time scale and NP (nannoplankton) zonation follows Berggren et al. (1995). Lithostratigraphy follows Insole and Daley (1985), Hooker (1986) and Hooker et al. (2004). figure is redrawn from Figs. 1 and 2 in Grimes et al. (2005).

values (see Fig. 2 for proxies and Fig. 4 for palaeotemperatures) and both should experience the same degree of isotope fractionation due to evaporation. However, Grimes et al. (2005) also noted that, if dietary effects were dominant over evaporative effects, this would probably result in overestimation of calculated summer season palaeotemperatures because only the calculated $\delta^{18}O_{lw}$ value would be affected by this process. In their specific study Grimes et al. (2005) argued that it was most likely (evidence in Section 4.1) that evaporative effects were dominant. Therefore, these summer season palaeotemperatures should be independent of any isotope fractionation due to ^{16}O removal resulting from either evaporation, or from an increase in ice volume in the primary source region of the local water (i.e., the oceans) linked to the early Oligocene Antarctic glaciation event (Oi-1).

5.2. Reconstructed palaeoclimates and palaeoenvironments

Although Grimes et al. (2003) were the first to demonstrate how the phosphate oxygen isotope values in fossil rodent tooth enamel could be reliably used to reconstruct palaeotemperatures, it was Navarro et al. (2004) who first used rodent teeth, albeit with whole teeth rather than just enamel, in a wide-ranging palaeoclimate study. In addition to providing a new rodent palaeothermometry equation derived from a wild population, Navarro et al. (2004) also applied the equation to the study of Quaternary climate change at Gigny, French Jura. They argued that calculated $\delta^{18}O$ values of Late Pleistocene meteoric waters from the Gigny cave, a world famous karstic sequence in France, varied sequentially through time from −14‰ to −6‰ and could represent alternating warm and cold glacial periods over the last 90,000 years. Furthermore, by correlating their data to the GISP 2 ice core, Navarro et al. (2004) suggested that the previously established chronology of the Gigny sequence should be treated with caution.

Grimes et al. (2005), on the other hand, were the first to use small rodents in a multipalaeoproxy approach, involving a combination of small mammal teeth and a collection of freshwater proxies (viz., gastropod shells, fish otoliths, and charophyte gyrogonites), to investigate if there were changes in the continental summer season palaeotemperatures of north-west Europe associated with the early Oligocene Antarctic glaciation (Oi-1, see Zachos et al., 2001). For reasons given in Section 5.1, the results, are independent of evaporative effects and ice volume changes, and indicate a fluctuating mesothermal climate with two possible minor cooling events not resolved in previous marine studies (Ivany et al., 2000; Kobashi et al., 2001). The younger of these two cooling events was argued to be related to the gradual build up towards Oi-1 (34.0–33.5 Ma) (Zachos et al., 2001), evident as a gradual increase in the marine benthic foraminiferal $\delta^{18}O$ values (Fig. 4). This preceded the sharp increase in $\delta^{18}O$, which marks the onset of Oi-1 (33.5 Ma, Zachos et al., 2001) (Fig. 4). Since the two cooling events were minor in scale and prior to the Oi-1 glaciation, Grimes et al. (2005) interpreted their trend in palaeotemperatures to be congruent with the marine Mg/Ca temperatures of Lear et al. (2000) in indicating no major temperature change across the Oi-1 glaciation itself. Furthermore, other continental palaeoclimate studies by Retallack et al. (2004) found (for Oregon, USA) no evidence for a 'terminal Eocene event' or for any other single abrupt palaeoclimate shift, whilst Kohn et al. (2004) reported climate stability across the Eocene–Oligocene transition in Argentina. All these results suggest that the majority of the isotope shift in the marine realm across the Oi-1 glaciation resulted from an increase in ice volume and that there was no significant global cooling at this time.

Tütken et al. (2006), in a multi-proxy palaeoenvironmental reconstruction of the Middle Miocene Steinheim Basin of SW Germany, were the first to use tooth enamel from both large and small mammals. Their results indicated that drinking water, calculated from the enamel of large mammals (proboscideans, rhinocerotids, equids, cervids, suids), had a $\delta^{18}O$ value of −5.9±1.7‰, (n=31), which they argued was typical for the Middle Miocene meteoric water of the area and corresponded to a MAT of 18.8±3.8 °C, calculated using a present-day regional $\delta^{18}O_{H20}$-MAT equation. In contrast, their results from the phosphate oxygen isotope analysis of small mammals, especially the abundant pika *Prolagus oeningensis* (order Lagomorpha), suggested that they drank from ^{18}O-enriched water sources (calculated $\delta^{18}O_{H20}$=+1.3±2.3‰, n=4), in a similar manner to some of the small mammals studied by Grimes et al. (2003, 2005). Tütken et al. (2006) analysed (using a total combustion–elemental analysis (TC-EA) technique) an Ag_3PO_4 precipitate obtained from complete incisor teeth of small mammals. Even though they provided some circumstantial evidence to suggest that there was little diagenetic alteration of the dentine, they also accepted that enriched $\delta^{18}O_{H20}$ values could be influenced by the incorporation of diagenetically altered dentine phosphate oxygen. In spite of this issue Tütken et al. (2006) further argued that differences in the composition of strontium and oxygen isotopes between large and small mammal teeth indicated different home ranges and drinking behaviour. This they argued suggested migration of some of the large mammals between the Swabian Alb plateau and the nearby Molasse basin, while the small mammals ingested their food and water locally.

Finally, in an ongoing research project Grimes et al. (2006) have extended the use of small mammals in palaeoclimate studies back to the Paleocene–Eocene transition in an attempt to discover the impact of the Paleocene–Eocene Thermal Maximum on continental climate change at the famous mammal site of Dormaal, Belgium. Initial results from the phosphate oxygen isotope analysis of mammalian tooth enamel (*Paschatherium*) and freshwater fish scale ganoine (*Lepisosteus*) show no shift in oxygen isotope composition contemporaneous with a dispersed organic carbon isotope excursion observed in the same section. Though further work is being undertaken, the most likely current explanation for this is that the vertebrate remains in higher levels at Dormaal are reworked from a basal gravel bed.

6. Conclusions

The phosphate oxygen isotope composition of tooth enamel from large mammals has been extensively used in continental palaeoclimate reconstructions. Advances in chemical separation techniques used to isolate phosphate oxygen from biogenic apatite and new analytical methodologies, including a direct laser fluorination technique, with a new pre-treatment stage, now allow the $\delta^{18}O_{PO4}$ values of small mammal teeth to be routinely determined. This, in conjunction with evidence for primary $\delta^{18}O_{PO4}$ values being maintained in the separated enamel (and in some cases, in whole teeth), means that relatively more abundant small mammal teeth may now be used in palaeoclimate reconstructions. If it can be demonstrated that there is a close association between small mammals and a single water source, plus if there is evidence that the small mammals obtained most of their water intake from drinking, then $\delta^{18}O_{lw}$ values can be combined with other oxygen isotope values from specific freshwater biota, using published thermometry equations. Owing to varying mineralization times between the different freshwater biota, the palaeotemperatures calculated using these equations represent summer season palaeotemperatures of varying climatic significance, which are independent of evaporative or ice volume effects. Mean annual temperatures, calculated using $\delta^{18}O_{lw}$ values, on the other hand should be treated with caution if evaporation has altered the local water isotope value such that it does not proxy meteoric water, or if there is evidence that the present-day $\delta^{18}O_{H20}$-MAT relationship does not apply to ancient environments.

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