Pergamon

Geochimica et Cosmochimica Acta, Vol. 65, No. 15, pp. 2535–2548, 2001

0016-7037/01 $20.00 + .00

PII S0016-7037(01)00606-8

Phosphate $\delta^{18}O$ determination of modern rodent teeth by direct laser fluorination: An appraisal of methodology and potential application to palaeoclimate reconstruction

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(*Received October* 9, 2000; *accepted in revised form March* 9, 2001)

Abstract—A direct laser-fluorination (DLF) method is presented for phosphate $\delta^{18}O$ analysis (mass 1 — 2 mg). The automated system heats samples in the presence of excess BrF_5 using a 25 W CO_2 laser, at 10.66 μm. $\delta^{18}O$ ratios of the liberated O_2 were measured using a dual inlet Optima mass spectrometer. As DLF measures whole apatite oxygen, non-phosphate bound oxygen must be removed by pre-treatment. Two methods were investigated: 1) heating to 1000°C; and 2) heating to 400°C followed by laser fusing. Method 2 is recommended as samples heated to 1000°C showed evidence of oxygen exchange with atmospheric water. To validate the DLF method, and show the potential of rodent teeth in palaeoclimate reconstruction, modern rodent teeth $\delta^{18}O$ results from 2 species are presented ($\delta^{18}O_p$). Large inter- and intra-jaw heterogeneity indicates that single teeth cannot be used for palaeothermometry. However, the overall standard deviations were low (*Glis glis* $\delta^{18}O_p = +10.4 \pm 0.7‰$ $n = 38$ and *Apodemus sylvaticus* $\delta^{18}O_p = +14.4 \pm 1.3‰$ $n = 24$). Using equations, derived from lab rodents, an ingested water value of $-5.6 \pm 2.2‰$ was calculated for *Apodemus sylvaticus*, only $-1.3‰$ lower than measured local water ($-4.3‰$). This suggests that the phosphate $\delta^{18}O$ of rodent teeth can be used as a proxy for palaeoclimate reconstruction. *Copyright © 2001 Elsevier Science Ltd*

1. INTRODUCTION

An understanding of palaeoclimates is critical to interpretation of earth history and biotic response to global change including former and future greenhouse and icehouse worlds (e.g., Collinson, 2000 and Hooker, 2000). The oxygen isotope composition of precipitation (meteoric water) provides an indicator of continental climate conditions because of the close relationship between mean annual temperatures (MAT) and average $\delta^{18}O$ of rainfall at mid to high latitudes (Dansgaard 1964; Rozanski et al. 1993). A proxy for meteoric water $\delta^{18}O$ values is the oxygen isotope composition of apatite in mammalian teeth and bones which is directly related to the $\delta^{18}O$ of body water, precipitated at a uniform temperature of $\approx$ 37°C. Body water is related to ingested water via metabolic isotopic fractionations that are species dependent, such that for a given species the $\delta^{18}O$ variation in tooth phosphate mirrors $\delta^{18}O$ variability of local meteoric water (Luz et al. 1984; D'Angela and Longinelli, 1990). As biogenic apatite, especially tooth enamel, is believed to be robust to diagenetic change, the $\delta^{18}O$ of fossil mammalian teeth holds considerable potential for tracking changes in the $\delta^{18}O$ of ancient meteoric water, and consequently palaeoclimate changes. This has been demonstrated previously by, among others, Fricke et al., (1998); Fricke et al., (1996); Kohn et al., (1998); and Stuart-Williams and Schwarcz, (1997).

Biogenic apatites contain three forms of oxygen: phosphate, carbonate, and hydroxyl. The carbonate component comprises up to 10% of the total oxygen and there is a fractionation of approximately 9‰ in $\delta^{18}O$ between CO_3^{2-} and PO_4^{3-} (Iacumin et al., 1996). In fossil material, the carbonate component of biogenic apatite is prone to diagenetic alteration (Fricke et al., 1998), and most analytical methods separate chemically the phosphate component before analysis to minimize diagenetic bias. The classical method for $\delta^{18}O$ analysis involves chemical purification of phosphate by dissolution of the sample and reprecipitation as Bi or Ag phosphate. The purified phosphate is then reduced to liberate oxygen for isotope analysis using fluorinating agents (e.g., Tudge, 1960; Kolodny et al., 1983; Luz et al., 1984), graphite reduction (O'Neil et al., 1994), or Br_2 reduction (Stuart-Williams and Schwarcz, 1995). These procedures are lengthy to perform and require relatively large amounts of sample for each analysis (5 — 30 mg). Studies using these methods restrict analysis to larger teeth and bone which do not occur as frequently in the fossil record as small teeth. Small teeth often have a total mass less than 5 mg which is at the lower end of the minimum mass for these chemical separation techniques.

Analytical methods for determining $\delta^{18}O$ of biogenic apatites have diversified in recent years through the use of laser assisted heating which permits rapid analysis on smaller samples. Techniques differ considerably in their mode of analysis (bulk versus in-situ or spot measurements) and, more significantly, in terms of which oxygen species are targeted for isotope analysis. Cerling and Sharp (1995) used an infrared laser to locally heat untreated tooth enamel to liberate CO_2 by thermal decomposition of the carbonate component. This method is potentially unreliable for fossil analysis as it targets carbonate oxygen which is prone to diagenetic exchange. Furthermore, being an in-situ technique, results may be influenced by the partly reacted thermal halo surrounding the ablation spot. Kohn et al. (1996) used an infrared laser-fluorination technique to obtain bulk $\delta^{18}O$ values of untreated biogenic

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apatite. However, Kohn et al. (1996) noted low yields and their $\delta^{18}O$ data were subject to a uniform offset of $\approx$ 1.7‰ relative to conventional data and carbonate-free standards. This offset may be due to an isotopically heavy oxygen component from carbonate or it may be related to low oxygen yields, which are known to affect measured $\delta^{18}O$ values in silicates (Mattey and Macpherson, 1993). Jones et al. (1999) used an ultraviolet laser-fluorination method to obtain apatite $\delta^{18}O$, which eliminated some of the uncertainties associated with infrared lasers. $\delta^{18}O$ values for untreated tooth enamel obtained by UV laser fluorination appear to be similar to conventional $\delta^{18}O$ values measured on separated Ag phosphate. However, as this UV laser technique targets all forms of available oxygen (carbonate, hydroxyl as well as phosphate) it is subject to error as the carbonate and hydroxyl groups are prone to fractionation and diagenetic alteration (Kohn et al. 1999). Even though Jones et al. (1999) and Kohn et al. (1998) stated that the fractionation of the $CO_3^{2-} - PO_4^{3-}$ and $OH^- - PO_4^{3-}$ would precisely compensate for each other, the effects of diagenetic change remain unconstrained using this technique unless the isotopic composition of the carbonate and hydroxyl components are independently determined.

In this paper we present a new method for the analysis of the phosphate only $\delta^{18}O$ component of biogenic apatite by direct laser fluorination (DLF) that requires sample masses of only ~1 to 2mg. We also present $\delta^{18}O$ data from modern rodent teeth (*Glis glis* (L.) and *Apodemus sylvaticus* (L.)) to validate the method and demonstrate its potential for palaeoclimate reconstruction.

Whilst large teeth are often readily available for collection in Tertiary badland landscapes e.g., of North America and Central Asia, this is by no means always the case. In most regions of the world there are not such extensive exposures to allow the ready recovery of many large mammals in long successions of strata. Therefore, the ability to use relatively more abundant, small mammals, e.g., rodents, would open a wider spectrum of opportunity for isotopic sampling. Our specific aim is to apply the method developed here to a palaeoclimate interpretation of the terrestrial realm across the Eocene/Oligocene transition in Europe. The sequence we have chosen is that of the Hampshire Basin, southern England, where we have a wealth of information on terrestrial palaeoenvironments (Collinson 1992, Hooker 1992, Hooker et al. 1995, Collinson and Hooker, 1987, 2000). Here large mammals are rare whilst small mammals, especially rodents, are abundant at numerous levels in a superposed sequence, time constrained by various correlation points and spanning the time interval of temperature change in the marine realm. One of these small mammals has been demonstrated to have lived in close association with the water bodies in which their remains were fossilised (Collinson and Hooker, 2000).

1.1. Sample Description

Two modern wild rodent species, the wood mouse, *Apodemus sylvaticus*, and the fat dormouse, *Glis glis*, were used in this study. *Glis glis* was chosen as a close living relative of some of the small mammals found in the British Tertiary. *Apodemus sylvaticus* was chosen as a small mammal known to be living in intimate association with a water body; a closely comparable situation to that known for the British Tertiary fossil assemblages that will be targeted in our palaeoclimate study.

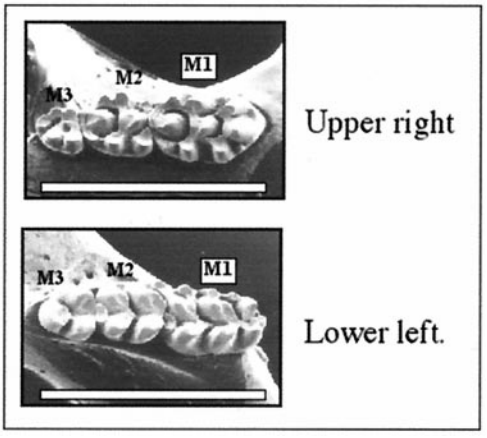

Fig. 1. Representative SEM images of cheek teeth of the wood mouse *Apodemus sylvaticus*. The scale bar on both images represents 3.8 mm.

1.2. Wood Mouse, *Apodemus sylvaticus*

The wood mouse, is classified in the order Rodentia, family Muridae. Wood mice inhabit most environments that are not too wet, i.e., woodland, arable land, ungrazed grassland, heather, blanket bog, and sand dunes. They feed on seeds, fruits, buds, stems, and invertebrates in varying proportions, depending on their season or habitat-related abundance, although seeds normally dominate the diet (Hansson, 1985). They obtain their water by drinking (Schröpfer, 1974) in common with other rodents that live in mesic environments (Wright, 1976). They are mainly ground dwelling but can also climb. The breeding season is between February/March and November, with usually one to two litters (although occasionally up to six) produced a year (Flowerdew 1991). Weaning takes place from between days 18 to 22 after birth.

Five specimens were collected from Dungeness, Kent, southeast England in 1992 by Royal Holloway Biology Department as part of a study of the intestinal parasites of wild wood mice. At this locality, freshwater ponds, ~100 m in diameter and 6 m deep, are surrounded by beach-pebble banks. These banks around the lakes act as efficient boundaries to the wood mouse range. Pond resident populations are restricted to narrow, dry, scrubby vegetation around the lake margins (typically 5 m wide). The pond was a ready source of drinking water. Water samples and wood mice were collected from around a single pond.

Apodemus sylvaticus only have 3 cheek teeth; all molars. The premolar has been lost in the course of evolution (Fig. 1.). Before wear, their teeth have cusps arranged in parallel mesiodistal rows, joined by high buccolingual ridges (Hillson, 1993). Wear converts this arrangement into a series of incipient lophs.

1.3. Fat Dormouse, *Glis glis*

The fat dormouse is also classified in the order Rodentia, but in the family Gliridae. It is a modern species, that inhabits

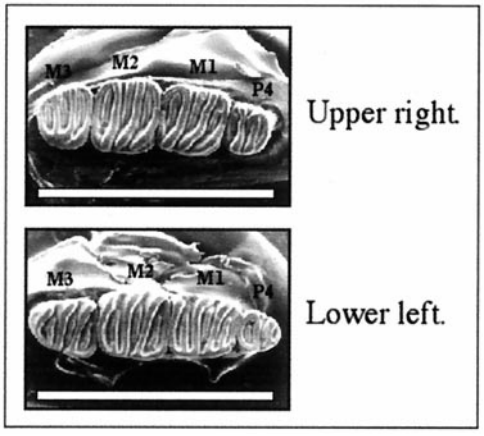

Fig. 2. Representative SEM images of casts of the cheek teeth of the fat dormouse *Glis glis*. The scale bar on both images represents 7 mm.

mixed and deciduous woodlands, orchards, and gardens. It is arboreal, spending most of its life in the canopy, but often entering buildings too (Jones-Walters and Corbet, 1991). The woodlands in which they live are generally dominated by hazel, oak, holly, and birch. They feed mainly on soft fruits, berries, nuts, seeds, buds, and bark and to some extent on insects, eggs, and nestlings of birds (Jones-Walters and Corbet, 1991; Nowak, 1999). They are recorded as drinking large quantities of water (Jones-Walters and Corbet, 1991). They possess four cheek teeth per jaw, three molars and one premolar (Fig. 2.). The cheek teeth are brachyodont (i.e., low crowned) and flat. The occlusal surfaces are crossed buccolingually by low parallel ridges, which are finely spaced (Hillson, 1993). As *Glis glis* have only one litter per year, generally giving birth after mid-July (Pat Morris, pers. comm.), mineralisation of the last molar (M3) and the premolar (P4) should take place between mid and late summer.

Three *Glis glis* specimens were obtained from an apple barn in Great Missenden, Buckinghamshire, UK, by a licensed pest controller using live trapping and exterminated by chloroform. As a result of the location in which they were captured, it is reasonable to assume that they may have obtained the majority of their ingested water from an apple diet rather than from a local water source. Two of the animals were relatively young (A and C) and still possessed their deciduous premolars (DP4), with their permanent premolars (P4) still unerupted within the jaw. The third dormouse (B) possessed fully erupted P4 teeth, and so was older.

2. MATERIALS AND METHODS

Presented here is a new method for the laser fluorination analysis of the phosphate oxygen component only of biogenic apatite.

2.1. System Details and Procedures

The DLF system uses a 25 W CO_2 laser, operating at 10.66 μm (Mattey, 1998). Samples are loaded in Ni sample trays within a chamber fitted with a chemical vapor deposition (CVD) diamond window. The laser heats and reacts the whole sample, rather than ablates a single pit, in the presence of BrF_5 ($\sim$0.2 atm). The liberated oxygen is cleaned cryogenically to remove excess reagents and passed over KBr at 100°C to remove free fluorine. Pure oxygen is collected using an automated VG-type cold finger packed with silica gel. Oxygen isotope ratios are measured directly on the O_2 gas, relative to an oxygen gas standard calibrated to SMOW, using a dual inlet Optima mass spectrometer. A schematic diagram of the system is outlined in Figure 3.

All samples, biogenic apatites, NIST 120c standards and QBLC quartz standards, are handpicked and range in mass between 1 mg and 2.0 mg. A single run consists of 1 biogenic apatite sample or a NIST 120c standard and two QBLC quartz standards (Grassineau, 1994). The first quartz standard is a sacrificial sample to allow thermal heating of the sample chamber, and the second for calibration (offset correction) of the $\delta^{18}O$ phosphate result. After analysis, the phosphate sample melt residue is removed from the sample chamber, sectioned and analysed by electron microprobe (EM). Results from a number of runs, which are reported in Lindars (1998), show that the residues from the laser fluorination of biogenic apatites are pure CaF_2. Kohn et al. (1996) reported similar findings.

2.2. Prefluorination Technique

Following evacuation of the sample chamber, and before analysis, a prefluorination procedure is conducted to remove contaminants (e.g., water) that remain after evacuation. Excess (0.4 bar) BrF_5 is admitted to the sample chamber for 30 min. Results, which are reported in Lindars (1998) and summarised in Table A1, show that this optimum period of prefluorination has been determined experimentally and is the maximum clean-up time permitted without the biogenic apatite samples undergoing premature fluorination.

2.3. Oxygen Yields

Results, which are reported in Lindars (1998) and summarised in Figure A1, show a decrease in oxygen yield and increased $\delta^{18}O$ values over time when more than one biogenic apatite sample was analysed per run. This indicates that when laser heating a biogenic apatite sample others were also prematurely reacting. Therefore, only one biogenic apatite sample was analysed per run. Oxygen yields on all the biogenic apatite samples analysed ranged from 95 to 100%.

2.4. Pretreatments Techniques

A problem with DLF oxygen isotope analysis is that it measures whole apatite oxygen as opposed to purely phosphate oxygen. The presence of labile, non-phosphate, oxygen (i.e., structural carbonate, the hydroxyl ion, water and organic matter (OM)) is not a particular problem for modern materials because their proportions are considered to be in internal isotopic partitioning equilibrium with the phosphate component during enamel precipitation (Kohn et al., 1996). However, in fossil material diagenetic fractionation of non-phosphate oxygen may occur as the $\delta^{18}O$ of carbonate and hydroxyl is more susceptible to diagenetic alteration (Lee-Thorp and van der Merwe, 1987; Shemesh et al., 1988; McArthur and Herczeg, 1990; Ayliffe et al., 1992; Kohn et al.1999), potentially leading to a

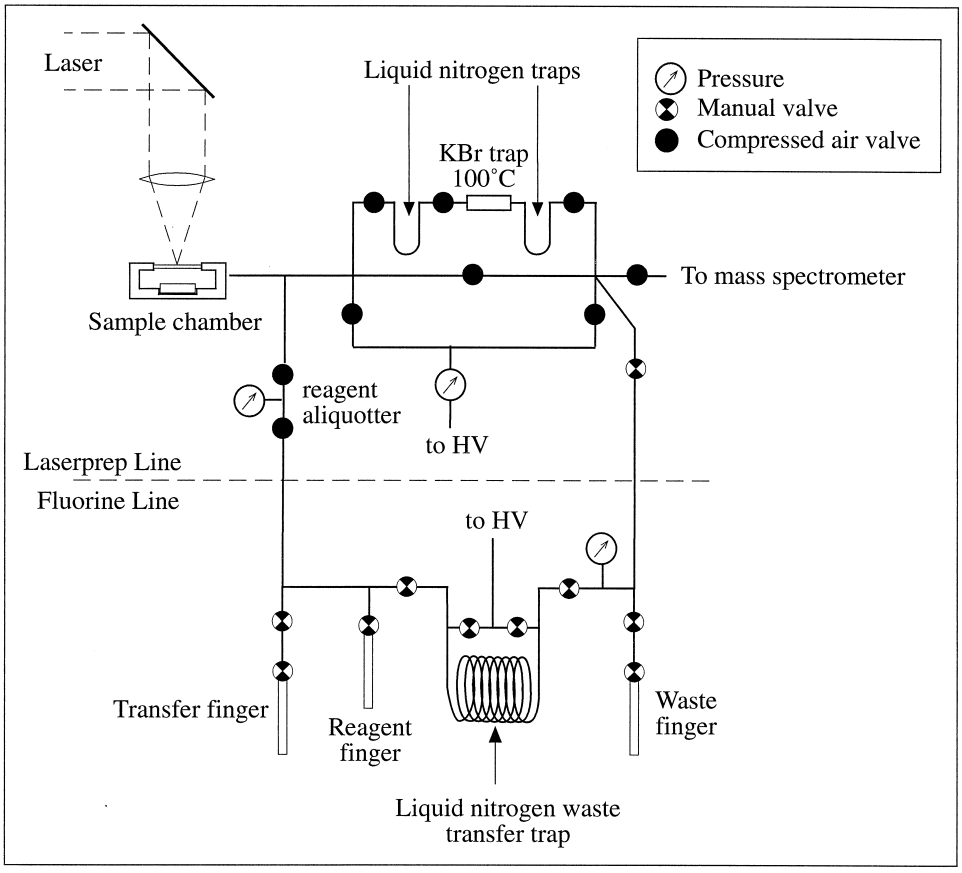

Fig. 3. Schematic diagram of the laser fluorination and cryogenic gas clean-up line of the CO_2 laser system at Royal Holloway.

compromised oxygen isotopic value. Therefore, a pretreatment to remove labile oxygen is required.

Two methods are presented here; 1) heating to 1000°C before loading and 2) heating to 400°C, loading, followed by fusing in situ using the laser, before prefluorination and analysis. All experiments were conducted upon pig bone, rather than tooth enamel, because one large single source of pig bone was available and also pig bone is considered more reactive, because of its larger surface area.

Hydrogen, carbon and nitrogen (HCN) analyses were conducted using a Fisons Instruments NA 1500 NCS with the aim of determining the effect of heating on the carbonate and water content of pig bone. Untreated samples and those heated to temperatures between 100 – 1000°C for 1.25 h were analysed. Results, which can be found in Lindars (1998) and summarised in Figure A2, show that up to 400°C, a reduction in H, N, C occurred as a result of the oxidation and removal of OM. Above 400°C carbon loss is the result of removal of structural carbonate. To confirm the removal of OM and carbonate on heating, the $\delta^{13}C$ values of the liberated CO_2 were measured to determine its source. $\delta^{13}C$ analyses were conducted using the stepped heating technique (SH) reported in Jackson et al. (1988), with detailed results reported in Lindars (1998) and summarised in Figure A3. $\delta^{13}C$ results indicate that below 400°C there was loss of both OM and carbonate, which agrees with results reported by Person et al. (1996). Above 400°C there was loss of carbonate only.

To ensure that pretreatment did not also modify the phosphate oxygen isotope signature, pig bone was heated from 100°C to 1000°C, in 100°C steps and the $\delta^{18}O$ of the residues measured. Results, which are reported in Lindars (1998) and summarised in Figure A4, indicate that above 600°C, the $\delta^{18}O$ values of the heated residues are constant with good reproducibility. This is attributed to the loss of OM and sorbed water below 600°C. A further improvement in reproducibility was observed around 1000°C as a result of the removal of OH^- and CO_3^{2-} groups.

The possibility of oxygen exchange between the heated phosphate residue and atmospheric oxygen ($\sim$ + 23.5 ‰, Galimov, (1985)) and water (~ -14‰, based upon a value for local surface water of $\sim$-6‰ quoted by Darling et al., (1992)) was also investigated. Pig bone was heated to 1000°C before being transferred to a sealable glass tube and stored in a desiccator until use. Results reported in Lindars (1998) and summarised in Table A2, indicate that there is no evidence of large positive or negative shifts in the oxygen isotopic composition of heated pig bone compared to an unheated equivalent, implying no atmospheric oxygen exchange during or after the heating.

In summary, heating pig bone to 1000°C significantly re-

duced extraneous oxygen from biogenic phosphate and did not appear to cause reaction with atmospheric water or oxygen. Therefore, this pretreatment was initially adopted for the analysis of the fat dormouse (*Glis glis*) tooth samples.

Long term data from the NIST 120c standard after 1000°C pretreatment, however, contrasted with the initial tests conducted on the pig bone. When compared to reported values ($\sim +21.4 \pm 0.4$‰ ($BiPO_4$, Bryant et al., 1994) and 21.7 ± 0.2‰ ($AgPO_4$, Lécuyer et al. 1993)) the isotopic composition of NIST 120c was significantly lower for those samples heated to 1000°C (12.5 ± 0.6‰). Therefore, other phosphates were heated to 1000°C and their $\delta^{18}O$ values measured. Results which are reported in Lindars (1998) and summarised in Table A2, show that cow bone, heated at 1000°C for 1 h also has significantly lower $\delta^{18}O$ values when compared to untreated samples, which could not be accounted for by the loss of structural carbonate. However, $\delta^{18}O$ results for heated Epiwala apatite crystals were similar to untreated samples. This could be due to the lack of carbonate substitution in the Epiwala apatite crystal. The large negative shifts in the $\delta^{18}O$ of heated cow bone and NIST 120c standard implies oxygen exchange with atmospheric water after removal from the furnace and loading into the sample chamber. Atmospheric water is assumed to be attracted to heated phosphates as a result of CO_2 release and the formation of hygroscopic CaO in the apatite lattice. Reversal of the oxygen isotopic exchange with atmospheric water was attempted by fusing with the laser while pumping the chamber to high vacuum to remove sorbed OH^-. However, results reported in Lindars (1998) and summarised in Table A2, indicate that it is not possible to reverse the oxygen exchange with atmospheric water, probably because, when laser heating the phosphates a melt is formed within which the OH^- dissolves and becomes an integral part of the residue.

Simply fusing untreated biogenic phosphates within the sample chamber, to remove carbonate, water and OM, produced varying results (Lindars, 1998, and Table A2). Although the NIST120c produced results (21.5 ± 0.5‰) not dissimilar to those reported by others ($\sim +21.4 \pm 0.4$‰ ($BiPO_4$, Bryant et al., 1994) and 21.7 ± 0.2‰ ($AgPO_4$, Lécuyer et al. 1993)), pig bone and cow bone produced much lower values, possibly due to incomplete oxidation of the OM within the bone.

As a final solution, biogenic apatite samples were combusted at 400°C, which removes all the OM but does not cause chemical changes which would allow any exchange with atmospheric oxygen or water. After loading into the sample chamber, the material was laser fused under pumped vacuum to remove the remaining water and carbonate. Results, which are reported in Lindars (1998) and summarised in Table A2, show that NIST 120c, which had shown evidence of exchange with atmospheric water after heating to 1000°C, produced $\delta^{18}O$ values similar to those reported by other labs. This pretreatment procedure was used in the analysis of the Wood mouse (*Apodemus sylvaticus*) samples.

2.5. Standards

32 analyses of the internal QBLC Quartz standard, not including the sacrificial quartz, were conducted and give a mean value of $+8.78$‰ $\pm$ 0.15 (1σ). The reported value is $+8.79$‰ (Grassineau, 1994). 33 analyses of the NIST 120c phosphate standard, were conducted on untreated material and gave a mean value of $+20.4$‰ $\pm$ 0.5 (1σ) (see Lindars, (1998), and Table A3). This is lower than the $\sim +21.4 \pm 0.4$‰ ($BiPO_4$, Bryant et al., 1994) and 21.7 ± 0.2‰ ($AgPO_4$, Lécuyer et al. 1993) reported by other laboratories using chemical separation techniques. However, 13 analyses of NIST 120c which were pretreated (heating to 400°C then fusing within the sample chamber) gave a mean value of $+21.3$‰ $\pm$ 0.4 (1σ), which is comparable to the chemical separation technique. It should be noted, however, that NIST 120c is not an accredited $\delta^{18}O$ isotopic standard, but rather a phosphate-bearing sediment. Therefore, it may not be comparable with the laser fluorination analysis of biogenic apatite. To tackle this problem, a large suite of modern tooth samples, covering a range of $\delta^{18}O$ values, is being sought. The $\delta^{18}O$ of the enamel carbonate will be measured and compared with the $\delta^{18}O$ of pretreated enamel (Phosphate is ~9‰ lower than carbonate). This should prove if the pretreatment technique is resulting in the measurement of only the phosphate component of biogenic apatite.

2.6. Thermometry Calculations

Longinelli and Nuti (1973) derived a phosphate-water isotopic temperature scale, which has been re-calibrated by Kolodny et al. (1983) (1), such that,

$$T°C = 113.3 - 4.38\,(\delta^{18}O_p - \delta^{18}O_w) \quad (1)$$

where $\delta^{18}O_p$ and $\delta^{18}O_w$ are the $\delta^{18}O$ values of phosphate and water, respectively. Assuming $\delta^{18}O_w$ is the $\delta^{18}O$ of a mammal's body water ($\delta^{18}O_{bw}$), from which all mammalian apatite is precipitated, Eqn. 1 can predict $\delta^{18}O_{bw}$ because mammals precipitate biogenic apatite in equilibrium with their body water under a constant temperature of 37°C, leading to the reduction of Eqn. 1 to:

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

Furthermore, the oxygen isotopic composition of mammal body water is controlled by its oxygen influxes (of which local meteoric waters predominate), 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). This leads to an enrichment of mammal body water, relative to meteoric water, by between $+23$ to $+30$‰ (Luz et al., 1990; Bryant, 1995). However, animals drinking plentifully, generally those eating semidry food, such as seeds, have a linear relationship between their $\delta^{18}O_{bw}$ and the $\delta^{18}O$ of their drinking water (Longinelli and Peretti Padalino, 1980; Luz et al., 1984; D'Angela and Longinelli, 1990; Ayliffe et al., 1992). As an example, Luz et al. (1984) reported the following equation for captive lab rodents (*Apodemus sylvaticus*) eating semidry food in a relative humidity of 50%:

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

where $\delta^{18}O_{lw}$ refers to the oxygen isotopic composition of drinking water. From Eqn. 2 and 3 it is possible to derive an equation that will predict $\delta^{18}O_{lw}$ (i.e., ingested water) from the oxygen isotopic composition of rodent tooth phosphate):

$$\delta^{18}O_{lw} = \{[\delta^{18}O_p - 17.42] - 0.24\}/0.59 \quad (4)$$

Eqn. 4 is derived from captive lab rodents (*Apodemus sylvaticus*) eating semidry food under controlled conditions and may not be representative of wild species. D'Angela and Longinelli (1990) proposed an Eqn. 5 based upon bone analyses of a wild population of wood mouse (*Apodemus sylvaticus*)

$$\delta^{18}O_p = 0.79\ (\delta_{18}O_{mw}) + 21.61 \quad (5)$$

However, little was known about the wood mouse habitat and diet in the D'Angela and Longinelli (1990) study, especially the location of a significant water body from which the rodents could have regularly drunk. As a consequence Eqn. 5 is calibrated to meteoric water and not a local water source. In addition, their calibration is based upon bone analysis rather than tooth analysis. In contrast, our study of *Apodemus sylvaticus* is based upon tooth analysis and there is clear evidence of a water source for the rodent's drinking water. This is more in line with the captive rodent study, and therefore Eqn. 4 was used. However, it should be noted that the water turnover by the wild *Apodemus sylvaticus* rodents in this study may have differed slightly from those in the Luz et al. (1984) study and that controlled conditions like humidity are also different in the wild.

3. RESULTS

Phosphate oxygen isotope analyses were carried out on the teeth of two modern rodent species, the fat dormouse (*Glis glis*) and the wood mouse (*Apodemus sylvaticus*) to validate the use of the DLF technique and to investigate if the phosphate oxygen isotopes from rodent teeth have potential for palaeoclimate reconstruction. Whole tooth samples were analysed. In these modern samples no attempt was made to separate the dentine from the enamel as they precipitated together and it was considered that there was no diagenetic alteration of the dentine, an assumption which cannot be made in the case of fossil rodent teeth.

3.1. *Apodemus sylvaticus*

As wood mice lack premolars (P4) only molars (M1, M2, and M3) could be analysed. These came from upper and lower right hand jaws. The oxygen isotopic results are summarised in Table 1. For *Apodemus sylvaticus* only inter jaw variations can be determined as the teeth were removed from the jaws without recording the individual source. The interjaw variations are ± 1.6‰ for M1, ± 1.1‰ for M2, and ± 0.48‰ for M3. The overall variation, taking into account all teeth, is 1.3‰ for *Apodemus sylvaticus.* Figure 4 indicates that this heterogeneity is inversely related to the order of mineralisation, i.e., the teeth to mineralise last (M3) are the most homogenous, with a standard deviation of 0.30‰, compared to 1.60‰ for M1 and 1.10‰ for M2. Even though not directly proven this may be ascribed to the influence of weaning over the isotopic composition of the maturing teeth. Once weaned a more isotopically stable water source is being ingested, as compared to ingestion of the mother's milk, which has its composition influenced by the mother's feeding habits and the fractionation experienced between ingested water and the milk. Alternatively, the overall variation could be due to climate variability, i.e., seasonality.

Table 1. Oxygen isotopic compositions of whole teeth from the rodent *Apodemus sylvaticus*. The teeth are from the right side of each mouse jaw. N/A: not analoged.

Tooth type.	M1 upper	M1 lower	M2 upper	M2 lower	M3 upper	M3 lower
	14.3	14.5	15.6	16.1	14.6	14.2
	12.6	11.4	13.9	15.2		15.0
	16.4	12.9	12.7	15.4		
	15.1	13.0		13.5		
	15.6	15.1		14.1		
				15.1		
				15.8		
Inter Jaw Mean	14.8 ±1.4	13.0 ±1.5	14.1 ±1.5	15.0 ±0.9	N/A N/A	14.6 0.5
Inter Jaw Mean	14.0 ±1.6		14.7 ±1.1		14.4 ±0.3	
Overall Mean			14.4 ±1.3			

The pond water collected at the same time as the rodents (summer 1992) was measured by the NERC stable isotope facility (Keyworth, UK) and had a mean $\delta^{18}O$ of −4.25 ‰.

3.2. *Glis glis*

A summary of all the oxygen isotopic results for *Glis glis* is given in Table 2. The oxygen isotopic composition of each tooth has been plotted against tooth position for each dormouse (Figs. 5 and 6). The intrajaw variation (i.e., variation between all teeth in a single specimen jaw) is ± 0.68‰ for specimen A, ± 0.80‰ for specimen B and ± 0.76‰ for specimen C. The interjaw variation (i.e., variation between the same tooth type in different specimens) is ± 0.64‰ for M1, ± 0.38‰ for M2, ± 1.18‰ for M3, (P4 teeth were only available from one specimen). The overall variation, taking into account all teeth is ± 0.81‰ for *Glis glis*. Figures 5 and 6 indicate that all the dormice appear to have similar $\delta^{18}O$ values for upper and lower teeth on one jaw side. However, there is heterogeneity when comparing left and right tooth $\delta^{18}O$ values, most noticeable in dormouse B. This could be a consequence of left and right teeth mineralising at different times. In addition, in contrast to the *Apodemus sylvaticus* teeth, there is no relationship between the timing of tooth mineralisation and increasing homogeneity in the $\delta^{18}O$ of the tooth, i.e., the M3 teeth, which are the last of those measured to mineralise, are not the most homogenous across the three specimens.

4. DISCUSSION

The oxygen isotope analysis of modern biogenic apatites (teeth and bone) has been reported previously by, among others, Fricke et al. (1998); Fricke et al. (1996) and Stuart-Williams and Schwarcz (1997). All suggest that biogenic apatites have the potential for use in palaeoclimate reconstruction. However, their analyses were conducted upon relatively large mammalian herbivore teeth, i.e., cows, pigs, and deer, and employed the classical chemical separation technique. Unfortunately, such large mammal teeth are not always common in the fossil record, whilst small mammal teeth are much more

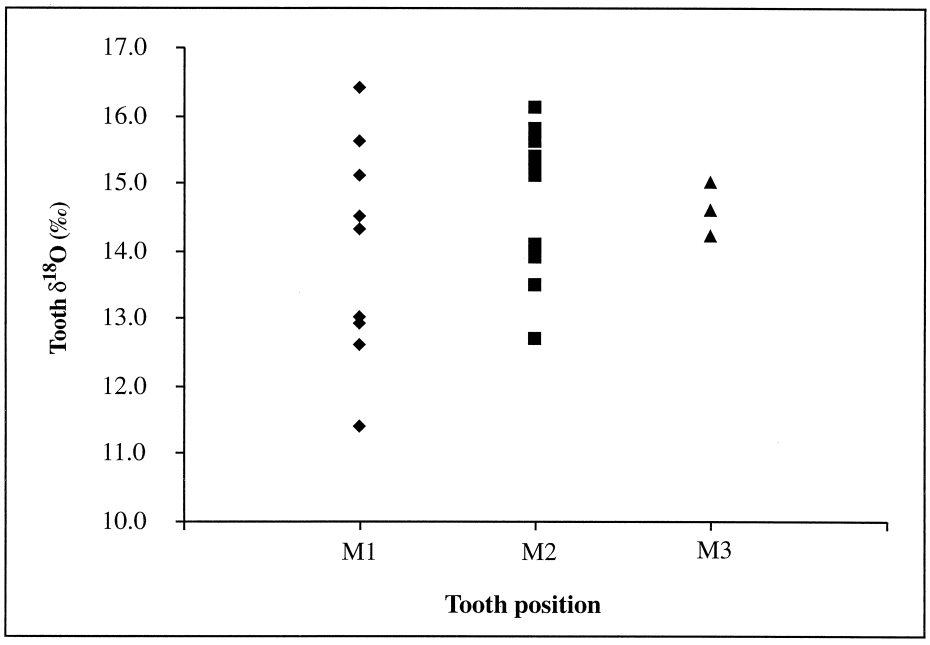

Fig. 4. Plots of oxygen isotopic composition against tooth position to illustrate interjaw variation within the *Apodemus sylvaticus*.

abundant (e.g., Collinson and Hooker, 1987). Another advantage in using small rather than large mammals when reconstructing palaeoclimates is their small home range. Provided postmortem transport is low, small mammals are likely to have formed their teeth in association with the water body in which the sediments containing their fossilised remains accumulated. In contrast, large mammals may have been reared many miles away from their site of entombment. Small teeth (e.g., rodents) could therefore provide a ready source for palaeoclimate reconstruction over important periods of past climate change such as the Eocene/Oligocene transition (Collinson, 1992; Hooker, 1992).

4.1. Inter and Intrajaw Variations

Both *Glis glis* and *Apodemus sylvaticus* teeth show $\delta^{18}O$ isotopic heterogeneity, with overall variations of ± 0.81‰ and ± 1.3‰ respectively. Sánchez Chillón et al. (1994) and Luz et al. (1990) report slightly lower variations (< ± 0.7‰) for modern bone and teeth of horses and deer. However, the overall variations in *Glis glis* and *Apodemus sylvaticus* teeth are less than the intratooth range in horses measured by Bryant (1995) (~± 2‰) and in artiodactyls measured by Fricke et al. (1996) (~± 3.5‰). Fricke et al. (1996) attributed their variation to seasonality, with low to high season-

Table 2. Oxygen isotopic compositions of whole teeth from the rodent *Glis glis*. Where A, B, and C are individual specimens. N/A: not analoged.

	M1 left	M1 right	M2 left	M2 right	M3 left	M3 right	P4 left	P4 right	Upper and lower intra jaw mean		Overall intra jaw mean	
Specimen A												
Upper jaw	10.1	9.2	9.5	10.4	10.9	10.2	N/A	N/A	10.4	±0.6	10.4	±0.7
Lower jaw	11.6	11.3	10.3	10.6	10.5	10.4	N/A	N/A	10.8	±0.5		
Specimen B												
Upper jaw	10.8	10.8	9.9	10.4	10.7	8.3	8.8	9.5	10.2	±1.0	10.0	±0.8
Lower jaw	10.2	10.0	10.7	10.8	10.3	8.7	9.7	9.9	10.1	±0.7		
Specimen C												
Upper jaw	10.5	10.2	10.5	lost	11.9	12.0	N/A	N/A	11.0	±0.8	10.8	±0.8
Lower jaw	10.4	10.4	10.1	10.1	lost	11.8	N/A	N/A	10.6	±0.7		
Left and right	10.6	10.3	10.2	10.5	10.9	10.2	N/A	N/A				
Inter jaw mean	±0.5	±0.7	±0.4	±0.3	±0.6	±1.5	N/A	N/A				
Overall Inter jaw mean	10.5 ±0.6		10.3 ±0.4		10.5 ±1.2		N/A N/A					
Overall Mean							10.3 ±0.8					

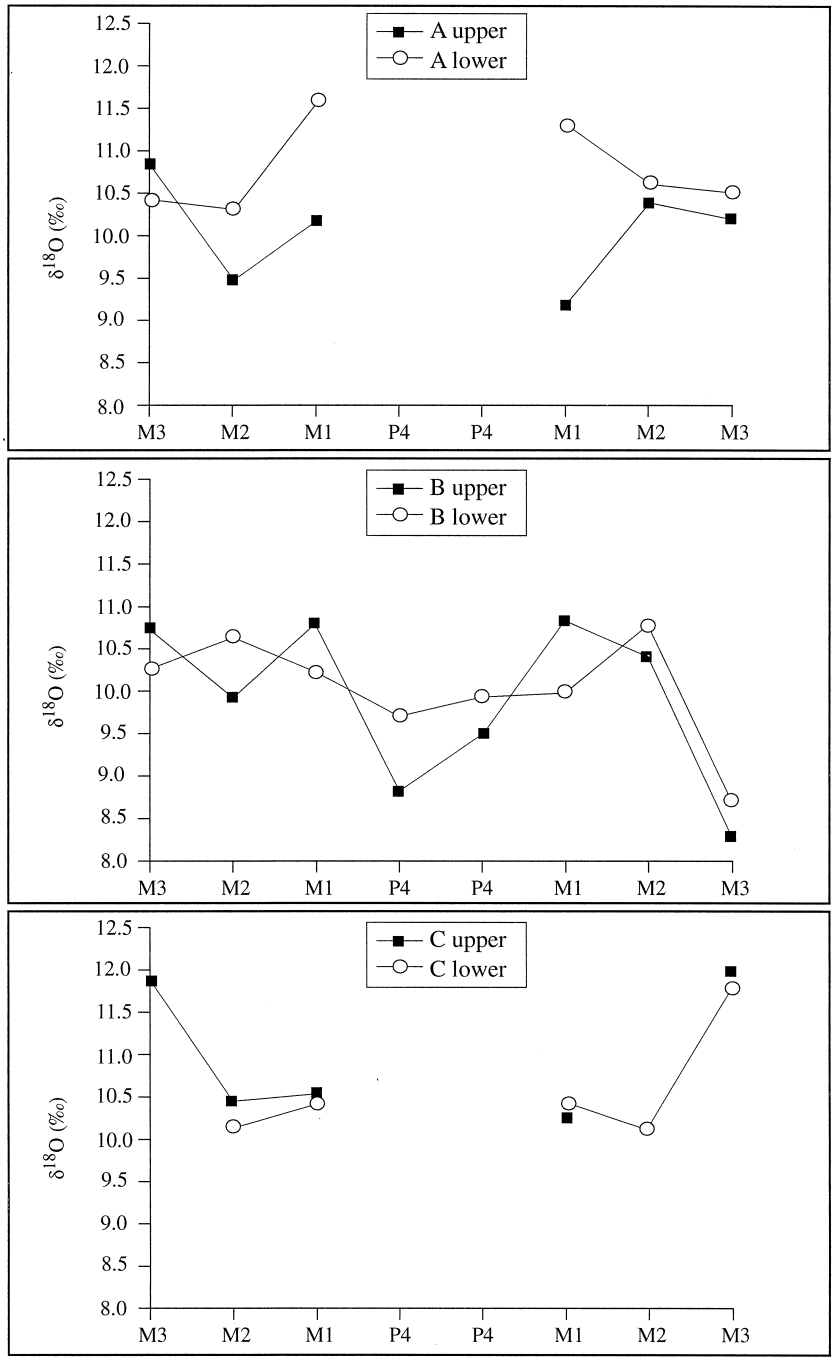

Fig. 5. Plots of oxygen isotopic composition against tooth position to illustrate intrajaw variation within the three *Glis glis* dormice A, B, and C.

ality giving variations of ~± 1 ‰ and ~± 3.5‰ respectively, rather than to natural variation, i.e., metabolism and timing of mineralisation. The teeth in this study are low-crowned molars which mineralise and erupt a short time after birth. Thus un-like high crowned teeth and those which continuously grow (e.g., rodent incisors), molars from one specimen record only a short climate period. However, in a study involving more than one specimen, individuals may have been born and their teeth mineralised and erupted at different intervals during the breeding season, thus recording seasonal temperature variations. Therefore the overall $\delta^{18}O$ variation in the teeth of *Apodemus sylvaticus*, whose breeding season lasts from March to October, could be attributed to variations in seasonality. This cannot, however, be the case with the $\delta^{18}O$ variation in the *Glis glis* teeth as they have one litter a year within a short breeding period.

With respect to their potential for future palaeoclimate reconstruction the large inter and intrajaw heterogeneity in the

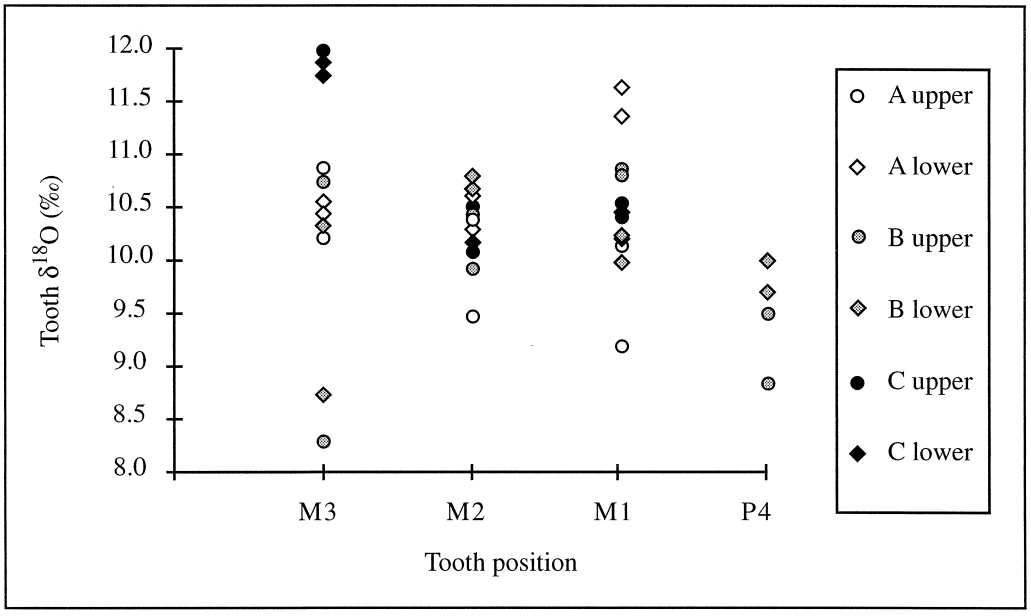

Fig. 6. Plots of oxygen isotopic composition against tooth position to illustrate interjaw variation within the three *Glis glis* dormice A, B, and C.

results from modern rodents *Glis glis* and *Apodemus sylvaticus*, indicates that single teeth cannot be used for isotope palaeothermometry because of the variations present within a single specimen and within a population. However, the overall standard deviation over both data sets was low (*Glis glis* = ± 0.81 ‰ and *Apodemus sylvaticus* = ± 1.3 ‰) indicating that a mean taken from the analyses of numerous rodents within a sampling program could be used for palaeoclimate reconstruction. In addition, in the *Apodemus sylvaticus* teeth there is a clear relationship between the timing of tooth mineralisation and increased homogeneity in $\delta^{18}O$ values. Even though not directly proven, this indicates that the $\delta^{18}O$ of early erupted teeth, i.e., DP4, M1, and M2, may be affected by weaning. This is logical because, before weaning, water sourced from mothers milk might involve different fractionations, hence affecting tooth isotopic composition. Therefore, based upon these results, we suggest that the sampling programme should involve the teeth of > 5 animals and also take into account rodent habitat (thus possible dietary influences) and the tooth types available (M3's and P4's, preferable as likely post weaning teeth). Ancient rodent habitat can be inferred from ecological diversity analyses and autecological study of the animals sampled (e.g., Collinson and Hooker, 1987, 2000).

4.2. Thermometry Calculations

As the $\delta^{18}O$ tooth phosphate results exhibit heterogeneity it is not possible to use one tooth analysis to obtain an estimate of ingested water (Eqn. 4). However, in *Glis glis* the standard deviation for the complete data set is relatively low (± 0.8‰). A mean $\delta^{18}O$ tooth phosphate value of +10.3‰ yields a $\delta^{18}O$ value of −12.4 ± 1.4‰ for ingested water (Eqn. 4). The measured mean $\delta^{18}O$ groundwater value for Wallingford, Oxfordshire is −7.2‰ (Darling et al., 1992). However, monthly rainfall records for the area show winter values normally around −11‰, with summer values around −4‰. Therefore the calculated meteoric water value of −12.4‰ is just outside the range for the groundwater measurements.

However, as the *Glis glis* samples were pretreated by heating to 1000°C, their individual $\delta^{18}O_p$ value and, therefore, the calculated body water and ingested water values, should be considered with caution. This is because results from the NIST 120c standard pretreated in the same manner indicated exchange with atmospheric water resulting in lower $\delta^{18}O$ values. Another potentially more important source of error, which could account for lower $\delta^{18}O$ values calculated for ingested water, is connected with the diet of *Glis glis*. As they were caught in an apple barn, it is possible that they obtained the majority of their ingested water from a diet of apples, rather than from a local water source. Therefore, their tooth $\delta^{18}O$ values might not be expected to directly reflect local meteoric water.

As with the *Glis glis* samples the average $\delta^{18}O$ tooth phosphate value for *Apodemus sylvaticus* was used (14.4± 1.3 ‰) in the calculation of body water $\delta^{18}O$. Eqn. 4 predicts an ingested water composition of −5.6 ± 2.2‰. However, the variation of ± 2.2‰ is large and may result from seasonal variations, because of the March to October breeding season. There are thus appreciable variations in seasonal temperature which will affect the $\delta^{18}O$ of local meteoric water. However, the $\delta^{18}O$ of local meteoric water, and more importantly the variation, is reduced to −5.2 ± 0.7‰ if only M3 teeth are used, which, because they are the last to mineralise, should be the most homogenous. However, only three M3 teeth were analysed. The oxygen isotopic composition of the water body around which *Apodemus sylvaticus* lived was measured at −4.3‰. This is −1.3‰ lower than that calculated from the rodent teeth, but is within the overall error range, i.e., −3.4 [−5.6] −7.8‰.

The mean summer rainfall $\delta^{18}O$ value for southern England is −4.5‰ (Darling et al., 1992). The pond water was sampled once for independent analysis during the summer months and should, therefore, be reflected by the rodent tooth $\delta^{18}O$, which are believed to mineralise at this time. This has been demonstrated as the measured pond water value (−4.3‰), the historical mean rainfall value (−4.5‰, Darling et al., 1992), are both within the range calculated for the rodent tooth ingested water (−5.6 ± 2.2‰). However, several other factors such as lake size (and, therefore, rates of evaporation), temperature, and amount and source of precipitation should also affect the lake water $\delta^{18}O$ value. In addition, as only one lake water sample and $\delta^{18}O$ value was obtained, and the breeding season for the resident *Apodemus sylvaticus* is March to October over which there is an appreciable temperature range, the comparability of the calculated and measured $\delta^{18}O$ results is promising for palaeoclimate reconstruction.

4.3. Application of Technique to Fossil Teeth and Palaeoclimate Reconstruction

The laser fluorination and pretreatment technique conducted on whole *Glis glis* and *Apodemus sylvaticus* teeth should be applicable to fossil rodent teeth with minor adjustments. For example, owing to the potential for postdepositional diagenetic alteration of biogenic dentine (among others, Wang and Cerling 1994; Kohn et al. 1999) only enamel should be used in fossil studies. In addition, there is the potential for postdepositional diagenetic alteration of the CO_3^{2-} and OH^- components of fossil enamel (Kohn et al. 1999). The phosphate component of biogenic apatite constitues the major source of oxygen, but there is the potential for oxygen exchange between CO_3^{2-} and OH^- and PO_4^{3-} during laser fusing. However, even though this should be taken into account when considering the $\delta^{18}O$ results from fossil enamel, it should also be noted that samples are heated to fusion temperatures progressively while the sample is being pumped to high vacuum. Therefore, labile oxygen components are removed under conditions that do not favor equilibrium isotopic exchange, thus reducing the potential for oxygen exchange with the PO_4^{3-}.

The study of the *Apodemus sylvaticus* teeth indicates that the $\delta^{18}O$ value of a shallow pond ~100 m diameter, in a temperate climate, can be predicted. However, the *Glis glis* study failed to predict the $\delta^{18}O$ value of local meteoric water, because there was the strong implication that they sourced their water intake largely from an apple diet. This highlights the importance for strong palaeoenvironmental evidence that indicates the association of fossil rodents with a source of drinking water. Such evidence is provided by Collinson and Hooker (2000) for one Eocene rodent.

This study also suggests that mammals as small as wood mice have a metabolism constant enough to allow their tooth phosphate isotopic composition to be used as a proxy for the isotopic composition of a palaeowater and therefore palaeoclimate. In addition this study suggests that M3 and P4 teeth, where available, should be used in palaeoclimate studies, as they are least likely to be affected by unpredictable metabolic fractionation effects before weaning. However, the $\delta^{18}O$ results from this study also indicate that even with these teeth there is likely to be a variation in the $\delta^{18}O$ results (± 1 – 2‰). This may be attributed to seasonal variations over the time period represented by the sample so that tracking palaeoclimate change over time, e.g., across the Eocene/Oligocene boundary, needs to be based upon average values from as many tooth samples as possible from each horizon.

5. CONCLUSIONS

DLF is an analytical technique which measures the phosphate oxygen only component of biogenic apatites which are too small (~1 – 2 mg) to be analysed using classical chemical separation techniques. The CO_2 laser operates at 10 μm, which is strongly absorbed by phosphates, allowing a thermal reaction in the presence of BrF_5, which releases 100% of the phosphate oxygen, leaving a pure calcium fluoride residue. However, to measure only the phosphate oxygen component of biogenic apatite, a pretreatment is required to remove non-phosphate oxygen (i.e., structural carbonate, the hydroxyl ion, water and organic matter (OM)). Heating of phosphate samples to 1000°C substantially reduces, if not completely removes, all non-phosphate oxygen. However, heating to 1000°C results in some samples being susceptible to oxygen exchange with atmospheric water, lowering the $\delta^{18}O$ value of the phosphate oxygen. To prevent atmospheric exchange, samples were heated to 400°C to oxidise and remove OM, before fusing within the sample chamber under high vacuum, to release CO_2. NIST 120c samples heated to 400°C and fused within the sample chamber before isotope analysis produced a value of +21.3 ± 0.4‰ in accordance with the reported values (+21.4 ± 0.4‰, $BiPO_4$, Bryant et al. (1994) and 21.7± 0.2‰, $AgPO_4$, Lécuyer et al. (1993)).

Although the results from the modern rodents *Glis glis* and *Apodemus sylvaticus* show large inter and intrajaw heterogeneity, the standard deviation over both data sets was low (*Glis glis* ± 0.81 ‰ and *Apodemus sylvaticus* ± 1.3 ‰). This indicates that whilst single teeth cannot be used for isotope palaeo-thermometry, because of the variations present within a single specimen and within a population, a mean taken from the analyses of numerous teeth may be useful. The minimum number of animals, not teeth, should be >5 and post weaning teeth, i.e., M3 and P4 mol/L, should be used.

Results from the molars of three wood mice (*Apodemus sylvaticus*) from a naturally bounded site, show an interjaw variation of ± 1.3 ‰. Using the equations, derived from lab rodents, an ingested water value of −5.6 ± 2.2‰, is calculated. This average value is 1.3‰ lower than that measured for the lake around which they lived, but within error. This suggests that mammals as small as wood mice have a metabolism constant enough to allow their tooth phosphate isotopic composition to be used as a proxy for the isotopic composition of a proximal water body and therefore to be used in palaeoclimate reconstruction.

Acknowledgments—This research was funded from a NERC CASE studentship awarded to Elaine Lindars and a NERC reserach grant, GRE/12864 (Mattey, Collinson, Hooker), employing Stephen Grimes. Many thanks to Joel Baker, Mark Brownless, Nathalie Grassineau, and David Lowry for assistance in the stable isotope analysis. We are indebted to and appreciative of Gordon Cressey, Andy Currant, John Spratt, Jo Cooper, and Terry Williams at the Natural History Museum for help and assistance in sample preparation. We would also like to thank Matthew Kohn and two anonymous reviewers whose comments

greatly improved the manuscript. The associate editor, Prof. David Cole is also thanked for his assistance in handling the review process.

Associate editor: D. R. Cole

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APPENDIX

Table A1. A summary of Electron Microprobe (EM) data showing compound percents of phosphorus (P) and fluorine (F) for several phosphates with various times of prefluorination.

	0h		0.75h		2.5h		48h	
Length of Pre-fluorination	P (%)	F (%)	P (%)	F (%)	P (%)	F (%)	P (%)	F (%)
Pig tooth	14.8 ± 2.3	0.0 ± 0.0	15.3 ± 0.6	6.9 ± 0.6	14.3 ± 0.5	6.6 ± 1.7	13.2 ± 0.8	11.2 ± 2.7
Epiwala Apatite Crystals	18.3 ± 0.1	1.6 ± 0.0	—	—	17.8 ± 0.5	1.7 ± 0.2	—	—
Pig Bone	—	—	—	—	13.0 ± 0.2	13.0 ± 0.7	—	—
Pig Bone	15.0 ± 1.6	0.2 ± 0.0	—	—	11.3 ± 1.1	3.5 ± 1.8	—	—
Pig Bone pre-treated with NaOCl	15.2 ± 1.8	0.3 ± 0.1	11.8 ± 2.2	13.2 ± 2.3	—	—	—	—

Table A2. The oxygen isotopic compositions of phosphate standards. CT refers to data obtained by conventional techniques and the values are means of data measured on pure phosphate; DLFT referes to the laser fluorination technique, values are means taken from measurements made on the same phosphate but before and after different pretreatments. The heat pre-treatment is the ignition of samples at 400 °C or 1000 °C for 1 hour, the fuse pre-treatment is the lasering of samples within the LF chamber whilst the chamber is pumping to high vacuum, and the ignition and fusing (*e.g.* 400 °C + fuse) pretreatment is the lasering of samples which have already been heated to 400 °C or 1000 °C conventionally then the lasering of the sample to try to reverse the CaO to CaOH reaction. (1) Bryant et al. (1994).

	CT	DLFT				
Phosphate	($BiPO_4$)	unheated	100 °C	100 SDC + fuse	fused	400 °C + fuse
Pig Bone	—	11.5‰	10.0‰	12.0‰	11.9‰	
		±4.0	±0.4	±0.1	±0.3	±0.1
		n = 11	n = 17	n = 3	n = 3	n = 3
Epiwala Apatite	—	12.0‰	11.6‰	12.4‰	—	—
Crystals		±0.4	±0.3	±0.2	—	
		n = 6	n = 2	n = 3		
Cow bone	—	17.4‰	9.5‰	11.4‰	20.3‰	16.4‰
		±0.5	±0.9	±0.6	±0.7	±0.5
		n = 19	n = 2	n = 2	n = 3	n = 10
NIST 120c	(a) 21.4‰	20.4‰	12.5‰	13.6‰	21.5‰	21.29‰
	±0.4	±0.5	±0.6	±0.04	±0.5	±0.4
	n = 15	n = 33	n = 4	n = 4	n = 8	n = 13

[a]Bryant et al.

Table A3. Mean isotopic values for the internal standard QBLC Quartz and the international standard NIST 120C with and without pre-treatment.

		$\delta^{18}O$ (‰)	n
Quartz			
QBLC Quartz		8.78 ±0.15	32
Phosphate			
NIST 120c	(all data)	20.4 ± 0.5	33
NIST 120c	(single day)	20.8 ± 0.3	6
NIST 120c	(1000°C)	12.5 ± 0.6	4
NIST 120c	(400°C + fused)	21.3 ± 0.4	13

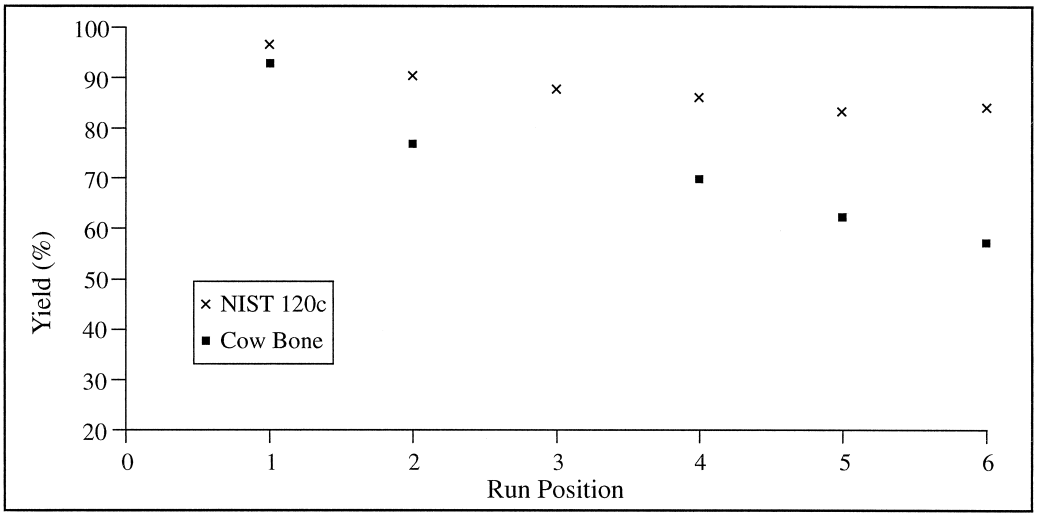

Fig. A1. A plot of oxygen yield against run position during two single days for phosphate NIST 120c, a biogenic phosphate, and a cow bone, showing the decrease in yield throughout a single day.

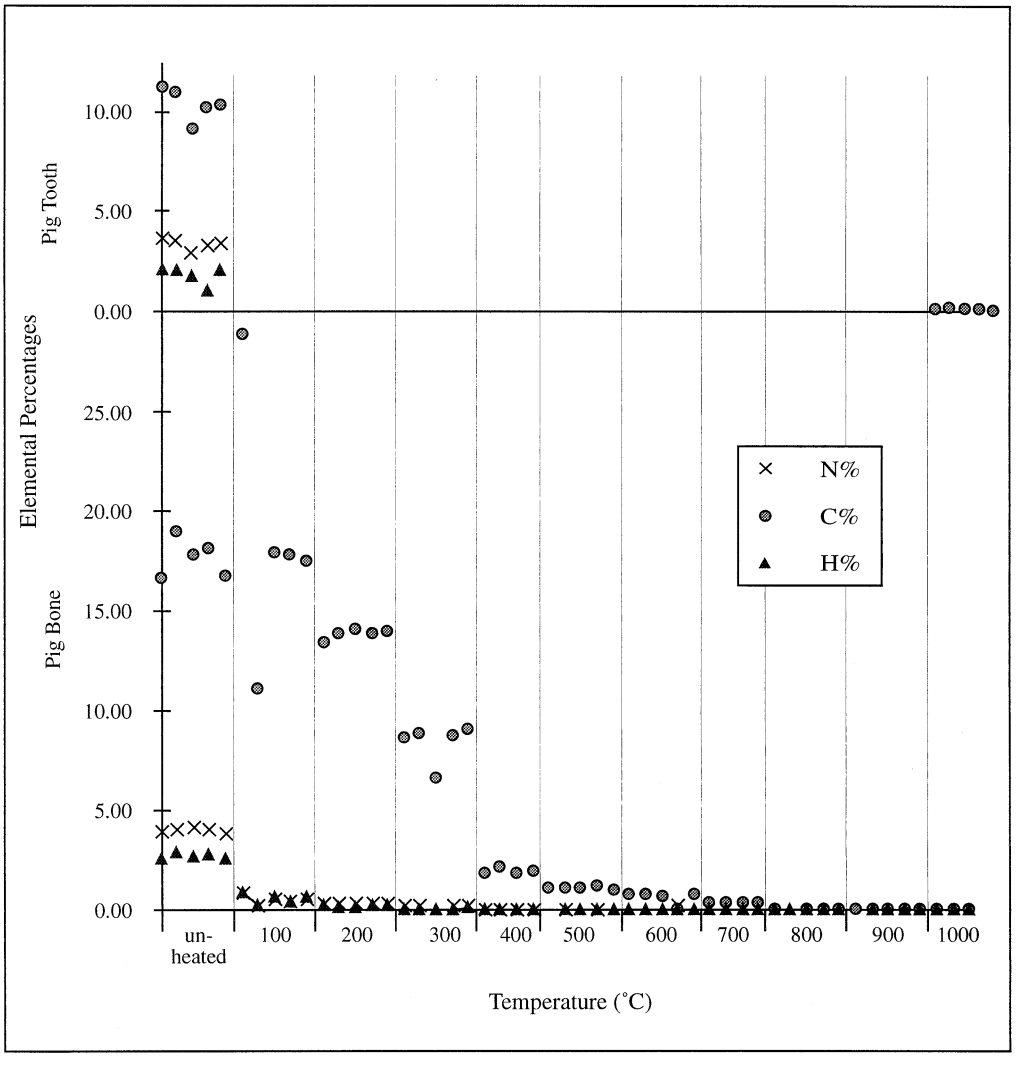

Fig. A2. A plot of carbon, hydrogen and nitrogen elemental percentages within pig bone sequentially heated to 1000 °C and pig tooth unheated and heated at 1000°C.

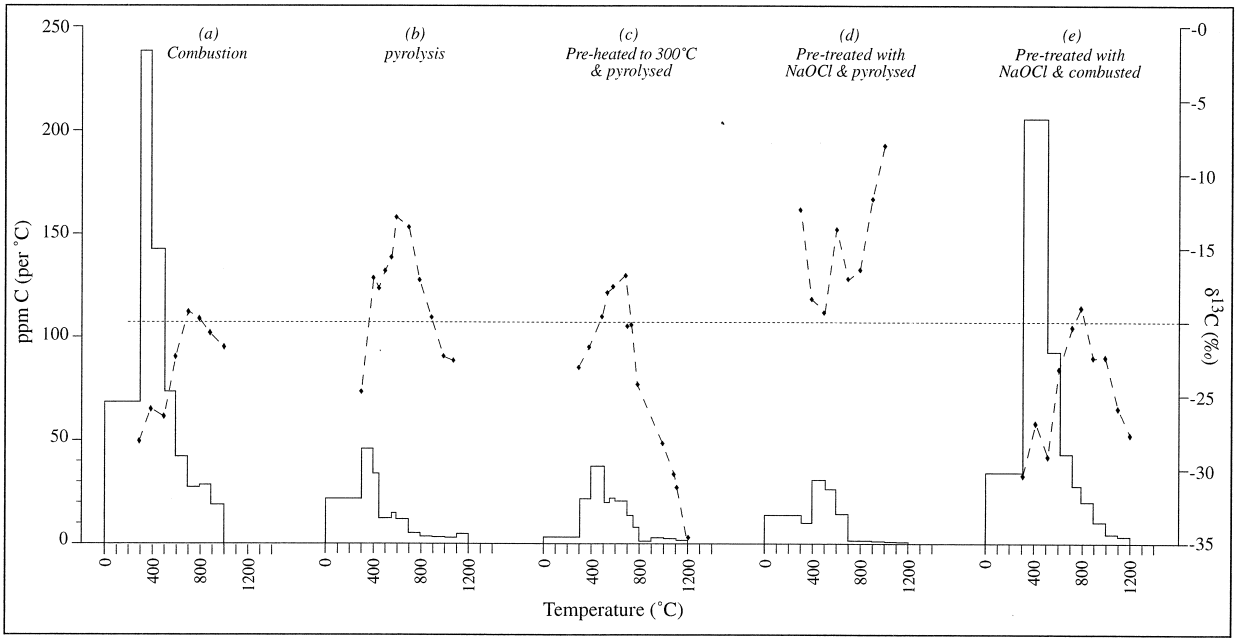

Fig. A3. Stepped heating profiles for pig bone. The histograms represent the amount of carbon in ppm released per °C (left axis) against temperature steps. The dashed lines represent $\delta^{13}C$ values (right axis) against temperature steps. Plot (a) shows data from the combustion of pig bone, (b) data from the pyrolysis of pig bone, (c) data from the pyroloysis of pig bone after its pre-heating to 300°C, (d) data from the pyrolysis of pig bone after pre-treatment with NaOCl, and (e) data from the combustion of pig bone after its pre-treatment with NaOCl.

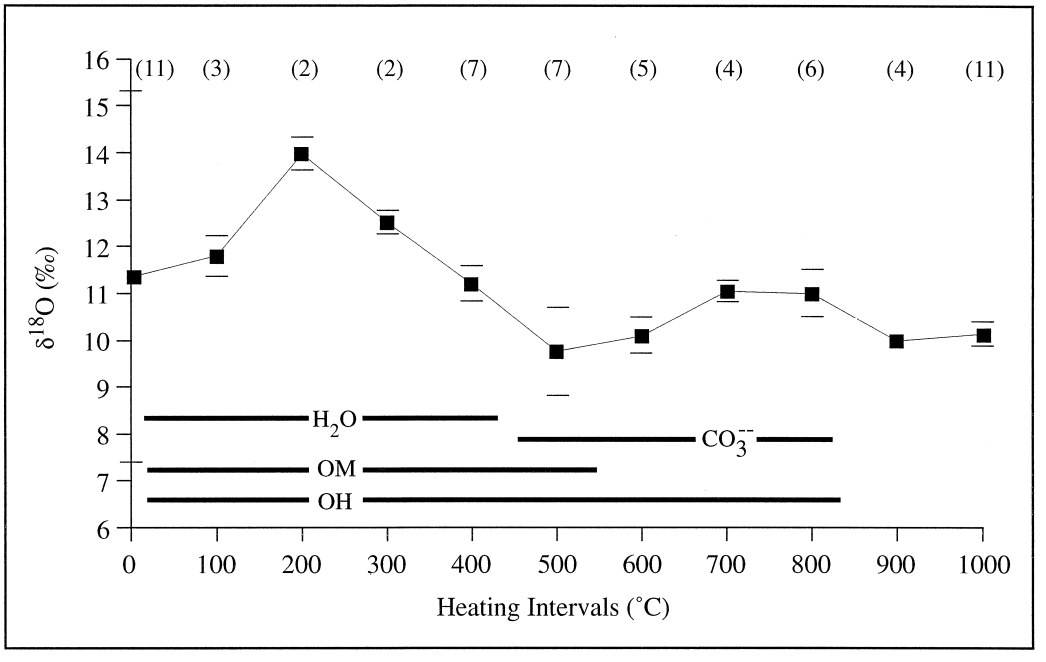

Fig. A4. A plot of oxygen isotopic composition against temperature for the sequential heating of pig bone. Where the values in brackets represent the number of analyses carried out, (■) represents the mean isotopic composition at that temperature, (−) represents the error (1σ) associated with those means. The labeled bars indicate the source of oxygen for given temperature.